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PLANT ECOLOGY

Floral differentiation and growth rhythm of rhizome buds of the spring ephemeroid plant *Adonis amurensis* Regel et Radde

Diferenciación floral y ritmo de crecimiento de las yemas de rizomas en la especie primaveral efímera *Adonis amurensis* Regel et Radde

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Running title: Differentiation and growth of *Adonis Amurensis* buds

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Abstract. Spring ephemeroid plants complete their aboveground reproduction and growth during the short growing season, and may go dormant subsequently underground. Little is known about the underground dormancy and biological activities of the plants. In this study, we observed organogenesis and growth rhythm of rhizome buds of *Adonis amurensis* Regel et Radde, a spring ephemeroid plant from the Changbai Mountains in northeastern China. Our results showed that *A. amurensis* did not go through summer dormancy, but started producing mixed buds on rhizomes

soon after the aboveground parts had died. The buds grew in length and diameter following an exponential model with significantly slower rates in the early organogenesis stages, and faster rates before winter dormancy. Allometric analyses of growth rates in length and diameter indicated that in general diameter increased with length at a slower rate. Energy allocation, and adaptation to the environment, might play an important role in the growth dynamics and allometric relationships in *A. amurensis*.

Keywords: *Adonis amurensis* Regel et Radde; Rhizome bud; Exponential growth; Allometric growth; Ecological dormancy.

Resumen. Las plantas efímeras de primavera completan su crecimiento y reproducción aéreas durante la corta estación de crecimiento, y subsiguientemente pueden entrar en dormancia en el suelo. Se conoce poco sobre la dormancia subterránea y las actividades biológicas de las plantas. En este estudio, observamos la organogénesis y el ritmo de crecimiento de yemas de rizomas de *Adonis amurensis* Regel et Radde, una planta efímera en primavera de las Montañas Changbai en el noreste de China. Nuestros resultados mostraron que *A. amurensis* no entró en dormancia durante el verano, sino que comenzó a producir yemas mixtas en rizomas poco después de la muerte de la parte aérea. Las yemas crecieron en longitud y diámetro siguiendo un modelo exponencial con tasas significativamente menores en los primeros estados de la organogénesis, y tasas mayores antes de la dormancia invernal. Análisis alométrico de tasas de crecimiento en longitud y diámetro indicaron que en general el diámetro incrementó con la longitud a una tasa menor. La distribución de energía, y la adaptación al medio, pueden jugar un rol importante en la dinámica del crecimiento y relaciones alométricas en *A. amurensis*.

Palabras clave: *Adonis amurensis* Regel et Radde; Yemas de rizoma; Crecimiento exponencial; Crecimiento alométrico; Dormancia ecológica.

INTRODUCTION

Spring ephemeroid plants in the Northern Hemisphere are perennial herbs with flowers emerging aboveground in the early spring, and vegetative growth and fruiting completed before the fully expanded leaves of the canopy block the light in late spring or early summer (Lapointe, 2001; Zheng & Hu, 2009). This unique life history strategy pertains to their ecological functions such as ecosystem productivity (Ye et al., 1992). Therefore, these plants are an important floristic element of the broadleaf deciduous forest, and have been extensively studied in terms of their floristic diversity and geographic distribution (Zhang, 2008; Zheng et al., 2014), community ecology (Schemske, 1978; Yu & Zhou, 2003), physiological ecology (Sohn & Policansky, 1977; Schemske et al., 1978; Givnish, 1982; Nishikawa & Kudo, 1995; Ohkawara et al., 1997; Kudo et al., 2001; Frenne et al., 2010) and reproductive strategies (Maslova et al., 2003; Constable et al., 2007; Tu et al., 2012).

Adonis amurensis Regel et Radde is one of the dominant spring ephemeroid plants in the forest of the Changbai Mountains in northeastern China, and has important ornamental and medicinal values. It is a geophyte with short rhizomes. Previous studies of *A. amurensis* have addressed issues in the areas of medicinal uses (Pauli & Junior, 1995; You et al., 2003; Kuroda et al., 2010; Shang et al., 2013) , landscape application (Kawano & Hayashi, 2004), reproductive biology (Chen, 2013; Sun et al., 2015), flowering phenology (He et al., 2014; Jia & Pu, 2015), and biological characteristics (Francis et al., 2005). However, there is no report about the

characteristics of floral differentiation. When the aboveground parts of *A. amurensis* die in the late spring, biological activities such as rhizome growth, bud formation, and floral differentiation happen underground, so plants can sprout again in the next spring. Although it is clear that soil freezes in the winter months and plants go dormant, little is known about whether there is dormancy in the summer and fall.

In a wide range of spring flowering perennial geophytes, floral bud initiation is completed within the renewal bud period in late summer and autumn. This development pattern has been reported in several spring- flowering bulbous plants, such as *Narcissus* (Noy-Porat et al., 2009; Feng et al., 2015), *Tulipa* (Kamenetsky et al., 2003), *Hyacinihus* (Hartsema, 1961), *Iris* (Le Nard & De Hertog, 1993) and *Crocus* (Wilkins, 1985; Molina et al., 2005); in these species floral differentiation occurs during summer, and then floral buds go into summer dormancy. However, no reports have described the morphological developmental traits of spring ephemeroid plants during summer and autumn, after the death of the aboveground plant parts. Studies of mature plants of *A. amurensis* suggested that there were two periods of dormancy (summer and winter) and growth (spring and fall) in their life history (Wan & Liu, 1996). However, the information about the growth patterns and dynamics of the underground activities on *A. amurensis* is unclear. Filling in this knowledge gap will contribute to increase our understanding of the life history strategies and the evolution of these spring ephemeroid plants.

In this study, we examined various developmental stages of *A. amurensis* to gain insights into the patterns of rhizome growth and floral differentiation. Specifically, we focused on the following questions: 1) is there a summer dormancy?; 2) when do flowers and leaves form before winter dormancy?; 3) is rhizome growth constant throughout the developmental stages?, and 4) what is the pattern of rhizome growth?

MATERIALS AND METHODS

Ethics statement. No specific permits were required for the described field studies. We confirmed that the location was neither privately-owned nor protected in any way. The experiment did not involve endangered or protected species.

Natural conditions of the research area. This research was conducted in the Tonghua Normal University (TNU) field station (41° 44’ 47.04” N, 125° 58’ 49.63” E, altitude 429 m.a.s.l.), located in the Tonghua section of the southeast Changbai Mountains. The area is of the north temperate continental monsoon climate with cold, dry winters and hot, humid summers. The annual average temperature is 5.5-6.0 °C, the average frost-free period is 140-171 d, and the mean annual precipitation is 893-1083.8 mm, which mostly falls from July to August (Song et al., 2011).

Research methods. In the natural forest of Tonghua, *A. amurensis* emerges aboveground in early March and blooms in mid-March. Leaves expand in early April, and fruits ripen in mid-May. At the end of May, the aboveground parts wither and die (He et al., 2014). Based on the phenology, we transplanted 300 plants that have completed their fruiting phase to a site at the TNU research station, which had similar environmental conditions to the natural forest. From 19 May to 21 July, rhizomes (each with 8-13 buds) were taken every three days. From 21 July to 4 November, the sampling interval was about 20 d. Growth in length and diameter was measured for each bud using a vernier caliper. Bud and floral differentiation determinations were made following Yang et al. (2004) and Li et al. (2012). The buds were first fixed in formalin-acetic acid-alcohol for at least 36 hrs. Then they were dehydrated using

absolute ethanol firstly, acetone secondly, and isoamyl acetate thirdly, 30 minutes per step. Thereafter, a critical point was to dehydrate buds. With this purpose, they were dried in carbon dioxide, vacuum evaporated and sprayed with metal. The observations of bud and floral differentiation were done under a Hitachi S-3000N scanning electron microscope.

Data analysis. One-way ANOVA analyses were conducted using the SPSS17.0 statistical software to test whether growth in bud length and diameter differed significantly across the various developmental stages. Levene's and T2 Tamhane's tests were used to test for heteroscedasticity (Búrquez et al., 2010). The coefficient of variation (*CV=SD/M*, where M is the average and SD is the standard deviation) was calculated to examine the relative variations among samples. Growth rates for bud length and diameters were formulated as total growth (μm) during any given period divided by the growth period (days). This is, $S_{t+1} = (LM_{t+1} - LM_{t})/T$, where S is the growth rate during a certain period; *LM* is the average bud length/bud diameter of each sample; *T* is the growth period in days, the interval between two subsequent samplings; *t* is the sampling time; and *t*+1 is the next sampling time.

In the regression of the bud growth rhythm, the first measurement was set as the first day of accumulated days. The correlation analyses for bud length and diameters were based on the 11 samplings. Of the following three functions, the one with the highest correlation was selected as the quantitative analysis model for regression and correlation analyses:

(1) Linear function: $y = a + b\,x$,

(2) Exponential function: $y = a\,e^{b\,x}$ and

(3) Power function: $y = a\,x^{b}$.

RESULTS

Differentiation processes and morphological changes in rhizome buds. The rhizomes of *A. amurensis* initiated new roots and buds at the end of the flowering phase in early April (Fig. 1: 1). The aboveground parts wither from mid to end of May. Instead of becoming dormant, the rhizome buds continue to grow, producing flowers and leaves. Floral morphogenesis was completed from mid to end of July (Fig. 1: 2-3). The differentiation of *A. amurensis* rhizome buds went through six stages. First, bud-scales formed from the flat shoot apical meristem acropetally and spirally between mid-April and late May (V) (Fig. 2: 1). The terminal flower primordium was small, flat and triangular initially (F1) (Fig. 2: 2). Second, as the volume of the flower primordium increased and elongated, 8 to 10 sepal primordia initiated in a clockwise fashion and were arranged in two whorls. Each primordium was crescent-shaped with a truncated apex (F2) (Fig. 2: 3). Third, 12 to 13 petal primordia were initiated in three whorls (Fig. 2: 4) in a sequential and centripetal manner (F3). Fourth, stamens formed sequentially in three or four whorls (Fig. 2: 5). After the initiation of the stamen primordia, the top of the receptacle became flat (F4). Fifth, the pistil primordia were initiated in a clockwise and centripetal manner filling the receptacle without any lacuna (Fig. 2: 6-7). Each carpel was plicately formed (Fig. 2: 8). Meanwhile, the stamen primordium differentiated into anther and filament (F5). Finally, all the floral organs developed and matured (Fig. 2: 9). The floral differentiation period lasted for 60 d. In addition, in the bud-scale axil, leaf primordium formed at the same time as the flower primordial (Fig. 2: 10), and later became pinnately divided when all the floral organs formed (Fig. 2: 11-12). After finished the differentiation, the buds continued development until the end of November, when soil froze and plants went

dormant for the winter (Fig. 1: 4-5); this growth can be divided into 5 stages (D1-D5). Flowers open in the early spring, after the snow starts melting (Fig. 1: 6).

FIGURE 1

FIGURE 2

Growth rhythm and plasticity analyses of rhizome buds. *Adonis amurensis* rhizome did not experience summer dormancy. Bud length and diameter did not change significantly in the early stages, but active floral differentiation occurred during this period of time (Table 1). Growth in both length and diameter generally increased significantly after floral differentiation was completed between stages F3/F4; D3/D4 in bud length, and F3/D1, D1/D3, and D3/D5 in bud diameter (Table 1). The coefficient of variation (CV) reflects the relative variation degree of a sample, which can reflect indirectly the growth plasticity of rhizome buds. During different growth stages, the CV of bud length was within the range of 9.5-31.8%, with an average of 16.7% (Table 1). The CV of bud diameter was within the range of 9.6-19.8%, with an average of 14.7%. The growth plasticity of rhizome bud length and diameter, as evaluated by the CV, showed great changes at different times. The shortest bud length was on 19 May (V), while the smallest bud diameter was on 7 August (D1). The maximum values of length and diameter were both on 20 October, with the bud length being nearly 1.6 times that of the diameter. This suggested that the growth plasticity of bud length was greater than that for bud diameter, but the growth processes were not synchronized.

TABLE 1

The growth rhythm and unsynchronized plasticity of growth (see Table 1) could be further analyzed using the growth rate (Fig. 3). The growth rate of bud length was 48.9 μm/d in the stage between bud-scale differentiation (V) and the initial stage of floral differentiation (F1). The rate then declined and reached its minimum of 5.8 μm/d during the sepal (F2) and petal (F3) differentiation stages. Then, the rate increased to 134.9 μm/d during the petal (F3) and stamen (F4) differentiation stages. After that, the rate declined slightly from 30 June (F4) to 7 August (D1), and then decreased dramatically to 38.9 μm/d by 9 September (D2). This was followed by a rapid increase to 801 μm/d in the stage between 22 September (D3) and 20 October (D4), before decreasing to 119.7 μm/d on 4 November (D5). In contrast, the bud diameter growth rate was not synchronized with bud length in most developmental stages. The lowest rate, 6.2 μm/d, was observed at the stamen and pistil differentiation stages; whereas, the highest rate of 72.6 μm/d occurred during the stage of 9-22 September (D2-D3). Thus, the results suggested that the *A. amurensis* rhizome buds displayed allometric characteristics, with periodic increases in elongation and diameter increase.

FIGURE 3

Growth model of rhizome buds. Growth of bud length and diameter in *A. amurensis* followed closely the exponential model ($P<0.001$) (Fig. 4). The growth rate of bud length ($b=0.0145$) was 1.88 times that of bud diameter ($b=0.0077$), There was a significant allometric relationship between the growth rates of length and diameter ($r=0.9071$, $P<0.001$), and the allometric equation was D (diameter) = 0.2809 L (length)$^{0.5321}$ (Fig. 5). During the underground development stages, the allometric

properties of bud growth were more distinct than that of floral differentiation.

FIGURE 4

FIGURE 5

DISCUSSION

Geophytes possess buds developing underground which help them to avoid damage from harsh conditions such as cold, heat, or drought (Yang & Zhu, 2011a; Volaire & Norton; 2006). The underground parts may enter dormancy for over nine months in summer and winter (Lapointe, 2001; Nishikawa & Kudo, 1995). As a spring ephemeroid geophyte, *Adonis amurensis* has been considered as a plant with two dormancy and two growing seasons (Wan & Liu, 1996). However, our observations of the developmental stages suggest that rhizome buds of *A. amurensis* do not go through summer dormancy. Instead, early summer is an important period for the differentiation and development of floral organ and leaf primordia (Fig. 2, Table 1). Bud scales form first followed by the initiation of floral organs in the order of sepals, petals, stamens, and carpels, each with multiple whorls. Leaf primordia form simultaneously with flower primordia (Fig. 2). The development pattern offers adaptive values to *Adonis* plants living in the understory environment of the cold temperate climate, where they have a short time period (40-60 days) to complete their reproduction and growth.

Adonis amurensis produces solitary terminal flowers. Therefore, its growth in the entire life history follows an S-shaped model as in many plants and animals with determinate growth (Yang & Zhu; 2011b). However, the rhizome buds of *A. amurensis* grow at an exponential rate in both length and diameter, fitting the early

phase of the S-shaped growth model (Fig. 4). The growth rates in both length and diameter are slow in the early floral differentiation and increase significantly later, especially in the period before winter dormancy (Figs. 2, 3, Table 1). The reasons may be complex, but energy allocation to floral development may delay growth of buds in length and diameter, while later, faster growth may help plants speed their sprouting in the early spring, when the snow starts melting and the top soil thaws.

Allometric relationships have been found among many quantitative characters of plants and animals, including reproductive and vegetative growth (Yang & Zhang, 2005), biomass allocation aboveground and underground (Zhang et al., 2002), and the biomass allocation of leaves and roots (Yang & Li, 2003; Zhou & Yang, 2006). In this research, the growth rate of bud length was always greater than that of bud diameter. However, asynchrony existed between the two at some specific times, leading to an allometric growth rhythm of periodic elongation and diameter increase. This may explain the relationship between characteristics of bud growth during underground development and the changes in those characteristics at different times.

During rhizome bud development, bud elongation and radial enlargement both increased rapidly with the gradually increasing temperature difference between day and night. The unsynchronized fluctuations in the growth rates revealed an allometric phenomenon in which the bud first elongated lengthwise, and then radially enlarged late in the growing season. This might be a response to a growth regulatory substance secreted by *A. amurensis* in reaction to the soil depth. This response is of great significance as it may control bud growth and developmental processes. This would be by regulating its vertical and radial allometric growth rates, making buds reach a certain size and be positioned close to the soil surface before winter dormancy. Further research is needed on the physiological regulatory mechanisms of allometric growth.

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Table 1. Quantitative characteristics of rhizome buds of *A. amurensis* at different sampling times.

Tabla 1. Características cuantitativas de yemas de rizomas de *A. amurensis* en diferentes fechas de muestreo.

Sampling date	Growth stage	Length (cm)		Diameter (cm)	
		M ± SD	CV（%）	M ± SD	CV（%）
19 May	V	0.530 ± 0.050 a	9.5	0.195 ± 0.026 a	13.2
28 May	F1	0.574 ± 0.083 a	14.4	0.202 ± 0.026 a	13.1
9 Jun	F2	0.600 ± 0.085 a	14.2	0.220 ± 0.033 a	15.0
17 Jun	F3	0.605 ± 0.084 a	13.9	0.228 ± 0.029 a	12.8
30 Jun	F4	0.780 ± 0.099 b	12.7	0.246 ± 0.027 ab	10.8
14 Jul	F5	0.940 ± 0.139 b	14.8	0.255 ± 0.046 ab	18.2
7 Aug	D1	1.225 ± 0.185 bc	15.1	0.282 ± 0.027 b	9.6
9 Sep	D2	1.353 ± 0.252 c	18.6	0.334 ± 0.059 bc	17.8
22 Sep	D3	1.542 ± 0.318 c	20.6	0.428 ± 0.073 c	16.9
20 Oct	D4	3.785 ± 1.204 d	31.8	0.557 ± 0.110 cd	19.8
4 Nov	D5	3.965 ± 0.733 d	18.5	0.655 ± 0.093 d	14.1

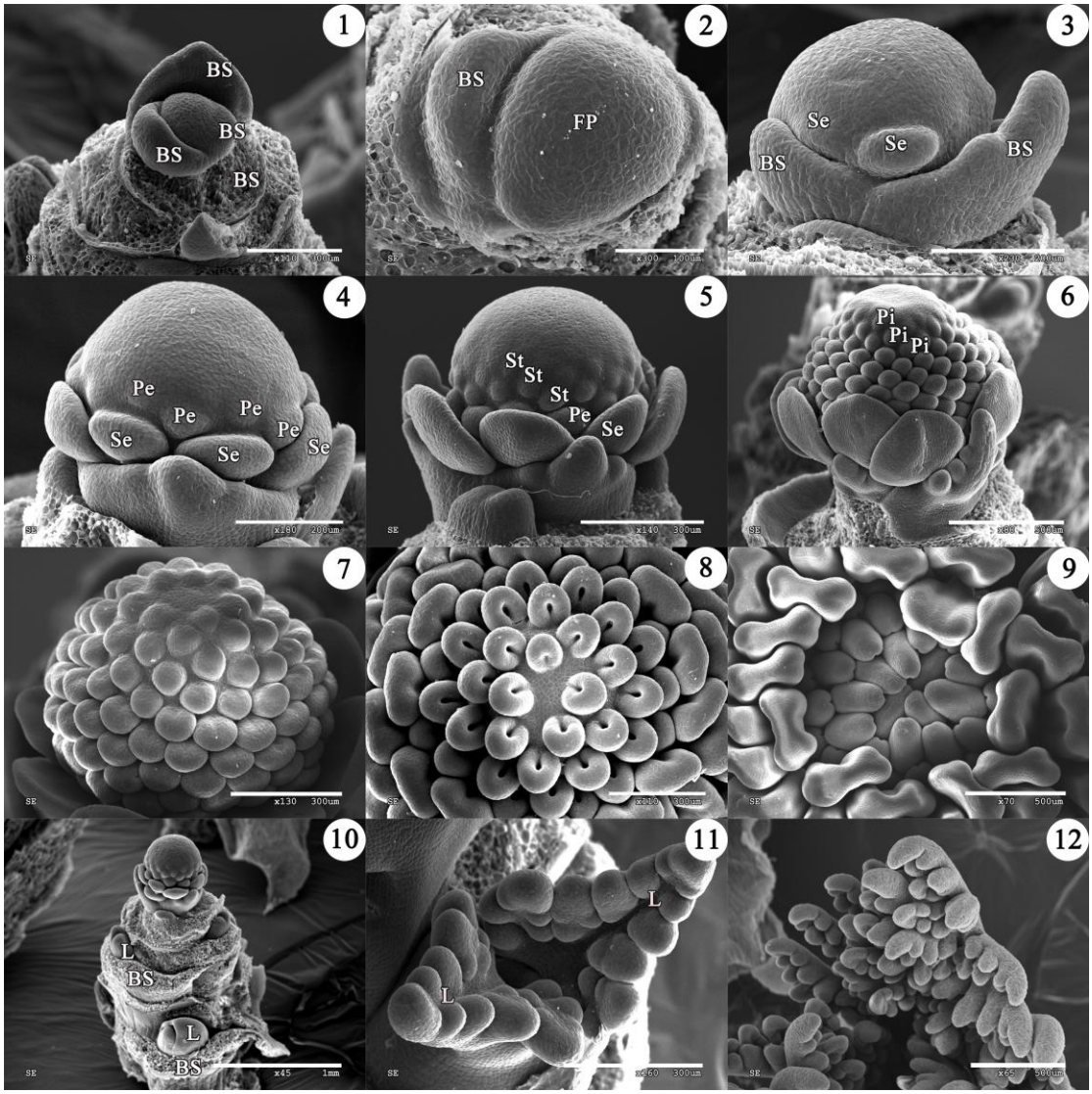
Note: Means followed by different letters are significantly different at the P<0.05 level. Abbreviations or symbols: M, Mean value; SD, Standard deviation; CV, Coefficient of variation; V, Bud scale differentiation stage ; F1, Initial stage of floral differentiation; F2, Sepal differentiation stage; F3, Petal differentiation stage; F4, Stamen differentiation stage; F5, Pistil differentiation stage; D1 to D5, Different developmental stages.

Nota: Promedios seguidos por letras diferentes son significativamente diferentes a un nivel de P<0,05. Abreviaturas o símbolos: M, valor promedio; SD, desviación estándar; CV, coeficiente de variación; V, Estado de diferenciación de escama de yema; F1, Estado inicial de diferenciación floral; F2, estado de diferenciación de sépalo; F3, Estado de diferenciación

de pétalo; F4, estado de diferenciación de estamen; F5, Estado de diferenciación del pistilo; D1 a D5, Diferentes estados del desarrollo.

Fig. 1. Development of rhizome buds on *A. amurensis.* (1) New roots and buds in early April. (2-5) Bud at different developmental stages. (6) Flowers at anthesis in mid-March. Scale bars: 1, 2, 3 = 1 mm; 4, 5, 6 = 1 cm.

Fig. 1. Desarrollo de yemas de rizomas en *A. amurensis*. (1) Nuevas raíces y yemas a principios de Abril. (2-5) Yema en diferentes estados de desarrollo. (6) Flores en antesis a mediados de Marzo. Escalas: 1, 2, 3 = 1 mm; 4, 5, 6 = 1 cm.



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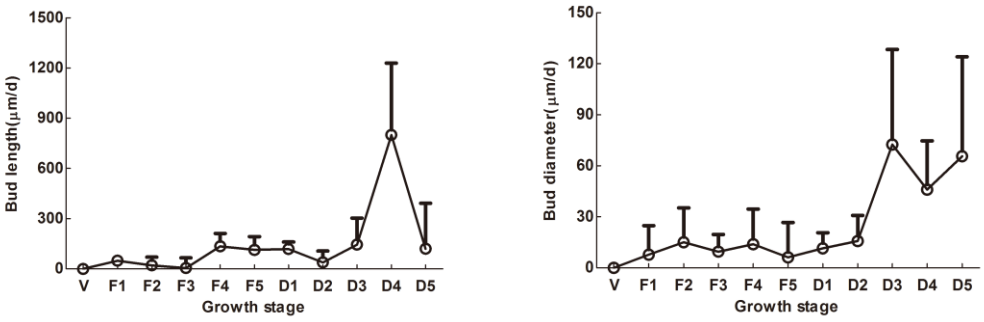

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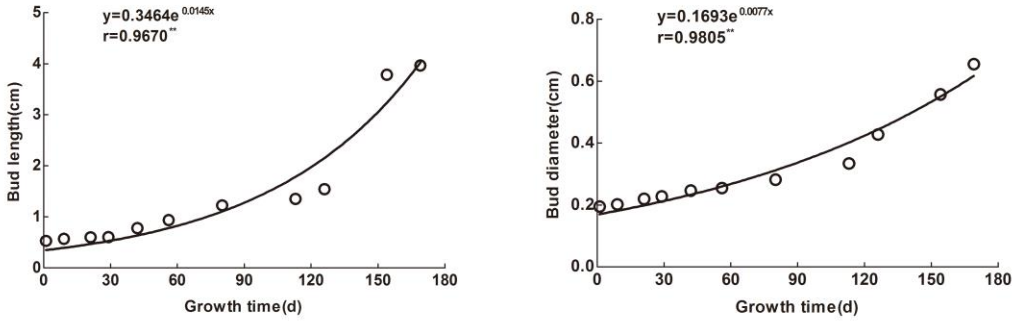

Fig. 4. Relationship between growth time with the length and diameter of rhizome buds of *A. amurensis*.

Fig. 4. Relación entre tiempo de crecimiento y longitud y diámetro en yemas de rizomas de *A. amurensis*.

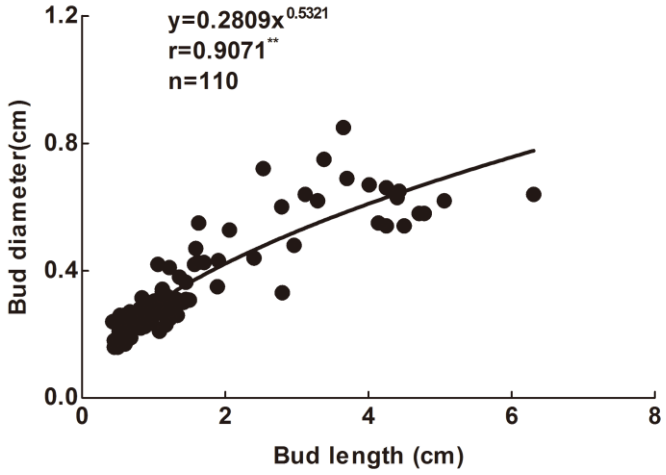

Fig. 5. Allometric relationship of rhizome buds of *A. amurensis* in diameter and length.

Fig. 5. Relación alométrica de diámetro y longitud en yemas de rizoma de *A. amurensis.*

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Floral differentiation and growth rhythm of rhizome buds of the spring ephemeroid plant *Adonis amurensis* Regel et Radde

Diferenciación floral y ritmo de crecimiento de las yemas de rizomas en la especie primaveral efímera *Adonis amurensis* Regel et Radde

Liu XL[1], JH Li[2, 3], JY Zhu[2], YF Yang[1]

Abstract. Spring ephemeroid plants complete their aboveground reproduction and growth during the short growing season, and may go dormant subsequently underground. Little is known about the underground dormancy and biological activities of the plants. In this study, we observed organogenesis and growth rhythm of rhizome buds of *Adonis amurensis* Regel et Radde, a spring ephemeroid plant from the Changbai Mountains in northeastern China. Our results showed that *A. amurensis* did not go through summer dormancy, but started producing mixed buds on rhizomes soon after the aboveground parts had died. The buds grew in length and diameter following an exponential model with significantly slower rates in the early organogenesis stages, and faster rates before winter dormancy. Allometric analyses of growth rates in length and diameter indicated that in general diameter increased with length at a slower rate. Energy allocation, and adaptation to the environment, might play an important role in the growth dynamics and allometric relationships in *A. amurensis*.

Keywords: *Adonis amurensis* Regel et Radde; Rhizome bud; Exponential growth; Allometric growth; Ecological dormancy.

Resumen. Las plantas efímeras de primavera completan su crecimiento y reproducción aéreas durante la corta estación de crecimiento, y subsiguientemente pueden entrar en dormancia en el suelo. Se conoce poco sobre la dormancia subterránea y las actividades biológicas de las plantas. En este estudio, observamos la organogénesis y el ritmo de crecimiento de yemas de rizomas de *Adonis amurensis* Regel et Radde, una planta efímera en primavera de las Montañas Changbai en el noreste de China. Nuestros resultados mostraron que *A. amurensis* no entró en dormancia durante el verano, sino que comenzó a producir yemas mixtas en rizomas poco después de la muerte de la parte aérea. Las yemas crecieron en longitud y diámetro siguiendo un modelo exponencial con tasas significativamente menores en los primeros estados de la organogénesis, y tasas mayores antes de la dormancia invernal. Análisis alométrico de tasas de crecimiento en longitud y diámetro indicaron que en general el diámetro incrementó con la longitud a una tasa menor. La distribución de energía, y la adaptación al medio, pueden jugar un rol importante en la dinámica del crecimiento y relaciones alométricas en *A. amurensis.*

Palabras clave: *Adonis amurensis* Regel et Radde; Yemas de rizoma; Crecimiento exponencial; Crecimiento alométrico; Dormancia ecológica.

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INTRODUCTION

Spring ephemeroid plants in the Northern Hemisphere are perennial herbs with flowers emerging aboveground in the early spring, and vegetative growth and fruiting completed before the fully expanded leaves of the canopy block the light in late spring or early summer (Lapointe, 2001; Zheng & Hu, 2009). This unique life history strategy pertains to their ecological functions such as ecosystem productivity (Ye et al., 1992). Therefore, these plants are an important floristic element of the broadleaf deciduous forest, and have been extensively studied in terms of their floristic diversity and geographic distribution (Zhang, 2008; Zheng et al., 2014), community ecology (Schemske, 1978; Yu & Zhou, 2003), physiological ecology (Sohn & Policansky, 1977; Schemske et al., 1978; Givnish, 1982; Nishikawa & Kudo, 1995; Ohkawara et al., 1997; Kudo et al., 2001; Frenne et al., 2010) and reproductive strategies (Maslova et al., 2003; Constable et al., 2007; Tu et al., 2012).

Adonis amurensis Regel et Radde is one of the dominant spring ephemeroid plants in the forest of the Changbai Mountains in northeastern China, and has important ornamental and medicinal values. It is a geophyte with short rhizomes. Previous studies of *A. amurensis* have addressed issues in the areas of medicinal uses (Pauli & Junior, 1995; You et al., 2003; Kuroda et al., 2010; Shang et al., 2013) , landscape application (Kawano & Hayashi, 2004), reproductive biology (Chen, 2013; Sun et al., 2015), flowering phenology (He et al., 2014; Jia & Pu, 2015), and biological characteristics (Francis et al., 2005). However, there is no report about the characteristics of floral differentiation. When the aboveground parts of *A. amurensis* die in the late spring, biological activities such as rhizome growth, bud formation, and floral differentiation happen underground, so plants can sprout again in the next spring. Although it is clear that soil freezes in the winter months and plants go dormant, little is known about whether there is dormancy in the summer and fall.

In a wide range of spring flowering perennial geophytes, floral bud initiation is completed within the renewal bud period in late summer and autumn. This development pattern has been reported in several spring- flowering bulbous plants, such as *Narcissus* (Noy-Porat et al., 2009; Feng et al., 2015), *Tulipa* (Kamenetsky et al., 2003), *Hyacinthus* (Hartsema, 1961), *Iris* (Le Nard & De Hertog, 1993) and *Crocus* (Wilkins, 1985; Molina et al., 2005); in these species floral differentiation occurs during summer, and then floral buds go into summer dormancy. However, no reports have described the morphological developmental traits of spring ephemeroid plants during summer and autumn, after the death of the aboveground plant parts. Studies of mature plants of *A. amurensis* suggested that there were two periods of dormancy (summer and winter) and growth (spring and fall) in their life history (Wan & Liu, 1996). However, the information about the growth patterns and dynamics of the underground activities on *A. amurensis* is unclear. Filling in this knowledge gap will contribute to increase our understanding of the life history strategies and the evolution of these spring ephemeroid plants.

In this study, we examined various developmental stages of *A. amurensis* to gain insights into the patterns of rhizome growth and floral differentiation. Specifically, we focused on the following questions: 1) is there a summer dormancy?; 2) when do flowers and leaves form before winter dormancy?; 3) is rhizome growth constant throughout the developmental stages?, and 4) what is the pattern of rhizome growth?

MATERIALS AND METHODS

Ethics statement. No specific permits were required for the described field studies. We confirmed that the location was neither privately-owned nor protected in any way. The experiment did not involve endangered or protected species.

Natural conditions of the research area. This research was conducted in the Tonghua Normal University (TNU) field station (41° 44' 47.04" N, 125° 58' 49.63" E, altitude 429 m.a.s.l.), located in the Tonghua section of the southeast Changbai Mountains. The area is of the north temperate continental monsoon climate with cold, dry winters and hot, humid summers. The annual average temperature is 5.5-6.0 °C, the average frost-free period is 140-171 d, and the mean annual precipitation is 893-1083.8 mm, which mostly falls from July to August (Song et al., 2011).

Research methods. In the natural forest of Tonghua, *A. amurensis* emerges aboveground in early March and blooms in mid-March. Leaves expand in early April, and fruits ripen in mid-May. At the end of May, the aboveground parts wither and die (He et al., 2014). Based on the phenology, we transplanted 300 plants that have completed their fruiting phase to a site at the TNU research station, which had similar environmental conditions to the natural forest. From 19 May to 21 July, rhizomes (each with 8-13 buds) were taken every three days. From 21 July to 4 November, the sampling interval was about 20 d. Growth in length and diameter was measured for each bud using a vernier caliper. Bud and floral differentiation determinations were made following Yang et al. (2004) and Li et al. (2012). The buds were first fixed in formalin-acetic acid-alcohol for at least 36 hrs. Then they were dehydrated using absolute ethanol firstly, acetone secondly, and isoamyl acetate thirdly, 30 minutes per step. Thereafter, a critical point was to dehydrate buds. With this purpose, they were dried in carbon dioxide, vacuum evaporated and sprayed with metal. The observations of bud and floral differentiation were done under a Hitachi S-3000N scanning electron microscope.

Data analysis. One-way ANOVA analyses were conducted using the SPSS17.0 statistical software to test whether growth in bud length and diameter differed significantly across the various developmental stages. Levene's and T2 Tamhane's tests were used to test for heteroscedasticity (Búrquez et al., 2010). The coefficient of variation (*CV=SD/M*, where M is the average and SD is the standard deviation) was calculated to examine the relative variations among samples. Growth rates for bud length and diameters were formulated as total growth (μm) during any given period divided by the growth period (days). This is, $S_{t+1} = (LM_{t+1} - LM_t)/T$, where S is the growth rate during a certain period; *LM* is the average bud length/bud diameter of each sample; *T* is the growth period in days, the interval between two subsequent samplings; *t* is the sampling time; and *t*+1 is the next sampling time.

In the regression of the bud growth rhythm, the first measurement was set as the first day of accumulated days. The correlation analyses for bud length and diameters were based on the 11 samplings. Of the following three functions, the one with the highest correlation was selected as the quantitative analysis model for regression and correlation analyses:

(1) Linear function: $y = a + b\,x$,

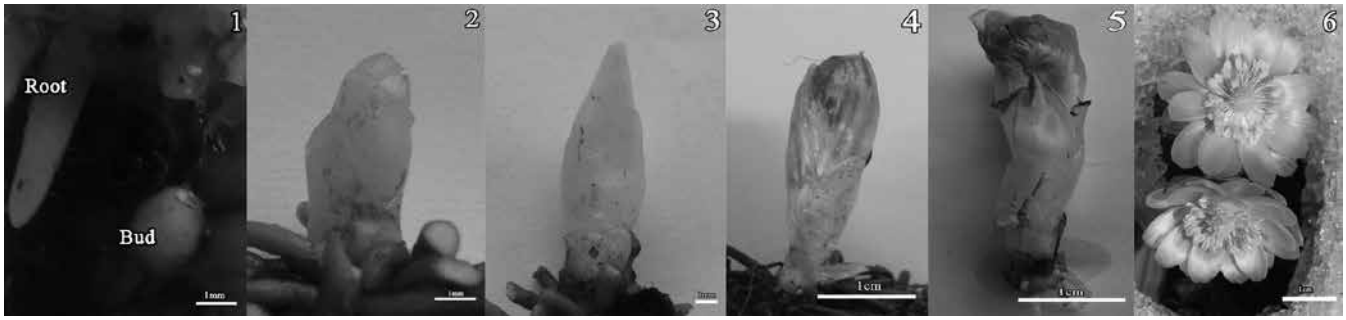
(2) Exponential function: $y = a\,e^{b\,x}$ and

(3) Power function: $y = a\,x^{b}$.

RESULTS

Differentiation processes and morphological changes in rhizome buds. The rhizomes of *A. amurensis* initiated new roots and buds at the end of the flowering phase in early April (Fig. 1: 1). The aboveground parts wither from mid to end of May. Instead of becoming dormant, the rhizome buds continue to grow, producing flowers and leaves. Floral morphogenesis was completed from mid to end of July (Fig. 1: 2-3). The differentiation of *A. amurensis* rhizome buds went through six stages. First, bud-scales formed from the flat shoot apical meristem acropetally and spirally between mid-April and late May (V) (Fig. 2: 1). The terminal flower primordium was small, flat and triangular initially (F1) (Fig. 2: 2). Second, as the volume of the flower primordium increased and elongated, 8 to 10 sepal primordia initiated in a clockwise fashion and were arranged in two whorls. Each primordium was crescent-shaped with a truncated apex (F2) (Fig. 2: 3). Third, 12 to 13 petal primordia were initiated in three whorls (Fig. 2: 4) in a sequential and centripetal manner (F3). Fourth, stamens formed sequentially in three or four whorls (Fig. 2: 5). After the initiation of the stamen primordia, the top of the receptacle became flat (F4). Fifth, the pistil primordia were initiated in a clockwise and centripetal manner filling the receptacle without any lacuna (Fig. 2: 6-7). Each carpel was plicately formed (Fig. 2: 8). Meanwhile, the stamen primordium differentiated into anther and filament (F5). Finally, all the floral organs developed and matured (Fig. 2: 9). The floral differentiation period lasted for 60 d. In addition, in the bud-scale axil, leaf primordium formed at the same time as the flower primordial (Fig. 2: 10), and later became pinnately divided when all the floral organs formed (Fig. 2: 11-12). After finished the differentiation, the buds continued development until the end of November, when soil froze and plants went dormant for the winter (Fig. 1: 4-5); this growth can be divided into 5 stages (D1-D5). Flowers open in the early spring, after the snow starts melting (Fig. 1: 6).

Growth rhythm and plasticity analyses of rhizome buds. *Adonis amurensis* rhizome did not experience summer dormancy. Bud length and diameter did not change significantly in the early stages, but active floral differentiation occurred during this period of time (Table 1). Growth in both length and diameter generally increased significantly after floral differentiation was completed between stages F3/F4; D3/D4 in bud length, and F3/D1, D1/D3, and D3/D5 in bud diameter (Table 1). The coefficient of variation (CV) reflects the relative variation degree of a sample, which can reflect indirectly the growth plasticity of rhizome buds. During



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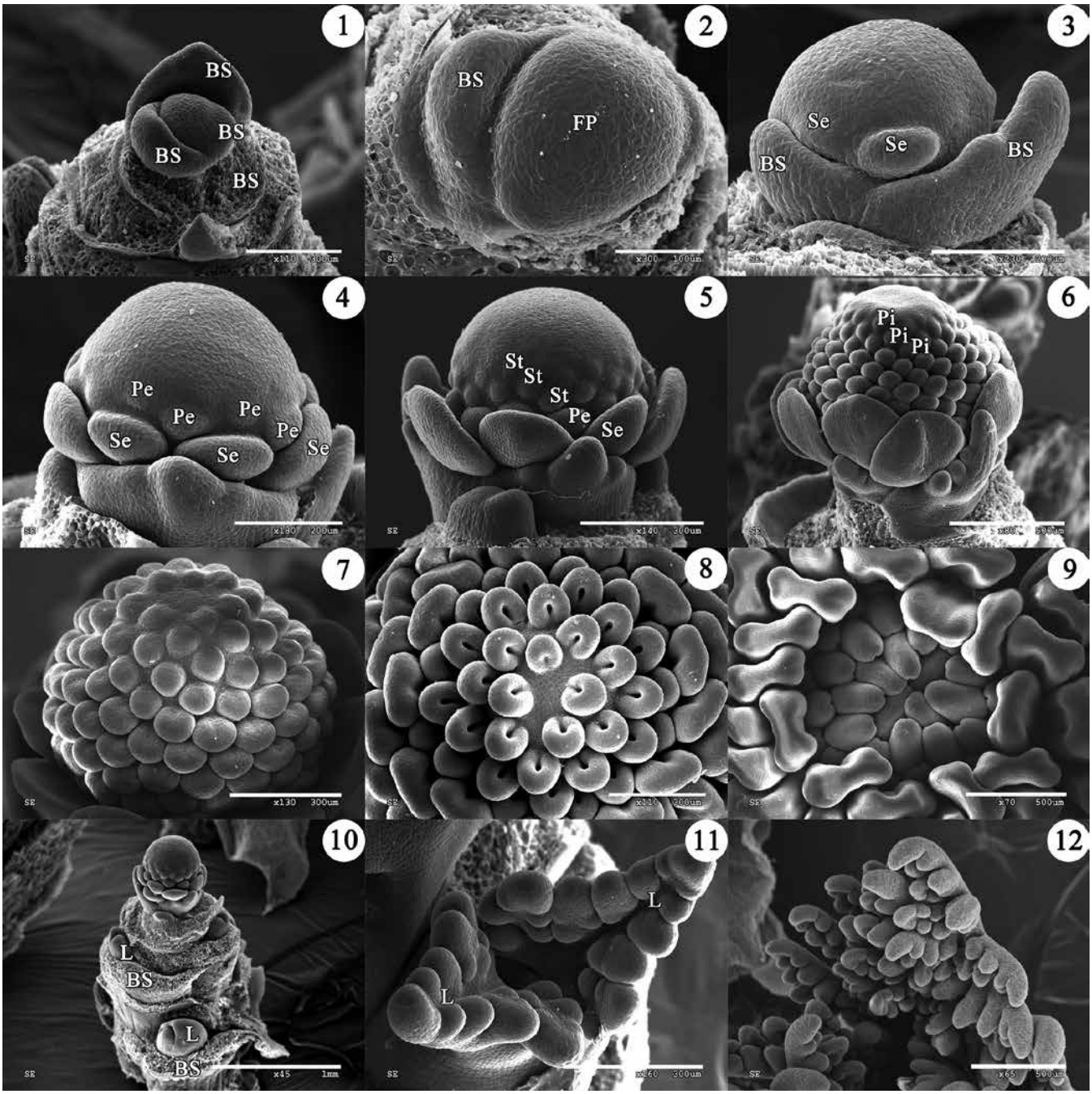

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different growth stages, the CV of bud length was within the range of 9.5-31.8%, with an average of 16.7% (Table 1). The CV of bud diameter was within the range of 9.6-19.8%, with an average of 14.7%. The growth plasticity of rhizome bud length and diameter, as evaluated by the CV, showed great changes at different times. The shortest bud length was on 19 May (V), while the smallest bud diameter was on 7 August (D1). The maximum values of length and diameter were both on 20 October, with the bud length being nearly 1.6 times that of the diameter. This suggested that the growth plasticity of bud length was greater than that for bud diameter, but the growth processes were not synchronized.

Table 1. Quantitative characteristics of rhizome buds of *A. amurensis* at different sampling times.
Tabla 1. Características cuantitativas de yemas de rizomas de *A. amurensis* en diferentes fechas de muestreo.

Sampling date	Growth stage	Length (cm)		Diameter (cm)	
		M ± SD	CV(%)	M ± SD	CV(%)
19 May	V	0.530 ± 0.050 a	9.5	0.195 ± 0.026 a	13.2
28 May	F1	0.574 ± 0.083 a	14.4	0.202 ± 0.026 a	13.1
9 Jun	F2	0.600 ± 0.085 a	14.2	0.220 ± 0.033 a	15.0
17 Jun	F3	0.605 ± 0.084 a	13.9	0.228 ± 0.029 a	12.8
30 Jun	F4	0.780 ± 0.099 b	12.7	0.246 ± 0.027 ab	10.8
14 Jul	F5	0.940 ± 0.139 b	14.8	0.255 ± 0.046 ab	18.2
7 Aug	D1	1.225 ± 0.185 bc	15.1	0.282 ± 0.027 b	9.6
9 Sep	D2	1.353 ± 0.252 c	18.6	0.334 ± 0.059 bc	17.8
22 Sep	D3	1.542 ± 0.318 c	20.6	0.428 ± 0.073 c	16.9
20 Oct	D4	3.785 ± 1.204 d	31.8	0.557 ± 0.110 cd	19.8
4 Nov	D5	3.965 ± 0.733 d	18.5	0.655 ± 0.093 d	14.1

Note: Means followed by different letters are significantly different at the P<0.05 level. Abbreviations or symbols: M, Mean value; SD, Standard deviation; CV, Coefficient of variation; V, Bud scale differentiation stage ; F1, Initial stage of floral differentiation; F2, Sepal differentiation stage; F3, Petal differentiation stage; F4, Stamen differentiation stage; F5, Pistil differentiation stage; D1 to D5, Different developmental stages.
Nota: Promedios seguidos por letras diferentes son significativamente diferentes a un nivel de P<0,05. Abreviaturas o símbolos: M, valor promedio; SD, desviación estándar; CV, coeficiente de variación; V, Estado de diferenciación de escama de yema; F1, Estado inicial de diferenciación floral; F2, estado de diferenciación de sépalo; F3, Estado de diferenciación

The growth rhythm and unsynchronized plasticity of growth (see Table 1) could be further analyzed using the growth rate (Fig. 3). The growth rate of bud length was 48.9 μm/d in the stage between bud-scale differentiation (V) and the initial stage of floral differentiation (F1). The rate then declined and reached its minimum of 5.8 μm/d during the sepal (F2) and petal (F3) differentiation stages. Then, the rate increased to 134.9 μm/d during the petal (F3) and stamen (F4) differentiation stages. After that, the rate declined slightly from 30 June (F4) to 7 August (D1), and then decreased dramatically to 38.9 μm/d by 9 September (D2). This was followed by a rapid increase to 801 μm/d in the stage between 22 September (D3) and 20 October (D4), before decreasing to 119.7 μm/d on 4 November (D5). In contrast, the bud diameter growth rate was not synchronized with bud length in most developmental stages. The lowest rate, 6.2 μm/d, was observed at the stamen and pistil differentiation stages; whereas, the highest rate of 72.6 μm/d occurred during the stage of 9-22 September (D2-D3). Thus, the results suggested that the *A. amurensis* rhizome buds displayed allometric characteristics, with periodic increases in elongation and diameter increase.

Growth model of rhizome buds. Growth of bud length and diameter in *A. amurensis* followed closely the exponential model (P<0.001) (Fig. 4). The growth rate of bud length (b=0.0145) was 1.88 times that of bud diameter (b=0.0077), There was a significant allometric relationship between the growth rates of length and diameter (r=0.9071, P<0.001), and the allometric equation was D (diameter) = 0.2809 L (length)$^{0.5321}$ (Fig. 5). During the underground development stages, the allometric properties of bud growth were more distinct than that of floral differentiation.

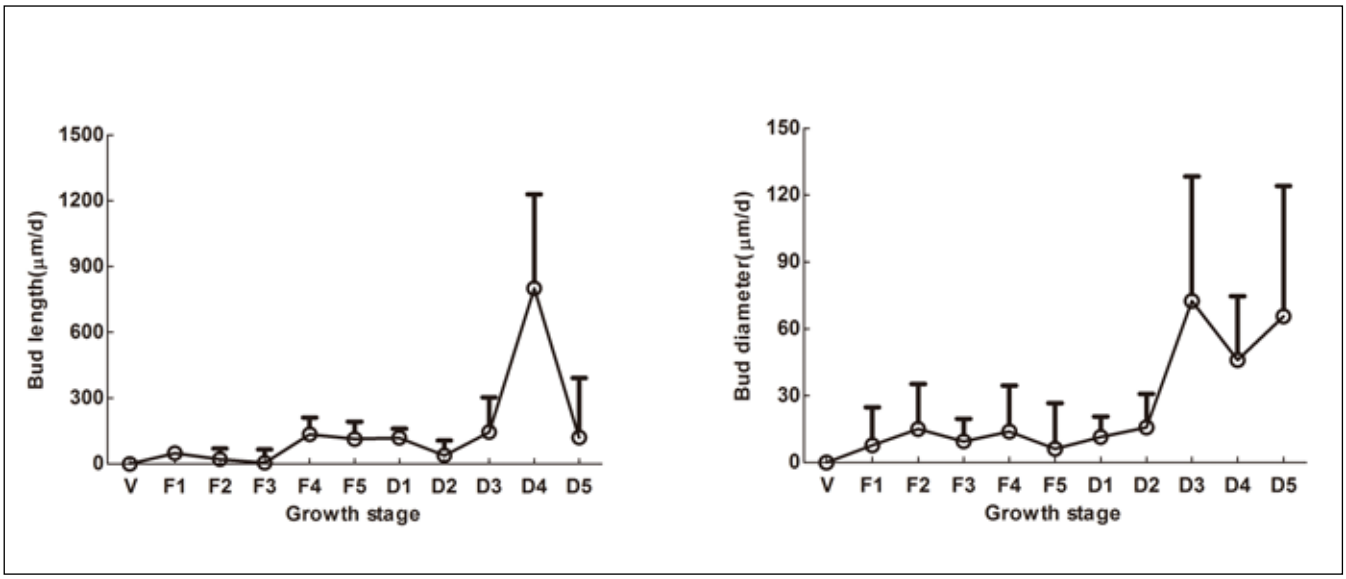

Fig. 3. Growth rate of rhizome buds of *A. amurensis* at different growth stages. Each value is the mean ± 1 S.D. of n = 11.
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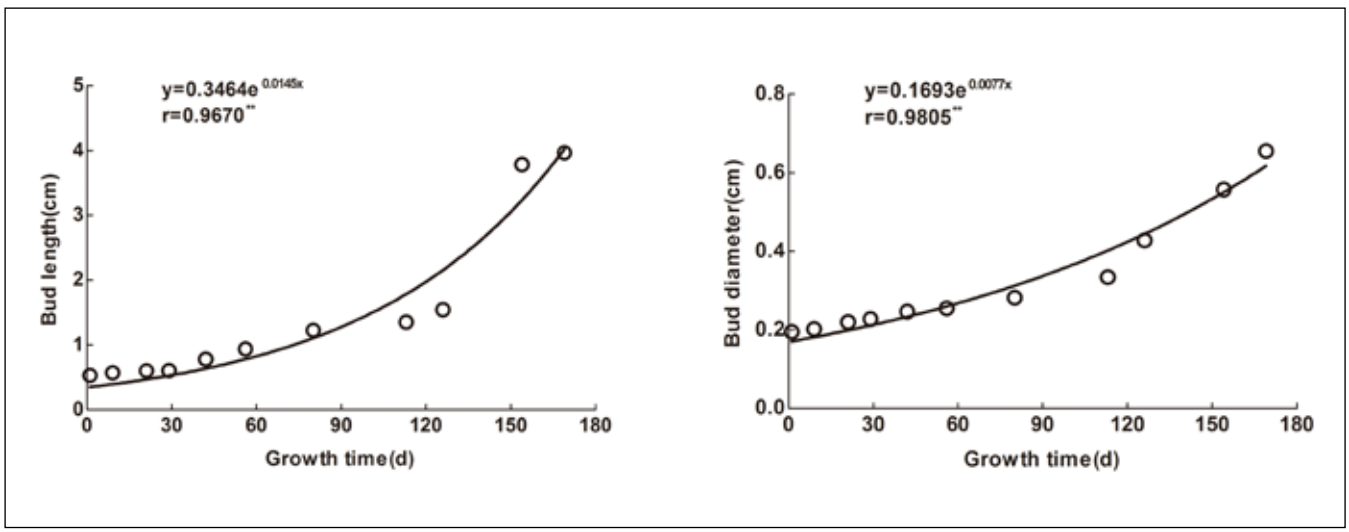

Fig. 4. Relationship between growth time with the length and diameter of rhizome buds of *A. amurensis*.
Fig. 4. Relación entre tiempo de crecimiento y longitud y diámetro en yemas de rizomas de *A. amurensis*.

DISCUSSION

Geophytes possess buds developing underground which help them to avoid damage from harsh conditions such as cold, heat, or drought (Yang & Zhu, 2011a; Volaire & Norton; 2006). The underground parts may enter dormancy for over nine months in summer and winter (Lapointe, 2001; Nishikawa & Kudo, 1995). As a spring ephemeroid geophyte, *Adonis amurensis* has been considered as a plant with two dormancy and two growing seasons (Wan & Liu, 1996). However, our observations of the developmental stages suggest that rhizome buds of *A. amurensis* do not go through summer dormancy. Instead, early summer is an important period for the differentiation and development of floral organ and leaf primordia (Fig. 2, Table 1). Bud scales form first followed by the initiation of floral organs in the order of sepals, petals, stamens, and carpels, each with multiple whorls. Leaf primordia form simultaneously with flower primordia (Fig. 2). The development pattern offers adaptive values to *Adonis* plants living in the understory environment of the cold temperate

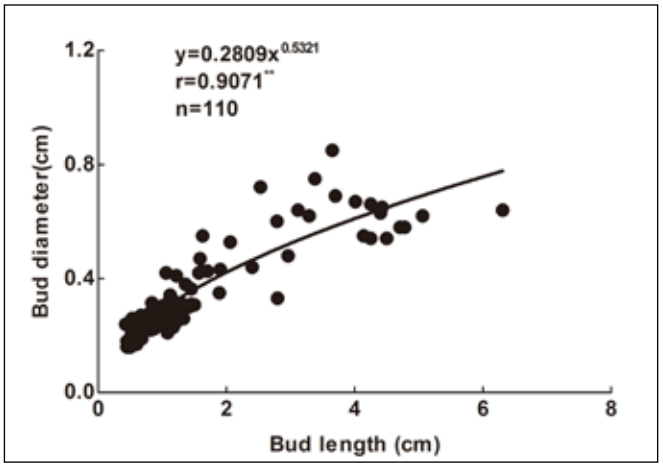

Fig. 5. Allometric relationship of rhizome buds of *A. amurensis* in diameter and length.
Fig. 5. Relación alométrica de diámetro y longitud en yemas de rizoma de *A. amurensis*.

climate, where they have a short time period (40-60 days) to complete their reproduction and growth.

Adonis amurensis produces solitary terminal flowers. Therefore, its growth in the entire life history follows an S-shaped model as in many plants and animals with determinate growth (Yang & Zhu; 2011b). However, the rhizome buds of *A. amurensis* grow at an exponential rate in both length and diameter, fitting the early phase of the S-shaped growth model (Fig. 4). The growth rates in both length and diameter are slow in the early floral differentiation and increase significantly later, especially in the period before winter dormancy (Figs. 2, 3, Table 1). The reasons may be complex, but energy allocation to floral development may delay growth of buds in length and diameter, while later, faster growth may help plants speed their sprouting in the early spring, when the snow starts melting and the top soil thaws.

Allometric relationships have been found among many quantitative characters of plants and animals, including reproductive and vegetative growth (Yang & Zhang, 2005), biomass allocation aboveground and underground (Zhang et al., 2002), and the biomass allocation of leaves and roots (Yang & Li, 2003; Zhou & Yang, 2006). In this research, the growth rate of bud length was always greater than that of bud diameter. However, asynchrony existed between the two at some specific times, leading to an allometric growth rhythm of periodic elongation and diameter increase. This may explain the relationship between characteristics of bud growth during underground development and the changes in those characteristics at different times.

During rhizome bud development, bud elongation and radial enlargement both increased rapidly with the gradually increasing temperature difference between day and night. The unsynchronized fluctuations in the growth rates revealed an allometric phenomenon in which the bud first elongated lengthwise, and then radially enlarged late in the growing season. This might be a response to a growth regulatory substance secreted by *A. amurensis* in reaction to the soil depth. This response is of great significance as it may control bud growth and developmental processes. This would be by regulating its vertical and radial allometric growth rates, making buds reach a certain size and be positioned close to the soil surface before winter dormancy. Further research is needed on the physiological regulatory mechanisms of allometric growth.

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