



Full Length Article

Effects of Exogenous Spermidine and its Synthetic Inhibitor on the Development of Bulbils on Herbaceous Peony (*Paeonia lactiflora*)

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Abstract

In this study, the herbaceous peony cultivar 'Zifengyu' was sprayed with the exogenous spermidine (Spd) and the spermidine biosynthesis inhibitor D-arginine (D-Arg). The bulbils of each treatment were selected every 10 days to investigate the dynamic changes of endogenous hormones and polyamines, the activity of enzymes and contents of soluble protein and soluble sugar. The development state of bulbils was observed with paraffin section in winter. Then the unearthed date, budding rate, blossoming season, plant height and flowering rate of the peonies in each treatment were statistically analyzed. The results showed that in petal primordium differentiation phase, Spd treatment increased the contents of endogenous Spd, putrescine (Put) and gibberellin A₃ (GA₃), whereas the D-Arg decreased their contents. But Spd and D-Arg treatments both accelerated the change speed of the antioxidant enzyme activities and reduced MDA content, enhanced the accumulation and utilization of soluble sugar and soluble protein, and raised the contents of zeatin (ZT) in the early development of bulbils and then declined later. At the same time, the contents of indole-3-acetic acid (IAA), abscisic acid (ABA), ethylene (ETH) were reduced and shorten petal primordium differentiation phase, extend stamen primordium differentiation phase. Spd treatment was not conducive to budding rate and blooming and plant development, D-Arg treatment had no significant effect on plant height, and could improve budding rate and blooming of herbaceous peony. © 2019 Friends Science Publishers

Key words: Herbaceous peony; Bulbils; Polyamine; Hormone; Antioxidant enzyme

Introduction

Herbaceous peony (*Paeonia lactiflora* Pall.) belongs to the Chinese peony branch, which is the herbaceous peony group perennial herb and a traditional Chinese famous flower, known as "flower phase", boasts graceful, rich color, shape and high ornamental value (Qin, 2004; Tang *et al.*, 2017). It has been widely used in medicine, garden and potted ornamental and become a hot spot in the domestic and international flower market of late years (Meng and Liu, 2005). The bulbils of herbaceous peony have a dormancy characteristic, which need to undergrowth 3 years before becoming the generation of bulbils (Cheng *et al.*, 2005). During the dormant stage, the development of bulbils is very slow, and it takes a period of low temperature in winter to break the dormancy. Therefore, the dormancy characteristic restricts the artificial regulation of herbaceous peony blooming season. Some studies indicate that polyamine is involved in regulating flower bud differentiation in apple, rape, chrysanthemum and other plants, but its mechanism is still unclear (Xu *et al.*, 2014). In

plants endogenous polyamine biosynthesis process, Put in the action of Spd synthase produce Spd, Spd again Spm generated under the action of Spm synthase (Xu *et al.*, 2014), among them Put is a precursor substance, there are two main biosynthetic pathways of Put: one is ornithine as a precursor, in the role of ornithine decarboxylase (ODC) decarboxylation directly formed; the other with L-arginine (L-Arg) as a precursor, arginine decarboxylase (ADC) decarboxylation formed indirectly. D-arginine (D-Arg) is a competitive inhibitor of ADC, inhibiting the biosynthesis of Put, further inhibiting the content of Spd and Spm (Shih and Kao, 1996). Numerous studies have shown that polyamines and their inhibitors are closely related to endogenous hormone levels (Xu *et al.*, 2014). Consequently, in-depth study of the development process of herbaceous peony bulbils and polyamines on its role, can further understand the mechanism of development of the bulbils, and provide a new method for the regulation of flowering stage, rather than low temperature and gibberellin. At present, the research on the herbaceous peony is mainly focused on the classification of resources, cultivation and management,

introduction of breeding, cell and molecular biology (Li and Guo, 2011), but the research on the effect of polyamine on the development of herbaceous peony's bulbils has not been reported. The purpose of this study is to determine the effect of exogenous polyamine and its inhibitor D-Arg on the growth of bulbils of herbaceous peony 'Zifengyu' and to understand its mechanism of action in order to provide new ideas and references for the regulation of flowering period in the herbaceous peony.

Materials and Methods

Plant Materials

The experiment was carried out from October 2014 to June 2017 at the Garden Center Laboratory of Horticultural Experiment Station of Shandong Agricultural University, the Gardening Center Laboratory and the Life Science Center, the Herbaceous Peony of the Horticulture Test Station and the Experimental Center of the Academy of Life Sciences. 'Zifengyu' (*Paeonia lactiflora* Pall.) was used as test material, and planted in the middle of October, 2014. After two years of growth and adaptation, healthy and no pests were selected and grown vigorously. On the 1st of November, 2016, "Zifengyu" was divided into three treatment groups: 0.3 mmol L⁻¹ Spd-treated group, 0.3 mmol • L⁻¹ D-Arg-treated group and control (water) group which sluiced the root, and the planting depth was about 40 cm. Each treatment group was divided into three 3 repeats and planted with 20 trees. The row spacing was 80 cm × 80 cm and the FPR (Fiber Reinforced Polymer) isolation plate with a depth of 60 cm was used to isolate each repeats. The bulbils were randomly sampled every 10 days starting from November 1, 2016 and every sample was repeated 3 times and the physiological and biochemical indexes were measured.

Quantification of SOD, POD, CAT Activity and CAT Content

Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activity and malondialdehyde (MDA) content were determined spectrophotometrically by a modified method of Cang and Zhao (2013).

Quantification of Soluble Protein and Soluble Sugar Content

Soluble sugar, soluble protein content were measured by anthrone colorimetric method and coomassie brilliant blue method (Cang and Zhao, 2013; Yu, 2014).

Quantification of Polyamine Content

The determination of spermidine (Spd), putrescine (Put), and spermine (Spm) was performed using the method of Liu

et al. (2002) using a Water 2487 HPLC column with a Novapak column. C18 column (250 mm × 4.6 mm, 5 μm), standard for Solarbio products.

Quantification of Hormones Content

The ethylene (ETH) content was measured by an external standard method (Liu *et al.*, 2006) using a GC Series Gas Chromatograph (GC-2014C) manufactured by Shimadzu Corporation, Japan.

High-performance liquid chromatography (HPLC) method (Zhang *et al.*, 2013) was used to measure the content of endogenous gibberellin, zeatin (ZT), indole-3-acetic acid (IAA) and abscisic acid (ABA). The measurements were performed on a Water 2487 high performance liquid chromatograph using a Novapak C18 (250 mm × 4.6 mm, 5 μm) column. The standard product was Sigma.

Observation on the Development Status and Progress of Bulbils in 'Zifengyu'

After Spd and D-Arg were used for watering the roots, the scale buds of each treatment group were sampled every 10 days, and then the outer scales of the bulbils were stripped off and paraffin sections were made with reference to Lv's method (Lv *et al.*, 2009). Then observations were taken with a Motic BA300 microscope, photographed with Motic Images Advanced 3.2 and Moticam 2006 to determine their developmental status. Observation records were made for each treatment group: the date of excavation was based on the time of the first scale bud emergence in each treatment group; the budding rate was the percentage of the number of buds out of the original buds (5); the flowering period was the first flower in each treatment group. The number of days from flower opening to the time of the last flower withered; flowering rate is the percentage of flowering buds as a percentage of total buds; plant height is the distance between the point where the stem intersects the ground to the highest point of the plant.

Statistical Analysis of Date

Data was processed and mapped using Microsoft Excel software, and data was tested for significance using SPSS Statistics V 19.0 software.

Results

The Development Process of Bulbils of 'Zifengyu'

According to the results of pre-test of paraffin section, the development process of 'Zifengyu' was completely divided into six stages: bract primordium differentiation phase, sepal primordial differentiation phase, petal primordium differentiation phase, stamen primordium differentiation

phase, pistil primordium differentiation phase and stamen petalody phase. Bract primordium differentiation phase, sepal primordial differentiation phase and stamen petalody phase did not appear in the sampling time because of the environmental factors and sampling interval time constraints.

From Table 1 and Fig. 1, it can be seen that unearthed dates of all groups are the same and the difference of blooming season are not significant ($P > 0.05$). Compared with the CK, the Spd-treated group's budding rate and flowering rate were reduced by 6% and 8%, respectively and plant height was 82.9% ($P < 0.05$) in the CK. In the D-Arg-treated group, the budding rate and flowering rate were increased by 38% and 15.3%, respectively, compared with the CK, and the plant height was 94.6% in the CK ($P < 0.05$). The results showed that exogenous Spd would reduce the budding rate, flowering rate, and plant height. D-Arg could increase the budding rate and flowering rate. The unearthed date and flowering period were less affected by exogenous Spd.

As shown in Fig. 2, both Spd and D-Arg treatments significantly shortened the petal primordium differentiation phase and prolonged the stamen primordium differentiation phase, but compared with D-Arg treated group, the shortening and prolongation of exogenous Spd was more significant ($P < 0.05$), indicating that exogenous Spd and D-Arg affect the development process of bulbils by affecting the length of the petal and stamen primordium differentiation phase, and the effect of exogenous Spd was more obvious.

Effect of Exogenous Spd on Antioxidant Enzyme Activity and MDA Content of Bulbils

The activity of SOD showed a decreasing trend overall, for the CK, it rose first and then decreased to 34.4% at the petal primordial differentiation phase (Fig. 3A). Finally, it was more stable and higher than the other two groups ($P < 0.05$). The Spd-treated group showed an upward trend while the D-Arg-treated groups showed a decreasing trend, but was significantly higher than the other two groups. In the stamen primordium differentiation phase, the CK first rose to its peak value and then rapidly decreased to 13.7%. The Spd-treated group showed a decreasing trend, and the later period increased slightly. D-Arg-treated group decreased first and then increased slowly; at the pistil primordium differentiation phase, CK and Spd treated group were at a low level, the difference was not significant ($P > 0.05$), D-Arg-treated group was lower, but slightly higher than the other two groups ($P < 0.05$). The results showed that the increase of SOD activity is conducive to the initiation of the differentiation of petals and stamen primordium. The low activity of SOD facilitates later development. Exogenous Spd and D-Arg will increase the activity of SOD in the petal primordium differentiation phase and accelerate the development of this stage. In the initial stage of stamen primordium differentiation, SOD activity was inhibited.

The POD activity in the primordial differentiation

Table 1 List for investigation of each treatment group while the bulbils was unearthed

Treatment	Unearthed Date	Budding rate (%)	Flowering rate (%)	Blooming Season	Plant height (cm)
Control	2017.3.21a	150b	74.7b	2017.5.1-5.8a	72.05c
0.3 mM Spd	2017.3.21a	144a	66.7a	2017.4.30-5.8a	59.70a
0.3 mM D-Arg	2017.3.21a	188c	90.0c	2017.5.2-5.8a	68.13b

The same alphabets in right side of the same list show no significance ($P > 0.05$), on the contrary, having significance ($P < 0.05$)

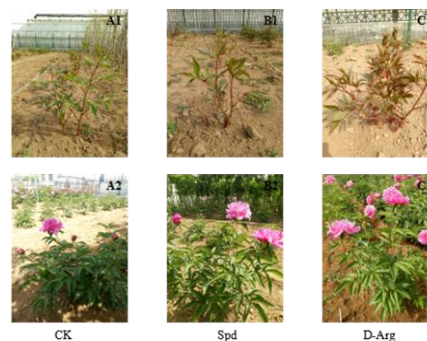


Fig. 1: The condition of unearthed and formation of flowers of each treatment group

Note: A1-A2: Excavation and flowering of bulbils in CK group;
B1-B2: Excavation and flowering of scale bulbils in Spd-treated group;
C1-C2: Excavation and flowering of bulbils in D-Arg-treated group

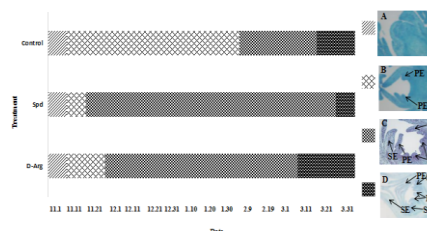


Fig. 2: The development process of the bulbils of each treatment group

Note: SE sepal primordium ; PE petal primordium ; ST stamen primordium ; PI pistil primordium

A: undifferentiation phase; B: petal primordium differentiation phase

C: pistil primordium differentiation phase; D: stamen primordium differentiation phase

stage of petals decreased in the CK and then decreased in the Spd-treated group (Fig. 3B). The Spd-treated group showed a stable trend in the early stage and increased in the later stage. The D-Arg-treated group was stable and lower than the other two groups ($P < 0.05$); The CK was more stable in the stamen primordium differentiation phase, and the Spd-treated group was more stable in the early stage of stamen primordium differentiation, and undulating in the late stage, and the D-Arg-treated group continued to fluctuate; the activity of POD in CK and D-Arg-treated group were stable in the pistil primordium differentiation phase, and peaked at the late stage of pistil primordial differentiation of Spd-treated group. The results showed that the exogenous Spd delayed the rise of POD during the petal primordium phase, and the POD activity of the stamen and

pistil primordium differentiation phase fluctuated. The D-Arg treatment maintained the POD activity in the petal primordium differentiation phase, and in the stamen primordium fluctuated and remains low during the pistil primordium differentiation phase.

The CAT activity in the primordial differentiation stage of petals was fluctuating in the CK, and significantly lower in the Spd-treated group than in the other two groups ($P < 0.05$) (Fig. 3C). After the D-Arg-treated group increased rapidly, the CAT activity decreased. In the basal differentiation phase, the CK rose first and then decreased, which was significantly higher than the other two groups ($P < 0.05$). The Spd-treated group continued to fluctuate less than the other two groups. The D-Arg-treated group decreased first, then rose, and then decreased. Compared with the other two groups ($P < 0.05$), the CK increased slowly during pistil primordium differentiation phase, while decreased in the Spd-treated group, which was significantly lower than the other two groups ($P < 0.05$). The D-Arg-treated group showed an upward trend in pistil primordium phase. The results showed that exogenous Spd treatment reduced the CAT activity in the primordial differentiation stage of petals and inhibited the change of CAT in stamen primordium differentiation phase and decreased the CAT activity in the pistil primordium differentiation phase. D-Arg treatment accelerated the process which was rose first and then down of CAT activity in the primordial differentiation stage of petals, and decreased the activity of CAT in the stamen primordium differentiation phase, and increased the activity of CAT in the early stage of pistil primordium differentiation, and caused it to rapidly decline.

From Fig. 3D, it can be seen that the MDA content in the petal primordium differentiation phase was rapidly decreased in the CK, then slightly increased, remained stable, and the Spd-treated group also decreased rapidly, and significantly lower than the other two groups ($P < 0.05$). The MDA content of D-Arg-treated group first decreased rapidly after the first rise; at other times, the difference between the three groups was not significant ($P > 0.05$). The contents of MDA were higher in the undifferentiated period of bulbils and decreased with the initiation of development. Exogenous Spd could significantly reduce the MDA content in the primordial differentiation stage of petals.

Effect of Exogenous Spd on the Metabolism of Soluble Protein and Soluble Sugar of Bulbils

The soluble proteins in all treatment groups were stable in the early stage of scale bud development, and both showed an upward trend in the late stage of stamen primordium differentiation, and there were large fluctuations ($P < 0.05$) (Fig. 4A). The results showed that the synthesis and distribution of soluble protein remained stable at petal and stamen primordium differentiation phases, while the synthesis rate and content increased at the later stage of stamen primordium differentiation.

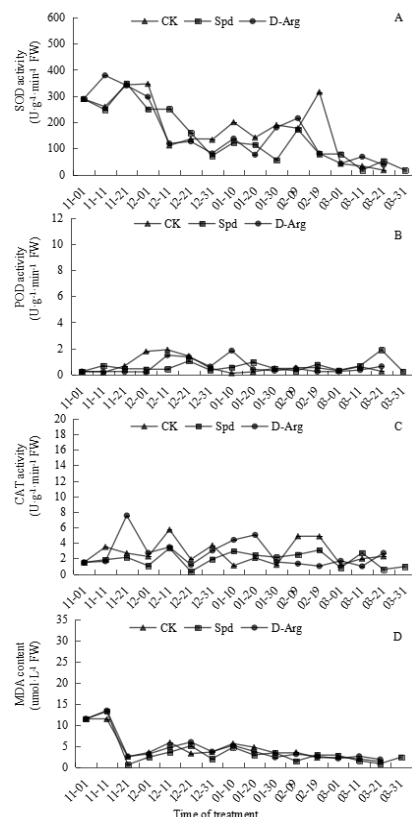


Fig. 3: Effects of exogenous Spd and D-Arg on the activity of SOD, POD, CAT and content of MDA in 'Zifengyu' during the development of bulbils

The soluble sugar content in the primordial differentiation stage was lower in the CK than in the other two groups ($P < 0.05$). Spd treated group quickly reached its peak value and then declined, and the D-Arg-treated group showed an upward trend. There were two peaks of soluble syger in the stamen primordium differentiation phase of CK and were higher than those in the other two groups ($P < 0.05$). The Spd-treated group first rose and then decreased and tended to be stable. The D-Arg treated group first rose and then decreased and was higher than the other two groups ($P < 0.05$) (Fig. 4B). The results showed that both the exogenous Spd and D-Arg could increase the soluble sugar content in the primordial differentiation stage of petals, and the exogenous Spd increased the times of the rise, and the D-Arg treatment increased the soluble sugar content. Both also increase the utilization of soluble sugars, and both the content of soluble sugars are in stabilization at the stamen primordium differentiation phase.

Effect of Exogenous Spd on the Content of Endogenous Polyamines of Bulbils

The content of endogenous Spd in the primordium differentiation phase of flower petal first stabilized and then decreased in the Spd-treated group and was lower than the

other two groups. The D-Arg-treated group increased rapidly and was significantly higher than the other two groups ($P < 0.05$); The CK group peaked at the stamen primordium differentiation phase and was higher than the other two groups. The Spd-treated group tended to stabilize at first and then decreased, and the D-Arg treated group decreased and was lower than the other two groups ($P < 0.05$). In the pistil primordium differentiation phase, the CK was significantly higher than the other two groups ($P < 0.05$), and both the Spd-treated group and the D-Arg-treated group remained stable (Fig. 5A). As the results, the content of endogenous Spd was increased in the early stage of petal and stamen primordium differentiation, and the content of endogenous Spd was decreased in the initial stage of petal primordium differentiation by treatment with exogenous Spd, and D-Arg treatment was increase endogenous Spd content at the initial stage of petal primordium differentiation and stamen primordium differentiation.

According to Fig. 5-B, the content of Put in petal primordium differentiation phase decreased slightly after CK first, and decreased in Spd-treated group, and was significantly lower than that in other two groups ($P < 0.05$). The D-Arg-treated group peaked. After the rapid decline, there are two peaks in the stamen primordium differentiation phase of CK, and was significantly higher than the other two groups ($P < 0.05$), the other two groups are constantly ups and downs. During the pistil primordium differentiation phase, CK group rose first and then decreased, while the other two groups remained at a low level. The results showed that the exogenous Spd treatment reduced the content of endogenous Put in the petal primordium differentiation phase, and D-Arg treatment increased the endogenous Put content in this period.

From Fig. 5C, Spm content in the CK has been at a relatively stable low level throughout the bulbils development. The Spm content in the Spd-treated group shows a steady change, and peaks in the later period, and increased in the D-Arg-treated group, but decreased rapidly during the petal primordium differentiation phase. In the stamen primordium differentiation group, the Spd-treated group first decreased, then rose and then decreased, then increased slightly. The Spm content of D-Arg-treated group increased slightly, and then remained stable. In the pistil primordium differentiation phases, Spm content of Spd-treated group maintained a low level and the D-Arg-treated group increased. As the results, the effect of endogenous Spm was weak in the development process of herbaceous bulbils, and endogenous Spm content fluctuates with exogenous Spd treatment, and D-Arg treatment. Increased the endogenous Spm content in the petal and pistil primordium differentiation phase.

Effect of Exogenous Spd on the Metabolism of Endogenous Hormones of Bulbils

The endogenous ZT content in the CK group of the

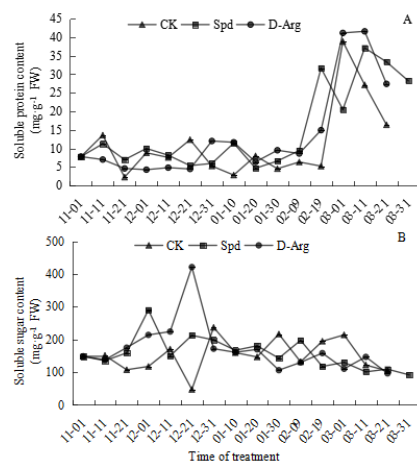


Fig. 4: Effects of exogenous Spd and D-Arg on the content of soluble protein and soluble sugar in 'Zifengyu' during the development of bulbils

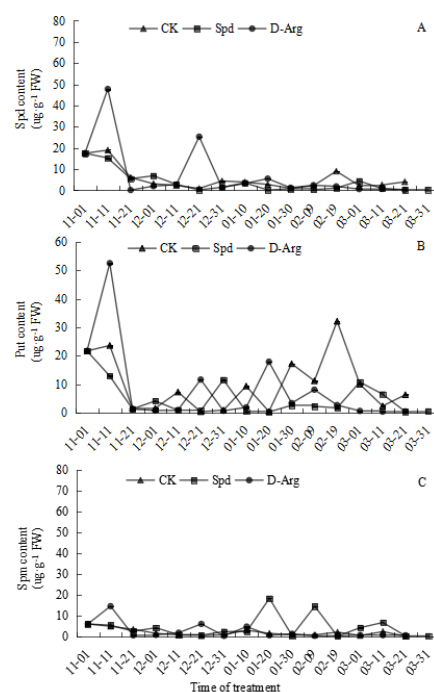


Fig. 5: Effects of exogenous Spd and D-Arg in the content of Spd, Put and Spm in 'Zifengyu' during the development of bulbils

primordial differentiation stage of petals was significantly increased at the early stage, then rapidly decreased to 2.24%, and then slightly undulated (Fig. 6A). The endogenous ZT was observed in the Spd-treated group before entering the petal primordium differentiation phase, but the ZT content first decreased and then rose. The content of ZT in the transitional stage of the D-Arg-treated group from the undifferentiated stage to the primordium differentiation stage of the petals showed a downward trend. In stamen

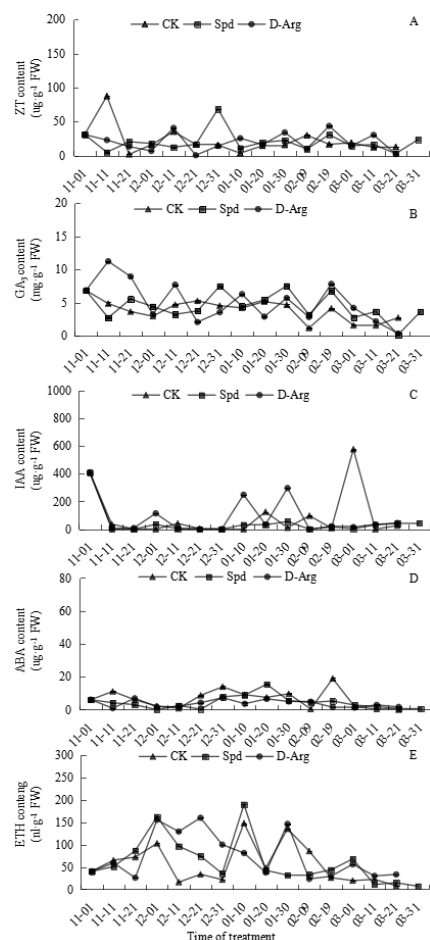


Fig. 6: Effects of exogenous Spd and D-Arg in the content of ZT, GA₃, IAA, ABA and ETH in 'Zifengyu' during the development of bulbils

primordial differentiation stage, the ZT content of CK group first rose and then decreased. The amplitude was small and the Spd-treated group fluctuates. The D-Arg-treated group first rose and then fallen at the beginning and then remains stable. In the pistil primordial differentiation phase, the CK slowly declines, and the Spd-treated group showed an upward trend. The D-Arg-treated group first rose then decreased and rose again. The results showed that the content of ZT increased at the initial stage of petal primordial differentiation, but then decreased, which favored the later development of bulbils. Spd and D-Arg treatments reduced the ZT content in the petal primordial differentiation phase, and delayed the increase of ZT content in the stamen primordial differentiation phase. D-Arg treatment has little effect on the ZT content during stamen primordial differentiation phase, and increased ZT content in the early stage of pistil primordial differentiation phase.

The content of GA₃ in the petal primordial differentiation phase was stable after the first decline in CK

group, and the content of GA₃ in the Spd-treated group showed a decreasing trend before entering into the primordial differentiation stage of petal, and was significantly lower than in the other two groups ($P < 0.05$). In the differentiation phase, the GA₃ contents increased in the early stage of petal primordial differentiation with D-Arg-treated group, which was significantly higher than the other two groups ($P < 0.05$), and then decreased rapidly; after the stamen primordial differentiation phase, the CK group decreased first and then increased. Afterwards, the Spd-treated group decreased at the beginning of the stamen primordial differentiation and then finally decreased to near zero, and the D-Arg-treated group continued to fluctuate; during the pistil primordial differentiation phase, the CK group was stable, and the Spd-treated group showed an upward trend. The D-Arg-treated group showed a downward trend (Fig. 6B). The results showed that exogenous Spd treatment decreased GA₃ content in the undifferentiated stage and increased GA₃ content in the primordial differentiation stage of petals. D-Arg treatment increased the content of GA₃ in the undifferentiated stage and decreased it in petal primordial differentiation stage, but remained high in the other two groups.

The IAA content in the CK group remained low at the early stage of petal primordial differentiation, and increased at the later stage (Fig. 6C). The Spd-treated group had lower IAA content before entering the petal primordial differentiation stage, and the D-Arg-treated group was in the petal primordial differentiation phase. The IAA content in the differentiation phase were also low; in the stamen primordial differentiation phase, the CK group increased, but the Spd-treated group also showed an increasing trend, but the amplitude was small; there were two peaks in the D-Arg-treated group. After the CK group recovered to a low level, the Spd-treated group showed a smaller upward trend, and the D-Arg-treated group remained stable and then rose rapidly. The results showed that low concentration of IAA facilitated the development of bulbils, and both exogenous Spd and D-Arg treatment could keep IAA at a low level during the primordial differentiation stage of petal, and at the same time increased the content of IAA in male and pistil primordial differentiation stage, in which the effect of D-Arg is more pronounced.

According to Fig. 6D, the ABA content in the CK group first decreased and then rose in the petal primordial differentiation phase. The ABA content in the Spd-treated group was lower, and the D-Arg-treated group showed a decreasing trend after a slight increase in the ABA content in the early stage of petal primordial differentiation. At the stamen primordial differentiation phase, the CK group first decreased, then peaked, and then decreased again. The Spd-treated group rose first and then decreased, but the amplitude was smaller. The D-Arg-treated group rose first and remained stable; during the pistil primordial differentiation phase, ABA content rose rapidly in the CK group, remained low in the Spd-treated group, and rose

rapidly the D-Arg-treated group. The results showed that the exogenous Spd treatment and D-Arg treatment both reduced the ABA content in the petal primordium differentiation phase and increased the ABA content in the stamen primordium differentiation phase.

In the petal primordium differentiation phase, the ETH content in CK group first rose and then decreased, then rose and then declined. Finally, the ETH content in the CK group increased. The Spd-treated group had a lower ETH content in the petal primordium differentiation phase, and ETH content of D-Arg-treated group in the early stage showed a decreasing trend and was low during the petal primordium differentiation phase. During the stamen primordium differentiation phase, it remained stable and then declined in the CK group, and the Spd-treated group continued to fluctuate at the early stage, and was stable at the later stage, and the D-Arg-treated group continued to fluctuate. During the pistil primordium differentiation phase, the CK group also decreased slightly, and the Spd-treated group maintained a low level, and the D-Arg-treated group showed a decreasing trend (Fig. 6E). As the results, exogenous Spd and D-Arg treatment could reduce the ETH content in the primordium differentiation phase and increase the ETH content in the stamen primordium differentiation phase.

Discussion

Polyamines (PAs), low-molecular-weight compounds, are some of the metabolites ubiquitously present in plants and play important roles in the plant response to various environmental stresses (Sharma *et al.*, 2013; Kotakis *et al.*, 2014; Kubis *et al.*, 2014). PAs are a class of aliphatic nitrogen-containing base compounds that possess two or more amine groups and widely present in higher plants, fungi, and prokaryotes. Plants have three major PAs, spermidine, putrescine, and spermine which play a multifaceted role in plant growth and development, and are related to cell division, embryogenesis, reproduction, flowering, seed germination, pollen tube growth, fruit formation and maturation. In addition, polyamines are closely related to various plant hormones, stress, and aging. A large number of studies have shown that there is a close relationship between polyamines and plant flower bud differentiation. Polyamines are involved in the regulation of flower bud differentiation in chrysanthemum, apple, rape and other plants (Cosat *et al.*, 1986; Yang and Jie, 1996; Ai *et al.*, 2011; Xu *et al.*, 2014); Applewhite *et al.* (2000) found that application of enzyme inhibitors to reduce the content of spermidine in the medium will inhibit Arabidopsis bolting and flowering, if it is moved to the non-inhibitor medium, it can continue to bolts and flowers. In this experiment, both exogenous Spd and D-Arg treatment could shorten the petal primordium differentiation phase and prolong the stamen primordium differentiation phase. Spd treatment was more effective, but both had little effect on the flowering

period. The results suggest that exogenous Spd treatment reduced budding rate, flowering rate, and plant height, while D-Arg treatment increased budding rate, flowering rate, and facilitated the growth of flowering and plant height of peony, indicating that in different plants, the content and function of polyamines were different, and the effects of external polyamines and their inhibitors were also different. Exogenous D-Arg treatment in *Paeonia lactiflora* Pall. could increase the budding rate and flowering rate.

The CAT, SOD and POD are the protective enzymes of active oxygen free radical scavenging system in plants. They synergistically prevent the damage of cell membrane by active oxygen radicals, inhibit membrane lipid peroxidation, and protect plants (Petricet *et al.*, 2013). It has been found that Spd can impair the supply of electrons that generate reactive oxygen species (ROS) (Shi *et al.*, 2010), and it can also eliminating ROS directly, and be combined with antioxidant enzymes or other antioxidant molecules (Shi and Chan, 2014). In melon seedlings, exogenous Spd treatment can increase the activity of antioxidant enzymes (Zhang *et al.*, 2017). Exogenous Put can improve the resistance of the tomato by increasing the activity of antioxidant enzymes in tomato, and then improve its resistance to stress (Slathia *et al.*, 2012). Under drought stress, exogenous Spd treatment can reduce the content of superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) in corn and tomato, have the effect of scavenging ROS, increase the antioxidant capacity of the plant and reduce the MDA content (Li *et al.*, 2018). Du *et al.* (2016) found that Spd can increase antioxidant enzyme activity and reduce MDA content. In this experiment, the activities of SOD, CAT, and POD increased in the early stage of petal primordium differentiation phase, decreased in later stages and were more stable, and also showed a trend of rising first and then decreasing in other stages, indicating that the activity of enzymes was increased during the development of scale buds. Similar to the above research results, it is presumed that the internal activation of the cells during the various stages of scale bud development may produce a series of oxides, peroxides etc. and the above enzymes need to play a role in reducing the damage to the peony. Exogenous Spd and D-Arg treatment reduced the activities of SOD, POD and CAT in the petal primordium differentiation phase, and accelerated the development of this stage, indicating that the internal ROS content of the scale bulbils was low, which favored their development. The activity of SOD, POD, and CAT increased and decreased in the stamen primordium differentiation phase, indicating that the active ROS in the bulbils is not conducive to its development. The content of MDA was high at the beginning of bulbils development, but it decreased rapidly and decreased, indicating that with the initiation of bulbils development, antioxidant enzymes played a role, and low levels of MDA reduce the damage to the plants.

Soluble protein and soluble sugar are important material basis for the development of bulbils. Put can

increase protein content in young leaves of tomato (Du *et al.*, 2016). Guo *et al.* (2018) found that low concentration of Spd will reduce the soluble protein content of belladonna. Ding *et al.* (2006) found that Spm increased nitrate reductase content by promoting the synthesis of protein in chrysanthemum, increased the ability of plants to absorbing and assimilating nitrogen, and increased the content of soluble protein in leaves, resulting in sufficient nutrition for buds. Material supply will accelerate the development of flower buds and advance the flowering period. In this experiment, the synthesis and conducive of soluble protein in the petal primordium differentiation phase and the early stage of the stamen primordium differentiation phase, but the synthesis rate was higher than the utilization rate in the later stage of stamen primordium differentiation, indicating that the treatment of exogenous Spd and D-Arg would increase the utilization rate of soluble protein in the primordial differentiation stage of petals, and then to promote its development, further illustrating that soluble proteins provide material basis for the development of bulbils.

Spd facilitates the accumulation of soluble sugar in corn, helps maintain cell turgor pressure and ensures cell function (Li *et al.*, 2018); in belladonna, low concentration of Spd reduces its soluble sugar content (Guo *et al.*, 2018). During the flower bud differentiation phase of tung oil tree, the soluble sugar content rise from the flower bud physiological development stage to the inflorescence differentiation stage reached the maximum value, then decreased (Sun *et al.*, 2014), indicating that the accumulation of soluble sugar facilitates the initiation of flower bud differentiation. However, with the differentiation of flower buds, soluble sugar will continue to be consumed. In this experiment, the treatment of exogenous Spd and D-Arg increased the utilization and synthesis efficiency of soluble sugar in the petal primordium differentiation phase, while inhibited the synthesis of soluble sugar in the stamen primordium differentiation phase, thus prolonging the development period of this stage. The same as the above findings, indicating that soluble sugar provide energy for bulbils development.

Filia *et al.* (1988) thought that Spd is a marker of the birth of an iris flower bud. Rey *et al.* (1994) thought that Spm accumulation is a physiological indicator of flower bud induction. In chrysanthemum, Spd regulates flower bud differentiation by generating Spm (Yang and Yang, 2009); when olive flower buds are morphologically differentiated that Spm content increased (Nasir *et al.*, 2007). Spraying polyamine synthesis inhibitors at the flowering stage of carrots and wheat can increase the Put content. Heby (1981) and Minnocha (1991) speculated that the accumulation of Put was due to the inhibition of Spd synthesis. In this experiment, the increase of endogenous Spd content favors the development of petal and stamen primordium differentiation. The content of endogenous Spd and Put in the early stage of petal primordium differentiation phase after exogenous Spd treatment is decreased. D-Arg

treatment increased the content of endogenous Spd and Put in the early stage of petal primordium differentiation phase, increased endogenous Spd content and increased the content of endogenous Put during stamen primordium differentiation phase. The effect of endogenous Spm on the development of the bulbils of *Paeonia lactiflora* was weak. After exogenous Spd treatment, the content of endogenous Spd decreased. It is presumed that the plant maintains the balance of polyamines in the body and negatively regulates the synthesis of endogenous Spd. However, after D-Arg treatment, Put cannot synthesize and cause positive feedback regulation. After the inhibition effect disappears, a large amount of synthesized Put and Spd content increase, indicating that the increase of endogenous Put, Spd is conducive to the development of the scale bud. Therefore, the treatment of exogenous Spd and D-Arg promoted the development of the petal primordium differentiation phase of the bulbils. However, Spd treatment resulted in a rapid development of the petal primordium differentiation phase, resulting in the nutritious material has not been effectively accumulated, which was detrimental to the budding rate and flowering rate, plant height. Changes in endogenous Spd and Put contents in plants affected endogenous Spm content, and endogenous Spm had no significant effect on the development of *Paeonia lactiflora* Pall. bulbils. At the same time, it was found that both Spd and Put exerted influence on the development of scale buds. At the same time, it was found that both Spd and Put exerted influence on the development of *Paeonia lactiflora* Pall. bulbils.

A number of studies have shown that polyamines and their inhibitors are closely related to endogenous hormone content (Gui *et al.*, 2003). Plant hormones are one of the regulatory pathways for the development of bulbils. In the flowering process of dianthus, exogenous Spd treatment will increase the content of endogenous ZR (Gui *et al.*, 2003); spraying exogenous Spd on the 'Fuji' apple flower can increase its endogenous ZR content (Xu *et al.*, 2001); during the differentiation of flower buds in citron, ZR content increased (Tian *et al.*, 2004). In this experiment, the increase of ZT content is conducive to the initiation of bulbils development, and the low concentration of ZT is conducive to the later development. Exogenous Spd and D-Arg treatment both reduce the ZT content in the petal primordium differentiation phase. It is speculated that exogenous Spd and D-Arg treatment speeds up the rate of endogenous ZT increase and decrease, which makes it ahead of the low level and thus conducive to the development of bulbils. Exogenous Spd treatment delays the increase of ZT content during stamen primordium differentiation phase. It is speculated that the increase of ZT content advances in time to accelerate the development of stamen primordium differentiation phase.

Spraying Spd to Red Fuji apple at full flowering stage can increase the endogenous GA₃ content of its flower, spraying polyamine synthesis inhibitor MGBG will reduce GA₃ content (Xu *et al.*, 2001). Smith (1985) suggested that

PAs play a role when GA₃ promotes internode elongation; GA₃ levels are inhibited in floral induction and initiation of olives, whereas low GA₃ levels play a promoting role (Cheng, 2008). In this experiment, both Spd and D-Arg treatment resulted in higher levels of GA₃ in the petal and stamen primordial differentiation phase than in the CK group, and a positive correlation between GA₃ content and endogenous Spd content, further demonstrating that high concentration of GA₃ favors the initiation of bulbils development, low concentration of GA₃ favors the whole process of *Paeonia lactiflora* Pall. bulbils development.

Feng and Yang (2011) found that the content of IAA in the apical bud first decreased during the flower bud differentiation process of chrysanthemum, and reached the minimum value in the early stage of differentiation of floret primordium, and reached the maximum in the middle stage of corolla formation. Johanna *et al.* (2003) thought that IAA is the main factor controlling flower bud differentiation, and high IAA inhibited flower bud differentiation. In this experiment, both exogenous Spd and D-Arg treatment affected endogenous IAA levels by affecting the endogenous Spd content, so that the endogenous IAA remained at a low level during the petal primordium differentiation phase, and during the differentiation of stamen and pistil primordium. It shows that low concentration of IAA facilitates the development of *Paeonia lactiflora* Pall. bulbils.

Exogenous polyamines can increase endogenous ABA concentrations (Serrago *et al.*, 2008); Johanna *et al.* (2003) believe that high levels of ABA inhibit flower bud differentiation; Wijayanri *et al.* (1997) and Ulger *et al.* (2004) both believe that ABA induces flower buds and IAA plays its role in the early stages. This shows that there are differences in the effects of ABA in different plant species. In this experiment, exogenous Spd treatment and D-Arg treatment decreased the ABA content in the petal primordium differentiation phase, increased the ABA content in the stamen primordium differentiation phase, and showed that ABA inhibited the development of the *Paeonia lactiflora* Pall. bulbils.

Some people think that spermidine and spermine can delay the aging of plants by inhibiting the biosynthesis of ethylene (Quinet *et al.*, 2010). The synthetic precursor for polyamines is methionine, which is the same as ETH, suggesting that the metabolism of polyamines may be closely related to ETH (Lv *et al.*, 2016). Exogenous application of Spd inhibits the production of ETH in tomato or plum fruits; ACC synthase inhibitor treatment can promote polyamine synthesis in rice plants (Zhang and Chen, 2005). The precursors of Spd and Spm and ethylene are both S-adenosylmethionine, and PAs and ethylene compete for S-adenosylmethionine, and the production of PAs inhibits the production of ethylene and slows down the rate of senescence in organs (Pan, 1985). In this experiment, exogenous Spd and D-Arg treatment decreased the ETH content in the petal

primordium differentiation phase, increased the ETH content in the stamen primordium differentiation phase, indicating that endogenous Spd affected ETH content, and low concentration ETH favored the development of bulbils, high concentration. ETH inhibits the development of *Paeonia lactiflora* Pall. bulbils.

Conclusion

In this experiment, the treatment of exogenous Spd and D-Arg significantly affected the petal and stamen primordium differentiation phase, and accelerated the development of *Paeonia lactiflora* Pall. bulbils. Spd treatment reduce budding rate, flowering rate, and plant height. D-Arg treatment increase budding rate and flowering rate, and facilitated the development of *Paeonia lactiflora* Pall., providing a new method for the cultivation and management of herbaceous peony and control of blooming season. And theoretical basis, formulate a more feasible comprehensive regulatory management plan to improve the competitiveness of the herbaceous peony fresh cut flowers in the flower market.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31670663), and Shandong Agricultural Engineering Forest Tree Breeding Project Corpus for High-grade Garden Flower Breeding (2130106).

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[Received 11 Dec 2018; Accepted 27 Feb 2019; Published (online) 21 Jun 2019]