

Article

Effects of Low-temperature accumulation on Flowering of *Prunus mume*

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Abstract: Low-temperature accumulation is one of the essential stages in the growth process of woody ornamental plants. In this study, two different low-temperature treatments, 6°C and 10°C, were used to analyze the effects of different low-temperature treatments on dormancy release and flowering of the 'Gulihong' plant using artificial low temperatures. Based on the experimental results, four typical early-blooming *Prunus mume* cultivars widely planted in Yangling area of Henan Province, China, including 'Zaoyudie', 'Zaohualve', 'Nanjing gongfen', and 'Gulihong', were selected as the experimental materials. The effects of low-temperature accumulation on the flowering characteristics of different cultivars were analyzed using a 6°C artificial low-temperature treatment. The suitable cultivation temperature for early-blooming *P. mume* cultivars was screened to provide a theoretical basis for further exploration of *P. mume* bonsai cultivation techniques. The results showed that the flowering rate, flower diameter, flowering quantity, flowering uniformity, and bud development in the 6°C treatment were significantly better than those in the 10°C treatment. The flowering rate and quality of different cultivars gradually increased with the accumulation of low-temperature. Therefore, chill accumulation plays a significant role in promoting flowering quality.

Keywords: *Prunus mume*, low-temperature, flower period regulation, dormancy release, flowering characteristics

1. Introduction

Prunus mume Sieb. et Zucc. is a traditional famous flower in China that blooms in late winter and early spring, making it one of the earliest flowering plants in the winter season. Due to its delicate fragrance, elegant color, and rich cultural heritage, it has been widely cultivated in China and has great market potential (Chu,1999). However, since *P. mume* bloom in winter, they cannot meet the specific viewing needs of other periods. Therefore, regulating the flowering time of *P. mume* can not only meet the viewing needs of the market and the public but also promote the development of the *P. mume* industry.

Natural dormancy is an adaptive mechanism for deciduous fruit trees to resist low temperatures in winter (Faust, M et al.,1997). Temperature is the most important climatic parameter affecting the release of bud natural dormancy in deciduous fruit trees. Under natural conditions, a certain accumulation of low temperatures is a necessary condition for the release of natural dormancy (Coville, 1920; Chandler, 1937; Dokoozlian,1999; Lavee and May,1997). Otherwise, plants cannot germinate and grow normally, and even cause flower organ deformities or severe sterility (Arora et al.,2003). Among them, chilling requirement refers to the effective hours of low-temperature required by deciduous fruit trees to break natural dormancy, and chilling accumulation is considered to be the most effective influencing factor for plants to break dormancy (Faust et al., 1997). Researches have shown that Li et al. (2020) believed that temperature mainly controls the duration of dormancy. Regardless of the photoperiodic conditions, low-temperature can induce growth cessation and control dormancy induction during artificial low-temperature induction of wax *P. mume*. Heide and Prestrud, (2005) found that the growth cessation and dormancy induction of apple and pear were not affected by photoperiod, and proved that low-temperature (<12°C) always induced these two processes regardless of the photoperiodic conditions. Therefore, sufficient low-temperature has a promoting effect on

the release of flower bud dormancy, and if the accumulation of chilling is insufficient, the purpose of early flowering cannot be achieved.

Currently, research on chilling requirements of woody plants mainly focuses on ornamental peach, pear, apricot, and other plants, and there is less relevant research on *P. mume*. However, like other deciduous fruit trees, *P. mume* are also sensitive to temperature and are usually controlled by regulating temperature to release flower bud dormancy. Therefore, in this study, four early-flowering *P. mume* cultivars widely cultivated in Henan Province were selected as experimental materials. Based on artificial low-temperature treatment, the effects of different low-temperature treatments and chilling accumulations on the release of dormancy and flowering were explored, in order to provide theoretical basis for *P. mume* cultivation technology.

2. Materials and Methods

2.1. Experimental materials

The experiment was conducted from September to December 2021 at the Yuling World Plum Garden in Yanling, Henan Province (34°09'N, 114°06'E). Four early-flowering *P. mume* cultivars, including 'Gulihong', 'Nanjing gongfen', 'Zaoyudie', and 'Zaohualve', were selected from the same batch of eight-year-old plants grown in pots, with 42 plants of 'Gulihong' and 24 plants of the other three cultivars, for a total of 114 plants. The experimental plants were robust, with a compact growth habit, no pests or diseases, uniform distribution of branches, plump and full buds, and consistent specifications, with a ground diameter of about 2.5 cm to 4.1 cm and a plant height of about 80 cm. The temperature controller of the cold storage was the XMK-7 type produced by Zhejiang Yuyao Mingxing Refrigeration Accessories Factory, with a temperature accuracy of 0.1°C.

2.2. Experimental Method

2.2.1. Methods to break bud dormancy under different low-temperature treatments

Eight-year-old potted 'Gulihong' were subjected to different artificial low-temperature induction treatments. Prior to the experiment, apical dominance was removed, and 1500mg/L paclobutrazol was sprayed to promote flower bud differentiation. After observing that the internal differentiation of the flower buds was basically completed under a microscope, the experimental materials were gradually cooled down. On September 6th, the samples were placed in two constant temperature cold storages at 6°C and 10°C for artificial low-temperature induction treatment, to induce the dormant buds to enter deep dormancy. The experiment was conducted under conditions of 2000Lx light intensity and 8/16 hours (light/dark) photoperiod, simulating the average light intensity in autumn at this latitude. The relative air humidity in the experimental environment was controlled at 80% by periodic watering of the ground. Starting from September 17th, three pots were taken from each cold room every 5 days, for a total of 7 times. After routine cultivation in the greenhouse for 30 days, the bud sprouting rate under different low-temperature treatments was observed, and the phenology and flowering conditions were recorded.

2.2.2. Experimental methods for the effect of low-temperature accumulation on flowering traits

In this experiment, potted plants of the four early-flowering cultivars of *P. mume*, 'Gulihong', 'Nanjing gongfen', 'Zaoyudie', and 'Zaohualve', all aged eight years old, were used. The control group of plants was placed outdoors under natural light and temperature during the experimental period. The low-temperature refrigeration environment in the cold storage was the same as that in 2.2.1. Starting from September 12, three pots were taken out from the cold storage every 5 days, and the frequency of removal was resumed to every 5 days after the sprouting rate stabilized at 50%. The plants were then moved into the greenhouse for regular management such as watering and fertilization.

2.3. Indicators measurement

2.3.1. Flowering rate

After being removed from the cold storage, the plants of each cultivar were observed daily at around 8 am. Five main stems were selected for each plant, each containing approximately 3 flower branches (about 150 flower buds). The buds were considered to have sprouted when the top of the flower bud cracked and began to show red. Observations began 30 days after sampling and the bud sprouting rate of each cultivar was recorded and calculated daily.

2.3.2. Flower diameter

Ten flowers from each of the different *P. mume* cultivars were selected and measured three times using a caliper with the cross method to determine the diameter at full bloom (precise to 0.01 cm). The flower diameter and other morphological data were recorded.

2.3.3. Bud morphology

Four robust and uniform cultivars in a 6°C cold storage were selected, with 3 plants per cultivar. Ten plump buds from the upper part of a one-year-old branch were marked for each plant, with 3 replicates taken from 3 different plants. Growth parameters were measured at 0 d (the day before the start of low-temperature induction) and every 10 d after treatment. The horizontal (W) and vertical (L) diameters of each bud were measured with an electronic caliper (to the nearest 0.001 cm) to observe changes in bud morphology during low-temperature release from dormancy.

3. Results

3.1. Effect of Different Low-temperature Treatments on Flowering Characteristics

3.1.1. Effect of Different Low-temperature Treatments on Flowering Rate

An experiment was conducted to investigate the effect of different low-temperature treatments on the flowering characteristics of 'Golrihong' plants. The plants were subjected to 6°C and 10°C treatments starting from September 12th, and were moved into a greenhouse for regular cultivation every 5 days until October 12th. The flowering rates of the plants were recorded once a certain chilling requirement was met, as shown in Table 1. Under natural conditions, the control group did not show any signs of bud break. Under the 6°C treatment, initial flowering was limited, with a chilling accumulation of 252 CU required for plants that were moved out on September 17th. However, the flowering rate exceeded 75% when the chilling accumulation reached 492 CU on September 27th. In comparison, under the 10°C treatment, plants moved into the greenhouse on September 12th did not show any signs of bud break initially. On September 17th, one to several flowers bloomed prematurely with a chilling accumulation of 126 CU, and the flowering rate increased gradually as the cold storage time extended. On October 12th, the flowering rate reached 60.07% when the chilling accumulation reached 426 CU. Overall, both low-temperature treatments were able to break bud dormancy, and the flower buds were able to grow and bloom normally. The flowering rate of the plants under both treatments increased gradually with the accumulation of chilling units, but the flowering rate of the 6°C treatment was significantly higher than that of the 10°C treatment.

Table 1. Effects of low-temperature treatments at 6°C and 10°C on the flowering rate of *P.mume*'Gulihong'.

Temperature Treatments (°C)	Flowering Rate (%)					
	Exit date					
	9-17	9-22	9-27	10-02	10-07	10-12
6	9.52	30.69	75.36	86.79	88.48	92.00
10	1.94	11.18	36.13	42.27	46.62	60.07

3.1.2. Effects of different low-temperature treatments on the flower diameter

The effects of different low-temperature treatments on the flower diameter of 'Gulihong' are shown in Table 2. After greenhouse cultivation, it was found that the control group without cold treatment did not flower, and the flower diameter under both low-temperature treatments increased to varying degrees as the cold accumulation increased. The flower diameter under the 6°C treatment was significantly higher than that under the 10°C treatment. In the batch moved out on October 12, the flower diameter under both the 6°C and 10°C treatments reached the maximum value in the corresponding treatment group, with values of 2.74 cm and 2.51 cm, respectively, which significantly increased by 27.44% and 31.41% compared with the batch moved out on September 17. On October 12, the flower diameter under the 6°C treatment increased by 9.16% compared with that under the 10°C treatment. After greenhouse cultivation, it was found that the flowers under the 10°C treatment were generally sparse, irregular, small and lightly fragrant, with poor flowering quality, slow development, and occurrence of flower drop and abortion, resulting in low ornamental value of the plants. It can be seen that the 6°C treatment had a significantly better effect than the 10°C treatment in improving the flowering quality in both treatments

Table 2. Effects of different low-temperature treatments on the diameter of *P.mume*'Gulihong'

Temperature Treatments (°C)	Flower diameter (cm)					
	Exit date					
	9-17	9-22	9-27	10-02	10-07	10-12
6	2.15±0.12a	2.13±0.20a	2.30±0.34a	2.51±0.15a	2.53±0.26a	2.74±0.20a
10	1.91±0.22b	2.05±0.23b	2.12±0.35b	2.30±0.25b	2.48±0.13a	2.51±0.18b

Note: Different lowercase letters in the same column indicate significant differences at the 0.05 level among different treatments ($P < 0.05$); data are mean \pm standard deviation.

3.1.3. Effects of different low-temperature treatments on the flowering quality

The effects of different low-temperature treatments on the flowering quality of 'Gulihong' are shown in Figure 1. After cultivation in the greenhouse, it was found that the overall flowering of the flowers treated at 10°C was sparse and irregular, the flowers were small and fragrant, the flowering quality was not high, the growth was slow and there were cases of bud drop and abortion, and the ornamental value of the plants was not high. It can be seen that among the two treatments

of 6°C and 10°C, the effect of 6°C on improving flowering quality was significantly higher than that of 10°C



Figure 1. The flowering quality of 'Gulihong' under the low-temperature treatment of 6°C and 10°C.

3.2. Effects of different low-temperature accumulations on flowering characteristics

3.2.1. Morphological changes of flower buds under different low-temperature accumulations

Table 3. shows the morphological changes of flower buds during the cold storage dormancy period. During the low-temperature induction to break the *P. mume* dormancy, the morphology of the flower buds also undergoes significant changes. The horizontal and vertical diameters of the flower buds increase with the continuous accumulation of low-temperature. During the dormancy period, the rate of morphological changes of flower buds in each cultivar is slow. After dormancy is released, the vertical diameter of the flower buds increases significantly. The horizontal diameter of 'Gulihong', 'Nanjing gongfen', 'Zaoyudie', and 'Zaohualve' increased by 30.24%, 35.40%, 44.59%, and 41.59%, respectively, compared to before low-temperature treatment. The vertical diameter also increased by 54.38%, 61.38%, 86.81%, and 86.09%, respectively. The horizontal diameter of 'Gulihong' reached the maximum value on October 16, increasing by 10.34%, 10.34%, and 5.96% compared to 'Nanjing gongfen', 'Zaoyudie', and 'Zaohualve', respectively. The vertical diameter of 'Gulihong' also increased by 18.25%, 7.19%, and 12.45% compared to 'Nanjing gongfen', 'Zaoyudie', and 'Zaohualve', respectively. Observation showed that the morphology of flower buds of each cultivar changed significantly before and after September 24, and it is speculated that this period may be a critical time for the physiological changes of flower buds, possibly related to dormancy release.

Table 3. Morphological changes of flower buds of different *P. mume* cultivars during low temperature-induced dormancy.

Date	'Gulihong'		'Nanjing gongfen'		'Zaoyudie'		'Zaohualve'	
	HD (mm)	VD (mm)	HD (mm)	VD (mm)	HD (mm)	VD (mm)	HD (mm)	VD (mm)
0904	1.16±0.13d	1.72±0.21e	1.07±0.13c	1.56±0.21d	1.00±0.12d	1.49±0.21d	1.13±0.22d	1.60±0.36d
0914	1.16±0.21d	1.97±0.26d	1.06±0.11c	1.51±0.21d	1.05±0.12d	1.49±0.23d	1.16±0.23c	1.61±0.26d
0924	1.29±0.10c	2.15±0.19c	1.12±0.17b	1.63±0.27c	1.13±0.12c	1.80±0.25c	1.17±0.16c	1.85±0.25c
1008	1.39±0.15b	2.52±0.18b	1.16±0.14b	1.88±0.26b	1.24±0.12b	2.25±0.24b	1.32±0.15b	2.51±0.24b
1016	1.60±0.11a	2.98±0.14a	1.45±0.16a	2.52±0.29a	1.45±0.10a	2.78±0.27a	1.51±0.01a	2.65±0.18a

Note: Different lowercase letters in the same column indicate significant differences at the 0.05 level among different treatments ($P < 0.05$); data are mean \pm standard deviation; HD represents horizontal diameter; VD represents vertical diameter.

3.2.2. Effects of Different Low-temperature Accumulations on Flowering Rate of Different *P. mume* cultivars

Under constant temperature treatment of 6°C, varying degrees of low-temperature accumulation have an impact on the flowering rate of different *P. mume* cultivars. The results are shown in Table 4. On September 6, 2021 at 10 a.m., different cultivars were placed in a 6°C cold storage, and then moved to the greenhouse for routine cultivation when the cold accumulation reached 132 CU, 252 CU, 372 CU, 492 CU, 612 CU, 732 CU, and 852 CU, respectively. Flowering rate was recorded and observed. Through observation, it was found that the control group without low-temperature treatment did not bloom. Until September 12, when the required cold accumulation was 132 CU, except for 'Gulihong' which showed a few early blooms, the flower buds of other *P. mume* cultivars did not sprout. On September 17, when the required cold accumulation reached 252 CU, flower buds began to sprout, with a flowering rate of 9.52% for each cultivar. Until September 22, when the cold accumulation reached 372 CU, 'Gulihong' and 'Nanjing gongfen' had a lower flowering rate, but 'Zaoyudie' and 'Zaohualve' had a flowering rate of more than 50%. Until September 27, the flowering rate remained above 60%. In conclusion, with the increase of cold accumulation, the flowering rate gradually stabilizes at more than 80%.

Table 4. Effects of Different Low-temperature Accumulation at 6°C on the Flowering Rate of Different *Prume mume* Cultivars.

Exit date	Chilling requirement	Flowering Rate (%)			
		'Gulihong'	'Nanjing gongfen'	'Zaoyudie'	'Zaohualve'
9-12	132	1.89	0	0	0
9-17	252	9.52	0	20.57	0
9-22	372	30.69	22.80	50.39	55.21
9-27	492	75.36	63.52	78.54	60.03
10-02	612	86.79	72.85	81.79	70.94
10-07	732	88.48	76.53	80.91	82.62
10-12	852	92.00	80.93	86.50	84.49

3.2.3 Effects of Different Low-Temperature Accumulation on Flowering Quality of Different *P. mume* cultivars

To investigate the effects of different levels of cold temperature accumulation on breaking dormancy and flowering traits, outdoor-grown plants were used as controls, and the flowering status of

four cultivars were observed in a greenhouse. After greenhouse cultivation, it was found that when the cold temperature accumulation was insufficient, the flowers were small and weakly fragrant, and even deformed with the phenomenon of single-petalled or heavily-petalled flowers blooming together. In addition, the flowering quantity was low, sparse and irregular, and the ornamental value of the plants was low. However, as the cold temperature continued to accumulate, the flower bud abortion of the four cultivars under cultivation was significantly improved, and the flowering quantity and uniformity were significantly increased, with the fragrance becoming more intense and the flowering quality significantly improved. The results showed that cold temperature accumulation played a certain promoting role in the flowering quality during cultivation. The mechanism behind this phenomenon may be related to the regulation of plant hormones, gene expression, and physiological and biochemical processes.

4. Discussion

Low-temperature is the key factor affecting the release of dormancy and flowering of plants. Different low-temperature treatments have effects on flowering traits such as flowering rate and flowering quality. Li et al. (2020) artificially low-temperature treated 'Suxin' wintersweet based on the Utah model and the 7.2°C model. In a low-temperature environment below 12°C, when the cooling requirement reaches 570 CU, the wintersweet flower buds can expand and open normally in advance. Heide and Prestrud, (2005) studied growth and dormancy of four apple cultivars and one pear cultivar and found that at least 6 weeks (about 1000 hours) of chilling at 6 or 9°C were required for release of dormancy and recovery of growth, while chilling at 12°C for 14 weeks treatment is almost ineffective. Ouyang et al. (2002) selected two nectarine cultivars 'Zaohongzhu' and 'Ruiguang 5' as test materials, and studied the effects of different temperature treatments on releasing the natural dormancy of peach buds. The results showed that the treatment at 6°C/15°C had the best effect. In this study, different low-temperature treatments of 6°C and 10°C were carried out on 'Gulihong', and it was found that the plants in the control group had no signs of germination under natural conditions. After cultivation in the greenhouse, both low-temperature treatments could break the dormancy of flower buds. The flower buds can also expand and open normally, but the flowering rate, flower diameter, flowering quantity, flowering uniformity and bud development of the 6 °C treatment group are significantly better than the 10 °C treatment group.

The accumulation of different low temperatures will have certain effects on flowering rate, flower diameter, flowering quality and other flowering traits of plants. Campoy et al. (2013) found that under different cold accumulations, the temperature efficiencies of apricot tree dormancy relief were different. With the accumulation of cold, the germination rate under all temperature treatments continued to increase. Nie et al. (2012) aimed at the problem of earlier flowering of *P. mume* in Kunming, and adopted low-temperature treatment for early, middle and late flowering cultivars in order to delay the flowering period. Low-temperature refrigeration treatment has a significant effect on delaying the flowering period of *P. mume*. In this study, the results of greenhouse cultivation showed that the plants in the control group without cold temperature treatment did not bloom at all. Under the constant temperature treatment at 6°C, the flowering rate showed an upward trend with the accumulation of cold. This is consistent with previous work by Gariglio et al. (2006) on peaches and Cook and Jacobs (2000) on apples. In addition, the flowering quantity and flowering uniformity of each cultivar are also significantly improved, the flower fragrance is more intense, and the flowering quality is significantly improved; when the cold accumulation is insufficient, the flowers are small and the fragrance is weak, accompanied by the phenomenon of single and double petals blooming at the same time, and the flowering is sparse and irregular, and even the flower buds will be aborted in severe cases, and flower development abnormalities such as 'leaf-wrapped flowers' will appear, which will affect the viewing effect. This is similar to the results of previous studies on pear (Jacobs et al.,2002), apple (Naor et al.,2003). The results showed that in the process of relieving flower bud dormancy at low-temperature, the accumulation of cold energy played a certain role in promoting flowering quality, otherwise it would affect the growth and development disorders in the later stage, even if the management in the later stage was perfect, it would not be able to develop normally.

5. Conclusions

The results of this research have significant implications for the cultivation of *P. mume*. By using artificial low-temperature to release dormancy, this study found that among the widely cultivated early-flowering cultivars in Henan Province, a treatment of 6°C can significantly improve the cultivation effect of *P. mume*. In addition, as the accumulated cold amount increases, the flowering rate and quality of *P. mume* cultivars under different temperature treatments gradually improve. These research results provide a basis for us to develop targeted cultivation management measures for *P. mume*, and help guide precise market control of flower production in Henan Province. In the future, we can further set up more temperature gradients to explore the optimal temperature for promoting the cultivation of *P. mume*, and analyze the dormancy mechanism of *P. mume* at the molecular level from transcriptome, metabolome, and other levels, in order to further study the effect of temperature on releasing dormancy of *P. mume*, and provide a more scientific basis for the development of *P. mume* cultivation technology. These research achievements are expected to promote the continuous improvement and perfection of *P. mume* cultivation technology, and also make a positive contribution to the development of the *P. mume* industry.

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