梅花‘南京红须’、‘南京红’花色的呈现特征

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摘 要：梅花‘南京红须’、‘南京红’的花色主要存在着花发育阶段导致的时间变化，反映其花色受花发育控制。二者的花色都在蕾期最浓艳，在初花期略淡，在盛花期又稍浓，在末花期最淡，尽管花瓣在花开放时便开始衰老；在整个花发育时期，同一朵花不同层次花瓣的颜色浓淡均为：外层花瓣＞中层花瓣＞内层花瓣，即花瓣在花冠中的具体排列位置决定着该片花瓣的特定颜色深浅；但不同层次花瓣颜色的变化趋势不完全一致。同时，两个品种外层花瓣的总黄酮含量变化与外层花瓣的色度变化成正相关。而花朵在树冠的着生部位导致的花色差异极不显著，表明‘南京红须’、‘南京红’的花色的空间变化极微。本文可为梅花红色花色的机理探索和花色色素生物合成关键酶基因 cDNA 克隆中的花朵选择提供参考。

关键词：‘南京红须’；‘南京红’；花色；呈现特征

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Expression characteristics of the flower colors of *Prunus mume* ‘Nanjing Hongxu’ and *Prunus mume* ‘Nanjing Hong’

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Abstract: The flower colors of *Prunus mume* ‘Nanjing Hongxu’ and *P. mume* ‘Nanjing Hong’ exist mainly temporal changes caused by different flower developing stages, reflecting that the flower colors are under the control of flower development. The flower colors of these two cultivars are all the strongest in Alabastrum period, thin in some sort in Initial flower period, thicken appreciably in Profuse flower period and thin furthest in Final flower period even if the petals senesce as soon as they begin to splay. At different flower developing stage, the chroma differences of petal colors of different layers are: outer layer＞middle layer＞inner layer, namely, the concrete collocating site of petal in the corolla decides the specific color chroma of this petal. But the change trends of the petal colors of different layers are not completely similar. In the mean time, the content changes of total flavonoids of the outer layers of two cultivars are positively correlated with the petal color changes of outer layers. On the other hand, the flower color difference induced by the inserting site of the flower in canopy is not remarkable at all, showing that the spatial variations of the flower colors are very petit.

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This paper could be a reference or a premise for the exploration on the flower color mechanism of red Mei flowers and the flower-selecting in the cDNA cloning of the key enzyme genes determining the biosynthesis of anthocyanins in Mei flower.

Key words: *P. mume* 'Nanjing Hongxu' (Nanjing red-bearded); *P. mume* 'Nanjing Hong' (Nanjing red); flower color; expression characteristics

Mei (*Prunus mume* Sieb. et Zucc.) flower is beloved deeply by Chinese for thousands of years because of its beauty, purity and quality of resisting cold. Today, it is one of the candidates of the national flower of P. R. China. It is well known that the scientific studies on Mei flower in China have made splendent achievements and Mei flower is the first horticultural plant which is accredited Chinese scientist, namely Chen Jun-Yu, as International Cultivar Registration Authority (Chen, 2002; Zhao *et al.*, 2003).

However, the study on the flower color of Mei has been a blank all the while (Chen, 2002; Zhang *et al.*, 2003).

The flower colors of Mei are very various, including mauve, pink, pure white, greenish white, light yellow or double color (Chen, 2001) and the flower color is the most pivotal characteristics determining the grace beauty and the aesthetic taste of Mei. *P. mume* 'Nanjing Hongxu' (Nanjing red-bearded) and *P. mume* 'Nanjing Hong' (Nanjing red) are the typical representatives of the mauve and the pink respectively (Chen, 1989). We have found that the red flower colors of these two cultivars result from the existence of the anthocyanins in their petals (Zhao *et al.*, 2004). But nobody has explored the variations of the flower colors of the cultivars till today.

This paper deals with the temporal and spatial variations of the flower colors of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' at flower developing stage and with the inserting site of the flower in canopy. The research results could provide a reference or a premise for the aesthetic appreciation of the flower color of Mei, the exploration on the flower color mechanism of red Mei flowers and the flower-selecting in cDNA cloning of the key enzyme genes determining the biosynthesis of anthocyanins in Mei flower.

1 Materials and methods

1.1 General

The layer classification of the petal was carried out according to the concrete and natural collocating site of the petal in the corolla. The open angle of the petal in the corolla was defined as the departure angle which was formed by the assumptive connecting beeline of the petal tip and the inserting point of the petal on receptacle vs the vertical axis passing through the inserting point.

The relative content of the anthocyanins of petal was mensurated with spectrophotometry. Anthocyanins of the petals were extracted with methanol containing 1% concentrated HCl (v/v) (Markham, 1982). Extracts were diluted properly with the same acidic methanol. A_{530} and A_{657} were determined at room temperature in a 1 cm path-length quartz cell using a Shimadzu UV-Vis spectrophotometer. The relative content of anthocyanin was calculated by a formula proposed by Rabino and Mancinelli (1986), namely ($A_{530} - 0.25 A_{657}$)/g(FW).

The content of total flavonoids of petal was determined according to the method developed by Zhao *et al.* (2004), and the formula ($C = 0.0749 \times A_{436} - 0.0024$) was selected as standard curve equation. The malondialdehyde (MDA) content of petal was determined with "Thiobarbituric acid (TBA) method" (Li, 2000). 0.5 g petals were ground with 5 mL phosphoric acid buffer (pH7.8) on ice and the TBA reaction was terminated by icy waterbath for 5 min. The absorbency was determined using above UV-Vis spectrophotometer.

All solvents used were of analytical grade.

1.2 Plant material

Flowers were obtained at Mei Flower Hill of Sun Yat-sen Mausoleum Administrative Office of Nanjing. The distance between plants of the trees is 3 m×3 m. The tree with well-balanced vigor and inerratic canopy was selected as sampling tree. On Feb 23 of 2004, just after the dew evaporated completely, various flowers were selected and picked according to different stages of flower developing and the Profuse flowers were picked according to different inserting site of flower in canopy. All flowers were encased in yellow kraft envelopes and immediately frozen at -20～-22℃ until analysis.

1.3 Temporal variations of the flower color: effects of flower developing stage on the flower color

1.3.1 Partition of the flower developing stage The developing course of Mei flower should be differentiated according to 5 concrete indexes including transverse diameter of corolla, flower shape, petal color, open angle of petal and stamen characteristics.

1.3.2 Determination of the variations of flower color From the outer to the inner, the petals of outer, middle and inner layers of the flowers of various developing stages were peeled carefully. Thereinto, the corolla of *P. mume* 'Nanjing Hongxu' usually consists of 3 layers of petals, there are 5 petals at outer, middle and inner layer respectively. *P. mume* 'Nanjing Hong' often possesses of 4 layers of petals. From outer to inner, the first and forth layer were respectively regarded as "outer layer" and "inner layer", and every layer composes of 5 petals. The second and third layers were generally regarded as "middle layer" composing of 10 petals. The flower color variations were quantified using the dynamic changes of the relative content of anthocyanins of petal. Synchronously, the contents of total flavonoids and MDA of petals were also determined.

1.4 Spacial variations of the flower color: effects of the inserting site of flower in the canopy on the flower color

The flower colors of the Profuse flowers of different inserting site of flower in canopy were also determined by the relative content of anthocyanins.

Table 1 Comparison of the visual characteristics of the flowers of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' at different developing stages

Cultivar	Developing stage of flower	Stamen	Petal color			Open angle of petal (°)		
			Outer layer	Middle layer	Inner layer	Outer layer	Middle layer	Inner layer
'Nanjing Hongxu'	Ⅰ	Hidden	Dark mauve	Mauve	Red	-10～-30	-10～-30	-10～-30
	Ⅱ	Golden anther	Mauve	Red	Red	10～45	10～40	10～30
	Ⅲ	Golden anther, light red filament	Mauve	Red	Red	45～60	40～50	30～45
	Ⅳ	Dark anther, erect filament	Red	Pink	Pink	60～100	50～90	45～90
'Nanjing Hong'	Ⅰ	Hidden	Dark mauve	Mauve	Red	-10～-30	-10～-30	-10～-30
	Ⅱ	Golden anther	Mauve	Red	Pink	10～45	10～40	10～30
	Ⅲ	Golden anther, white filament	Mauve	Pink	Pink	45～60	40～50	30～45
	Ⅳ	Dark anther, erect filament	Red	Pink	Faint red	60～120	50～90	45～90

2 Results and analyses

2.1 Visual characteristics of the flowers changed with flower developing stages

The flower developing of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' could be divided into 4 stages, namely Stage Ⅰ (Alabastrum period), Stage Ⅱ (Initial flower period), Stage Ⅲ (Profuse flower period) and Stage Ⅳ (Final flower period) (Table 1), and the flower shapes are "bead", "cup", "bowl" and "dish" respectively. Thereinto, the integrated expression of flower shape and flower color is the culmination at Stage Ⅲ which can fully unfurl the aesthetic merit of the cultivar.

Though the transverse diameters of the corollas of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' are almost the same at Stage Ⅳ, the splaying speed of the corolla of *P. mume*'Nanjing Hongxu' is quicker all the times (Fig. 1), showing that the flower of *P. mume*'Nanjing Hongxu' is bigger and more flamboyant than that of *P. mume* 'Nanjing Hong'. At Stage Ⅳ, many of the petals of outer layer of *P. mume*'Nanjing Hong' are prone to slouch, droop and abscise.

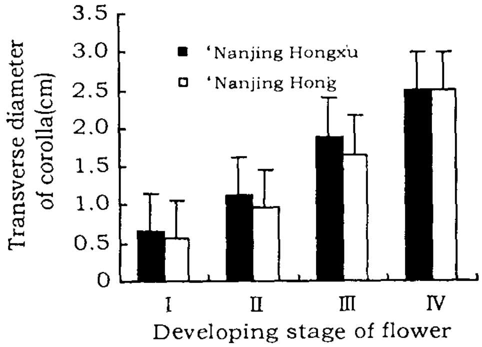

Fig. 1 Changes of the transverse diameters of corollas of *P. mume*'Nanjing Hongxu' and *P. mume*'Nanjing Hong' at the flower developing stage (The transverse diameter is the average value of 30 flowers)

2.2 Effects of flower developing stage on the flower color

2.2.1 Variations of the flower color at flower developing stages The flower colors of *P. mume* 'Nanjing Hongxu' and *P. mume*'Nanjing Hong' all reach the climax at Stage Ⅰ and begin to fade gradually soon after the petals spread despite the possible second peak at Stage Ⅲ (Figure 2, 3 and 4). It is speculated that the anthocyanins of the petals start to decompose ceaselessly as soon as the petals outspread because of light (Sweeny *et al.*, 1981; Rabino and Mancinelli, 1986; Beckwith *et al.*, 2004), which results directly in the fading of the flower color.

The change trends of the petal colors of different layers of *P. mume*'Nanjing Hongxu' at flower developing stage are almost the same: The relative contents of the anthocyanins of 3 layers are all the strongest at Stage Ⅰ, thin in some sort at Stage Ⅱ, thicken appreciably at Stage Ⅲ and thin furthest at Stage Ⅳ (Fig. 2). At Stage Ⅱ and Ⅳ, the colors of middle and inner layers are feckly uniform. However, the petal color of outer layer is much stronger than those of middle and inner layers at different flower developing stages (Fig. 2).

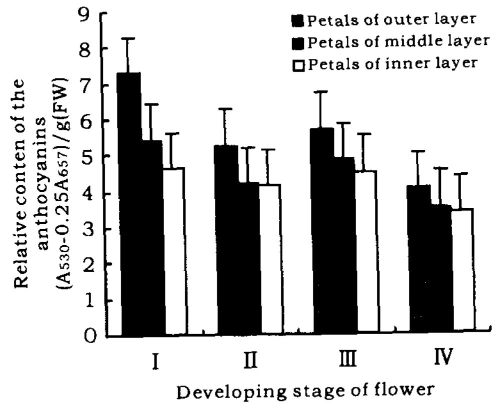

Fig. 2 Variations of the petal color of the different layers of *P. mume*'Nanjing Hongxu' at flower developing stage

The color of the whole corolla of *P. mume* 'Nanjing Hongxu' is the strongest at Stage Ⅰ, and the color at Stage Ⅲ is the next. The relative contents of anthocyanins at Stage Ⅲ, Ⅱ and Ⅳ are 86%, 78% and 63% of that at Stage Ⅰ respectively (Fig. 3).

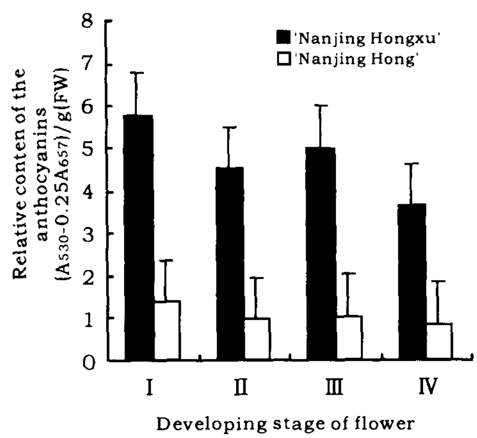

Fig. 3 Variations of the flower colors of *P. mume* 'Nanjing Hongxu' and *P. mume*'Nanjing Hong' at flower developing stage (The value of relative content of the anthocyanins is the average of those of three layers of petals)

The relative contents of the anthocyanins of 3 layers of *P. mume* 'Nanjing Hong' are also the strongest at Stage Ⅰ. The change trends of the petal colors of middle and inner layers at flower developing stages are almost the same: The relative contents thin at Stage Ⅱ, thicken appreciably at Stage Ⅲ and thin furthest at Stage Ⅳ. But the relative contents of outer layer decrease continuously with the flower developing stages (Fig. 4). The relative contents of anthocyanins at Stage Ⅲ, Ⅱ and Ⅳ are 75%, 70% and 61% of that at Stage Ⅰ. As to the whole corolla, the chroma difference of the flower color at 4 stages is not remarkable although the color is also the strongest at Stage Ⅰ, and the color at Stage Ⅲ is the next (Fig. 3).

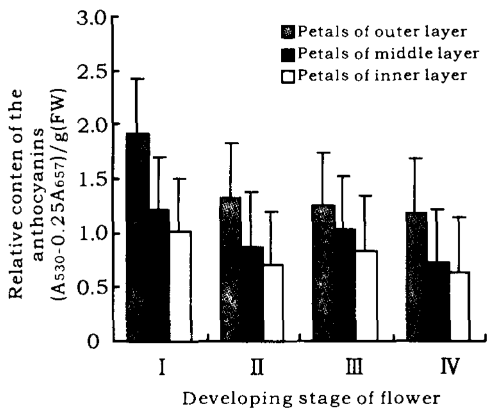

Fig. 4 Variations of the petal color of the different layers of *P. mume*'Nanjing Hong' at flower developing stage

At different flower developing stages, the chroma differences of petal colors of different layers of *P. mume*'Nanjing Hong' and *P. mume*'Nanjing Hong' are: outer layer > middle layer > inner layer (Fig. 2,4). It has been found to be the most important phenomena that the petals of the different layers of one corolla of *P. mume* 'Nanjing Hongxu' or *P. mume*'Nanjing Hong' are provided with obvious chroma difference. However, the inserting sites of all petals of one corolla on the receptacle are almost located in the same plane. The imbricate collocation of the petal in the corolla results in the specific site where the petal is situated in different layer and expresses the chroma difference. As a result, the concrete collocating site of petal in the corolla is the special characteristic of petal developing, deciding how the petal expresses specific chroma under the control of internal and external factors.

2.2.2 Variations of the content of total flavonoids of the petals at flower developing stage The contents of total flavonoids of 3 layers of petals of *P. mume* 'Nanjing Hong' and *P. mume* 'Nanjing Hong' all decrease with flower development, and the decreases of the contents of total flavonoids of outer layers exist the "tardiness point" at Stage Ⅲ (Fig. 5), which is consistent with the second peak of the petal color of outer layers. It can be thought that the changes of the contents of total flavonoids of outer layers of *P. mume*'Nanjing Hong' and *P. mume*'Nanjing Hong' are positively correlated with the petal color changes of outer layers (Fig. 2,5). This means that the flower colors of *P. mume* 'Nanjing Hongxu' and *P. mume*'Nanjing Hong' probably result from the interaction of the anthocyanins and the coexisting copigments, e. g. flavonols, because the flower colors of these two cultivars of Mei are mainly displayed by the petals of outer layers. On the other hand, the contents of total flavonoids of middle and inner layers of two cultivars almost decrease point-blank (Fig. 5).

2.2.3 Variations of the MDA content of the petals at flower developing stage The MDA contents of 3 layers of petals of *P. mume*'Nanjing Hong' and *P. mume*'Nanjing Hong' all increase with the flower development (Fig. 6), which implies that the petals senesce as soon as they begin to splay although their senescence is concealed by the flamboyant red. Furthermore, the changes of MDA contents also show that the senescence of the petals of outer layers is much quicker, being evidenced by the rapid depigmentation of outer petals after Stage Ⅰ (Fig. 2, 4). However, as to *P. mume* 'Nanjing Hong', it is unknown why the senescence of the petals of inner layer is quicker than that of middle layer after Stage Ⅱ (Fig. 6, B).

2.3 Effects of the inserting site of flower in the canopy on the flower color

The corolla chromas of the flowers inserting at different sites of canopy of *P. mume* 'Nanjing Hongxu' or *P. mume*'Nanjing Hong' were not accordant (Table 2). The higher the inserting site of the flower was, the thinner the flower color was. The flower color of the outer layer of canopy was thinner than that of the inner layer. However, va-

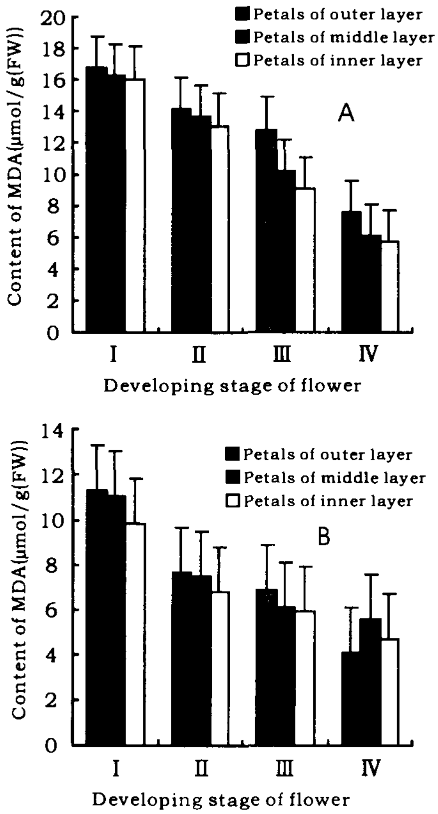

Fig. 5 Changes of the total flavonoids content in the petals of *P. mume* 'Nanjing Hong' and *P. mume* 'Nanjing Hong' at flower developing stage

A: *P. mume* 'Nanjing Hongxu'; B: *P. mume* 'Nanjing Hong'.

riance analysis reflects that the "F values" of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' are 3.402 3 and 2.845 3 ($<4.39=F_{0.95}(5, 6)$), respectively. Therefore, the flower color difference caused by the inserting site of flower in canopy was not remarkable at all, showing that the spatial variations of the flower colors are very petit. It has been confirmed that light is the most primary factor which influences the biosynthesis and decomposition of anthocyanins (Sweeny *et al.*, 1981; Rabino *et al.*, 1986; Beckwith *et al.*, 2004).

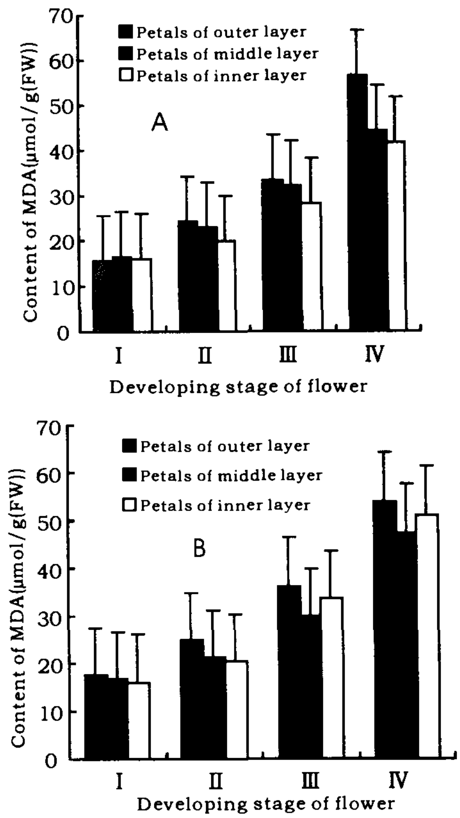

Fig. 6 Changes of the MDA content in the petals of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' at flower developing stage

A: *P. mume* 'Nanjing Hongxu'; B: *P. mume* 'Nanjing Hong'.

Table 2 Changes of the flower colors of *P. mume* 'Nanjing Hong' and *P. mume* 'Nanjing Hongxu' because of the inserting sites of the flowers in canopy (Relative content of the anthocyanins, $(A_{530}-0.25A_{657})/g \cdot FW$; The average value of two experiments)

Cultivar	Flowers on the top of canopy		Flowers in the middle part of canopy		Flowers on the lower part of canopy	
	Outer layer	Inner layer	Outer layer	Inner layer	Outer layer	Inner layer
'Nanjing Hongxu'	4.975	4.982	5.021	5.028	5.084	5.122
'Nanjing Hong'	1.170	1.187	1.225	1.231	1.247	1.255

That Mei effloresces before leaves bourgeon leads to the nicer and even conditions of natural ventilation and daylight illumination in different sites of one canopy, which results in the almost even contents of anthocyanins in flowers owning different inserting sites in the same canopy.

3 Discussion

The flower colors of *P. mume* 'Nanjing

Hongxu' and *P. mume* 'Nanjing Hong' are mainly under the control of flower developing, which is accordant with the research result of Martin and Gerats(1993). The colors of the petals of different layers or the whole corolla all change with flower developing stages. The colors are the strongest in Alabastrum period, thin in some sort in Initial flower period, thicken appreciably in Profuse flower period and thin furthest in Final flower period even if the petals senesce as soon as they begin to splay. At different flower developing stages, the chroma differences of petal colors of different layers are: outer layer > middle layer > inner layer, namely, the concrete collocating site of petal in the corolla decides the specific color chroma of this petal. But the change trends of the petal colors of different layers of one flower are not completely similar. And the flower colors result from the interaction of the anthocyanins and the coexisting copigments. On the other hand, the flower color difference caused by the inserting site of flower in canopy is not remarkable at all, reflecting that the spatial variations of the flower colors are very potty just because of the unconspicuous difference of ventilation and illumination in different sites of one canopy.

It is estimated that, as to Mei efflorescing in early spring, the red flower color possesses of special biological significance except luring pollinators, namely it can strengthen the ability of Mei to effloresce against cold. For many years, flower color of higher plant is thought to lure pollinators, such as insects and birds(Harborne, 1973; Driver*et al.*, 1998). But it has been found that the stamens and gynoecia of Mei flower are usually petalized and the Mei which flower is appreciated specifically by people is not generally fructiferous(Chen, 1989; Chen, 2001). Furthermore, three remarkable phenomena were observed in our study. (1) The flower colors of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' have been reached the climax in Alabastrum period when the petals do not spread at all, and begin to fade as soon as the petals spread. So, the period when the flower color is the strongest is not the period when the flower is waiting for pollination. (2) After the petals outspread, the petals of the outer layer often press close to branch and can not become the first point of the line of sight of animals. So, it is apparently impossible that the remarkable flamboyant of the petal color of the outer layer exists specially because of luring animals. (3) Mei flower is provided with much more characteristics of anemophilous flowers, e. g. the stamens protrude far-forth outside the corolla after the petals spread. On the other hand, it has been found that, in many plant tissues, the structure and control genes controlling the biosynthesis of anthocyanins can be induced to express by low temperature (Christie *et al.*, 1994; Leyva *et al.*, 1995; Shvarts *et al.*, 1997). Therefore, from the evolutionary viewpoint, the production and accumulation of the anthocyanins in petals of Mei may be the result of cold induction and selection of a very long period of time. As a result, the red flower color may play a role in protecting the flower of *P. mume* against cold in winter and early spring (Driver*et al.*, 1998; Leng *et al.*, 2003), which directly determines the survival possibility of this plant on earth.

The identifications of the molecular structures of the anthocyanins of the flower color pigments of *P. mume* 'Nanjing Hongxu' and *P. mume* 'Nanjing Hong' reveal that the key enzyme determining the red flower color of Mei is flavonoid 3'-hydroxylase (*F3'H*)(Zhao *et al.*, 2004a, b). It has been found that the transcription of *F3'H* and the activity of *F3'H* in the flower of *Petunia hybrida* 'Old Glory Red' (Brugliera *et al.*, 1999) and *Torenia hybrida* 'Summerwave Blue' (Ueyama *et al.*, 2002) all reach the climax in Alabastrum period. Therefore, in cDNA-cloning of the *F3'H* of Mei with red flower color, the flowers being in Alabastrum period should be the first choice.

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