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百合花瓣衰老过程中的蛋白质变化研究

薛秋华[a*]，林 香[b]，潘东明[b]
(福建农林大学, a. 园林学院; b. 园艺学院, 福州 350002)

摘要：以5个百合品种为材料,对百合切花花瓣衰老过程中可溶性蛋白的变化进行了研究。结果表明,5个品种的百合切花都具有相同的蛋白质条带,如49.9 kDa、45 kDa、32.8 kDa、22.1 kDa;但品种间也有明显差异,如38.4 kDa是'黄色风暴'特有的;40 kDa为'铁炮百合'特有;'金百合'没有26.5 kDa的条带,但是相同的杂交品系之间蛋白条带的相似性要明显高于不同杂交品系;在百合花花瓣衰老过程中有的蛋白质的含量不断下降,而有的保持不变;在花瓣衰败期有新的蛋白条带出现,如'西伯利亚'、'黄色风暴'和'索邦'均出现58.3 kDa条带、'金百合'出现67 kDa的条带,这些条带可能与花瓣衰老相关,是衰老特异蛋白。

关键词：百合；切花；衰老；蛋白质
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Changes in Protein during Senescence of Lily Cut Flowers

XUE Qiu-hua[a*], LIN Xiang[b], PAN Dong-ming[b]
(a. *College of Landscape Architecture*; b. *College of Horticulture, Fujian Agriculture and Forestry University*, Fuzhou 350002, China)

Abstract: The changes of proteins in cut flowers of five cultivars of lily *(Lilium* spp.) during senescence were studied using the improved polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that there were some similar protein bands, i.e. 49.9 kDa、45 kDa、32.8 kDa、22.1 kDa, in five lily cultivars, but there also were clear difference among the varieties, for example, the band of 38.4 kDa was characterized for 'Aziatische Gr. Yelloween', 'Longiflorum' with 40 kDa, but no 26.5 kDa. However, the similarity of protein band between the same hybrid strains was obviously higher than that between different ones. During the process of senescence, the contents of some protein decreased constantly, but some keep stablity. There were new protein bands occurred during petal degrade stage, such as 58.3 kDa for 'Oriental Gr. Siberia', 'Oriental Gr. Sorbonne', and 'Aziatische Gr. Yelloween', 67 kDa for 'Aziatische Gr. Trompeten'. It suggested that these specific proteins were related to petal aging possibly.

Key words: Lily; Cut flower; Senescence; Protein

Lilium spp., belong to family *Liliaceae*, are fragrant, bulbous, herbaceous perennials. Because of the availability in a wide variety of cultivars, colors and fragrance, lily flowers are used by many people as traditional flowers in wedding, Easter holiday in many countries. In ancient times, many cultures considered lilies to be signs of fertility and a pure life, and the flowers were used as offerings to appease the gods. In China, lily flowers were pronounced as "*Baihe*" in Chinese which means "love for all seasons", hence it is a "must have" wedding flower[1].

Many studies in post-harvest physiology,

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* Corresponding author

transportation and preservative technology of lily cut flowers around the world were carried out[2-8]. However, there was not report about the relationships between senescence and protein changes of cut flowers. Woodson et al.[9] had analyzed total soluble protein in *Hibiscus rosa-sinensis* L., Wang et al.[10] had studied the changes in soluble protein during the senescence of Chinese rose petal, and Xu et al.[11] exmined the changes in proteins during petal senescence of sweet osmanthus (*Osmanthus fragrans*). In this study, we used five cultivars of lily species as materials, 'Aziatische Gr. Trompeten', 'Oriental Gr. Siberia', 'Oriental Gr. Sorbonne', 'Oriental Gr. Yelloween' and 'Longiflorum', to discusse the changes of protein contents during petal sencscence, which could provide theoretical evidence for prolonging the life of lily cut-flowers.

1 Materials and methods

1.1 Materials

Flowers of the five lily cultivars, 'Aziatische Gr. Trompeten', 'Oriental Gr. Siberia', 'Oriental Gr. Sorbonne', 'Oriental Gr. yelloween' and 'longiflorum', were harvested and transported to our laboratory at October, 2006. Upon arrivals, the flowers were cut under deionized water and kept with average 40 cm long in plastic buckets filled with deionized water. The laboratory conditions were 20 ± 2℃, about 50% humidity and interior florescence light. The flowers during the senescence were divided into five stages, green bud, color change, tentative blossom, full bloom and degrade, respectively. At each stage, the middle section of the petals were weighed and cut into small pieces, then immediately put into liquid nitrogen for 3 minutes. The petals were then stored in ultra-low temperature freezer for use.

1.2 Methods

SDS-PAGE was performed based on the protocol described by Wan et al.[12] with changes in the gel recipe and preparation.

Protein extraction The middle sections of the outermost portals were sampled, and one gram of flower petals was mixed with 5 ml buffer (5 mmol/L Tris-HCl buffer, pH 6.8, 0.5% SDS, 10% glycerol and 5% β-mercaptoethanol) and grounded on water bath. The mixture was then centrifuged with 15 000 × g for 20 minutes. The suspension solution was poured into a clean beaker.

Protein Electrophoresis Twenty μl of suspension solution was mixed with 20 μl 2 × loading buffer and then boiled for 5 minutes. The mixture was then centrifuged with 18 000 × g for one minute. 20 μl of the resulted suspension was loaded to the gel. The standard Laemmli electrophoresis system was performed with 1.5 mm in thickness, 12.5% separating gel and 4% stacking gel under 100 constant Voltage. After electrophoresis, the gel was stained using silver-staining technique and immediately photographed.

2 Results

2.1 Vase life

The vase life of lily cut flowers were differet greatly among cultivars. In deionized water, 'Oriental Gr. Siberia' had the longest vase life of 12 d, both 'Oriental Gr. Sorbonne' and 'Aziatische Gr. Yelloween' 10 d, and both 'Longiflorum' and 'Aziatische Gr. Trompeten' 8 d.

2.2 Comparison of protein patterns at initial stage of cut flowers

Four polypeptide bands with molecular weights of 49.9, 45, 32.8, and 22.1 kDa, respectively, were all shown in the five cultivars (Fig. 1), while there were great variances of other bands presented in different flowers. The cultivar 'Longiflorum' had the most polypeptide bands, and the 'Aziatische Gr. Trompeten' had the fewest. A polypeptide band between molecular weights of 38.4 kDa and 32.8 kDa was only presented in 'Aziatische Gr. Trompeten'. 'All cultivars' had a 26.5 kDa polypeptide band except 'Aziatische Gr. Trompeten'. There was a 38.4 kDa polypeptide band only in 'Oriental Gr. Yelloween' and a 40 kDa only in 'Longiflorum'.

Three cultivars, 'Longiflorum', 'Oriental Gr. Siberia' and 'Oriental Gr. Sorbonne', had the highest contents of protein with molecular weight of

22.1 kDa, and the ‘Oriental Gr. Sorbonne’ had more 14.4 kDa proteins than other cultivars. The results indicated that both protein contents and types of proteins in flowers of the five cultivars were different.

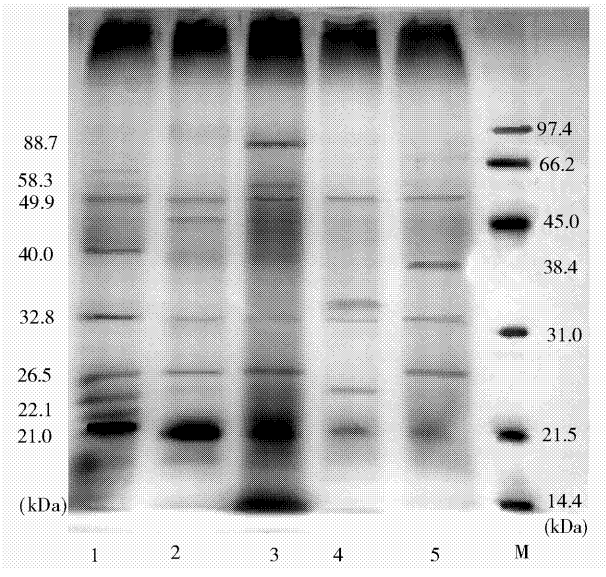

Fig. 1 Contrast of protein pattern in green bud stage of the five different cultivars of *Lilium* spp.

1. ‘Longiflorum’; 2. ‘Oriental Gr. Siberia’; 3. ‘Oriental Gr. Sorbonne’; 4. ‘Aziatische Gr. Trompeten’; 5. ‘Aziatische Gr. Yelloween’.

2.3 Changes of proteins patterns

Protein patterns of flower petals during the senescence process were shown in Fig. 2 ~ 6. The protein contents in ‘Longiflorum’ gradually decreased during preservation time. Some of the protein bands, especially with higher molecular weight protein between 41 and 97 kDa, dramatically decreased at full bloom stage (stage 4) and even disappeared at the last stage (stage 5). No new protein bands appeared in the electrophoresis profile in this cultivar (Fig. 2).

The contents of proteins in ‘Oriental Gr. Siberia’ with molecular weights ranging from 63.2 to 97.2 kDa and 39.1 to 52.4 kDa decreased or even degraded completely in the process of senescence (Fig. 3). However, Protein bands of 52.4 kDa, 25.8 kDa and 27.2 kDa, did not change at all stages. The protein contents of the low molecular weight bands , such as 14.4 and 16.9 kDa, increased slightly. There is a new band with molecular weight 58.3 kDa appearing at the degrade stage.

Protein contents in ‘Oriental Gr. Sorbonne’ during the aging process steady decreased (Fig. 4). Protein bands with molecular weight in the range from 39.9 to 96.3 kDa disappeared, while a new 58.3 kDa band appeared in the last stage of the flower senescence.

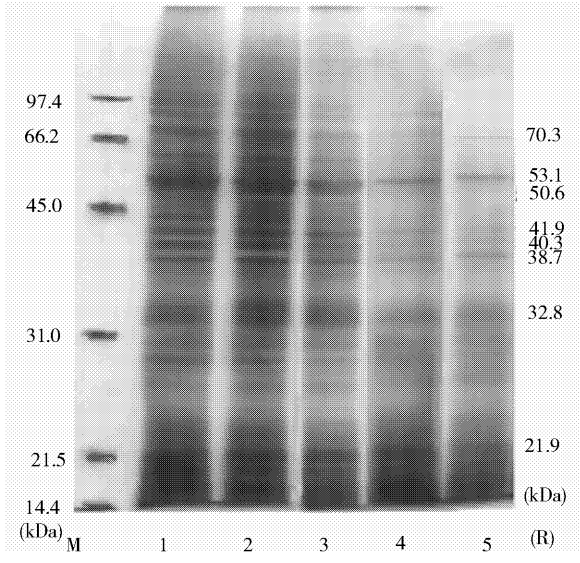

Fig. 2 Change of protein pattern in cut flowers of *Lilium* ‘Longiflorum’ during senescence

1. Green bud stage; 2. Color change stage; 3. Tentative blossom stage; 4. Full bloom stage; 5. Degrade stage.

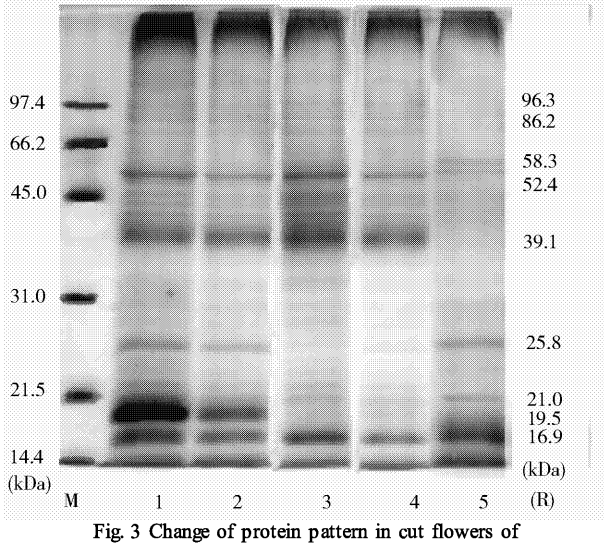

Fig. 3 Change of protein pattern in cut flowers of *Lilium* ‘Oriental Gr. Siberia’ during senescence

1. Green bud stage; 2. Color change stage; 3. Tentative blossom stage; 4. Full bloom stage; 5. Degrade stage

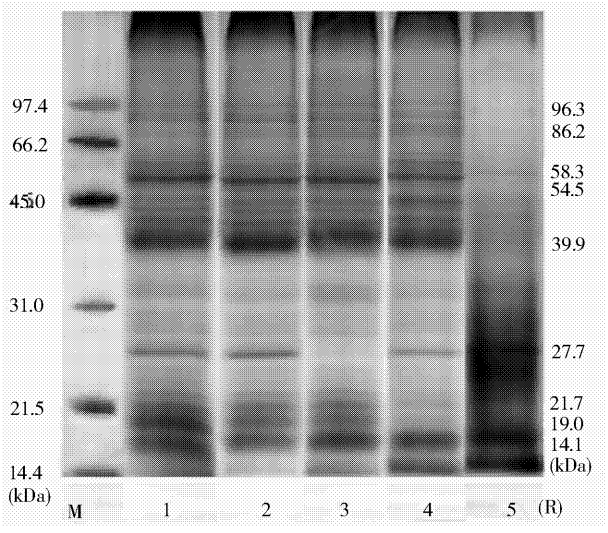

Fig. 4 Change of protein pattern in cut flowers of *Lilium* 'Oriental Gr. Sorbonne' during senescence

1. Green bud stage; 2. Color change stage; 3. Tentative blossom stage; 4. Full bloom stage; 5. Degrade stage.

During the senescence process of 'Aziatische Gr. Trompeten' flowers, proteins of polypeptide bands with molecular weights of 83.4 kDa and at the range from 38.5 to 51.8 kDa became weaker or even disappeared, except the polypeptide bands with molecular weights of 16.3 and 20.3 kDa. A new 67 kDa polypeptide band appeared at the decline stage during flower senescence (Fig. 5).

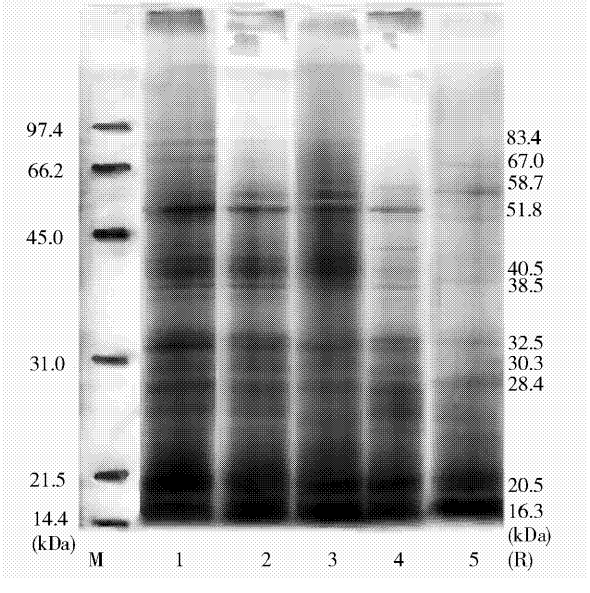

Fig. 5 Change of protein pattern in cut flowers of *Lilium* 'Aziatische Gr. Trompeten' during senescence

1. Green bud stage; 2. Color change stage; 3. Tentative blossom stage; 4. Full bloom stage; 5. Degrade stage.

The changes in protein profile of 'Oriental Gr. Yelloween' cut flower was showed in Fig. 6. The proteins with molecular weights at ranges from 72.6 to 96.1 kDa, 38.4 to 51.3 kDa、28.1 to 32.1 kDa、18.6 to 25.9 kDa decreased during the flower senescence, while the bands about 14.4、25.9、28.1、32.1、38.4、51.3 kDa, appeared at all stages, but decreased in quantity. A new polypeptide band with molecular weight of 58.3 kDa appeared at the last stage of the senescence.

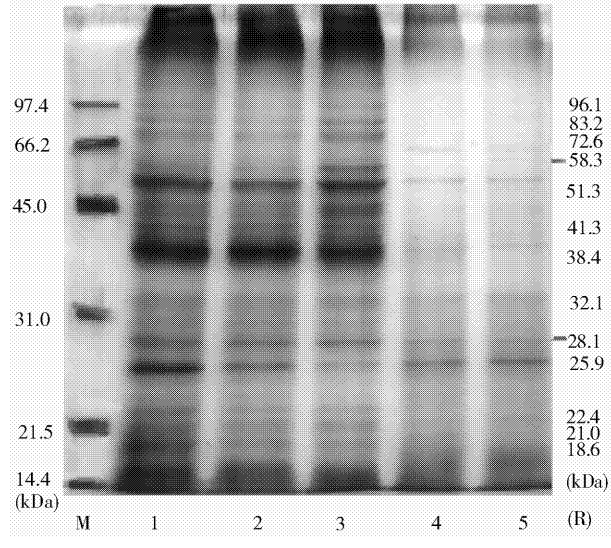

Fig. 6 Change of protein pattern in cut flowers of *Lilium* 'Oriental Gr. Yelloween' during senescence

1. Green bud stage; 2. Color change stage; 3. Tentative blossom stage; 4. Full bloom stage; 5. Degrade stage.

3 Discussion

Lilium spp. species consists of nine strains with hundreds of varieties or cultivars. Popular *Lilium* strains used for cut flowers are *Aziatische* hybrid, *Oriental* hybrid and *Longiflorum* hydrid[13]. Three cultivars ('Oriental Gr. Siberia', 'Oriental Gr. Sorbonne', and 'Oriental Gr. Yelloween') in our study are Oriental hybrid. The 'Aziatische Gr. trompeten' is *Aziatische* hybrid and 'Longiflorum' is *Longiflorum* hybrid. SDS-PAGE patterns of proteins in petals of these cultivars showed differences in number, position and thickness of polypeptide bands in the gels. The flower petals of 'Longiflorum' had the most polypeptide bands while those of 'Aziatische Gr. Trompeten' had the

fewest. All of these cultivars displayed four polypeptide bands with molecular weights of 22.1, 32.8, 45.0, 49.9 kDa. Only 'Oriental Gr. Yelloween' and 'Longiflorum' have a band of 38.4 kDa and 40 kDa, respectively, but 'Aziatische Gr. Trompeten' lacks of 26.5 kDa band.

Wang et al.[10] studied the changes in soluble proteins of rose flower petals during senescence and categorized these changes as three different groups, the stable proteins which maintained the same content level during senescence, degradable proteins which decreased during the senescence and the increasable proteins which increased from stage to stage during senescence. The last two protein groups were likely related to flower senescence. Our results revealed the similar patterns of rose cut-flowers, but also showed a new group of proteins which appeared at the last stage of senescence in some lily cultivars, i. e., 58.3 kDa protein band in the three *Oriental* cultivars and a 67 kDa band in 'Aziatische Gr. Trompeten'. As we have known that protein changes occur during senescence in many plant organs, the protein degradation and synthesis might play important roles during the senescence process[14]. From the perspective of vase life, 'Oriental Gr. Siberia' has the longest vase life, 'Oriental Gr. Sorbonne' and 'Aziatische Gr. Yelloween' are the second, and 'Longiflorum' and 'Aziatische Gr. Trompeten' are the shortest. According to the changes in component and content, it suggested that the protein of 58.3 kDa was possibly senscence-related specific protein, which did not occurred in 'Longiflorum' and 'Aziatische Gr. Trompeten'. The further study is expected. Zhou et al.[15] studied the leaf of caraway and identified one senscence-related protein (SRP63) of molecular weight about 63.1 kDa.

The new 58.3 kDa polypeptide bands appeared during the senescence of harvested flowers in the three *Oriental* cultivars, it indicated that these cultivars have the closest relationship. This newly synthesized protein may be worth further investigation of its impact on flower senescence. The protein profiles in petals during the senescence of post-harvested flowers, along with the gene expression pattern can provide theoretical evidence to extent the life of cut flowers of *Lilium* spp.

References

[1] Chen J Y, Cheng X K. China Floral Encyclopaedia [M]. Shanghai: Shanghai Culture Press,1990: 180.(in Chinese)

[2] Xue Q H, Sun L, Pan D M, et al. Physiological changes in senescence of lily cut flowers [J]. Chin Agri Sci Bull, 2005, 21(11): 179-182.(in Chinese)

[3] Yuan Q S, Huang X S, Ji Y, et al. Effects of temperatures on changes of hormone levels in lily flowers [J]. J Henan Agri Univ, 1996, 30(3): 203-206.(in Chinese)

[4] Liu Y Q, Wang F, Ding Q, et al. Physiological effects of blooming stimulators on *Lilium* flower preservation [J]. J Northwest Agri Univ, 2000, 28(6): 89-95.(in Chinese)

[5] Ye M Q. Effects of different preservatives on cut flowers of *Lilium longiflorum* [J]. Guangxi Agri Sci, 2001(4): 180-182.(in Chinese)

[6] Xin G, Hou D Y, Zhang W H, et al. Effect of package with polyethylene plastic film under cool storage on fresh flower-lily [J]. J Shen yang Agri Univ, 1999, 30(4): 426-429.(in Chinese)

[7] Van Doorn W G, Stead A D. Abscission of flower and floral paets [J]. J Exp Bot, 1997, 48(309): 821-837.

[8] Ranwala A P, Miller W B, Littlejohn G. Effects of gibberellin treatments on flower and leaf qualityof cuthybrid lilies [C]// Proceedings of the Eighth International Symposium on Flowerbulbs. Cape town, South Africa, 2000: 28-31.

[9] Woodson W R, Park K Y,Drory A, et al. Expression of ethylene biosynthesis pathway transcripts in senescing carnation flowers [J]. Plant Physiol, 1992, 99(2): 526-532.

[10] Wang R, Wang C R, Luo S, et al. SDS-PAGE analysis of soluble proteins of cut rose petals during senescence [J]. Acta Hort Sin, 1998, 25(3): 306-307.(in Chinese)

[11] Xu X F. Studies on the protein changes during petal senescence of *Osmanthus fragrans* [D]. Wuhan: Huazhong Agriculture University, 2003.(in Chinese)

[12] Wang J Z, Fan M. Handbook of Protein Technology [M]. Beijing: Science Press, 2005: 89-100.(in Chinese)

[13] Li Y B, Zhang J P, Li J H, et al. Prospect of fresh cut-flower of lily and the cultivation techniques for fine quality and high yield [J]. Southwest China J Agri Sci, 2004, 17(suppl.): 204-207.(in Chinese)

[14] Woodson W R. Changes in protein and mRNA population during the senescence of camation petal [J]. Physiol Plant, 1987, 71: 495-502.

[15] Zhou X J, Jiang W B, Zhao Y M, et al. The anglysis and survey of protein of 63 ku concerning senescence in leaf of *Coriandrum sativum* L. [J]. Chin Sci Bull, 2002, 47(9): 693-696. (in Chinese)