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# Effect of pre-harvest application of promalin and 1-MCP on preservation of cut lily and its relationship to energy metabolism

Fujuan Wei[a,1], Jin Wang[b,1], Shuai Huang[c], Bi Gong[a,*]

[a] College of Landscape Architecture and Arts, Northwest Agriculture and Forestry University, China
[b] Department of Bioresource Engineering, Faculty of Agricultural and Environmental Sciences, McGill University, Canada
[c] College of Life Science, Northwest Agriculture and Forestry University, China

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ABSTRACT

Lilies (*Lilium* spp.) are in demand worldwide because of their superior commercial and ornamental value. To develop new and practical preservation technologies, effects of pre-harvest treatment of Promalin (a.i. 1.8% each of 6-benzyladenine (6-BA) and gibberellic acid ($GA_{4+7}$)) and 1-Methylcyclopropene (1-MCP) on cut lily under cold storage were investigated. Oriental lily 'Sorbonne' was selected for this study. The field plants at coloring stage were sprayed with 25 mg $L^{-1}$ Promalin, 30 μL $L^{-1}$ 1-MCP additionally, their combination (1-MCP + Promalin) was also tested. Deionized water was used as control. The flower stems were harvested 24 h later and stored at 3 ± 1 °C. Preservation effect and physiological changes, e.g. inflorescence life, flower life, opening rate, dry weight loss rate, respiration rate, ethylene production rate, malondialdehyde (MDA) content, lipoxygenase (LOX) activity, energy metabolism related indexes including adenosine triphosphate (ATP) content, energy charge, activities of ATP synthase (ATPase), succinate dehydrogenase (SDH), cytochrome oxidase (CCO) and $Ca^{2+}$-ATPase were determined. The results showed that 1-MCP or Promalin treatment extended the vase life of stored cut lilies and inhibited the decline of the dry weight of petals, reduced respiration rate, ethylene production rate, LOX activity and the accumulation of MDA. They also maintained the activity of ATPase, $Ca^{2+}$-ATPase, SDH, CCO, ATP content and energy charge of cut lilies at a higher level than control. 1-MCP + Promalin exhibited an additive effect. Combination treatment maintained the flower opening ability as high as 89.3% after five-week storage, control merely maintained it till 3 weeks. These data indicate growth regulators (e.g. 1-MCP or Promalin) can extend the storage life of cut lily 'Sorbonne' through cutting down respiration consumption and manipulation of energy balance.

## 1. Introduction

Lily (*Lilium* spp.) is one of the commercially popular flower grown throughout the world. Hybrid lilies are usually bred for use as cut flowers due to its superior ornamental value which is generally based on features like attractive shape, color, and fragrant perfume (Liao et al., 2012; Woolf et al., 2012) and demanded increasingly worldwide. Ornamental value of the postharvest lilies declines quickly due to nutrition shortage, water balance disturbance and accelerating senescence of the flower (Waithaka et al., 2001; Liao et al., 2012). However, cut lilies are concentration harvested and there is usually a price difference between different market periods. Storage practice is necessary to preserve excess flowers or maximum the profit for either the grower or seller. Finding solutions to inhibit the postharvest senescence, extending the storage longevity is thereby an everlasting need and challenge for the research community.

A wide range of studies on the impact of preservatives on vase-life of cut lilies and their physiological influence have been well documented (Peng et al., 2007; Arrom and Munné-Bosch, 2012; Prisa et al., 2013). Wu et al. (2013) reported that the combination of modified atmosphere packaging and 1-MCP gas prolonged the postharvest vase-life of 'Activa' lily by 8.8 d with delayed flower opening and senescence. Whereas, investigations for cut lily preservation are less reported. If they are exposed at low temperature for too many days, cut lilies would undergo leaf yellowing and abscission, reduce flower opening rate, shorten vase life, which could decrease the ornamental and commercial values, thus, a shorter storage period (as long as 2 weeks) is suggested (Ranwala and Miller (2005). Previous studies on lily plant revealed that leaf chlorosis was related to darkness and hormone balance, whereas, flower senescence was associated to the regulation of ethylene (Ranwala and Miller,

* Corresponding author at: No. 3 Taicheng Road, Yangling District, Xian Yang, Shaanxi Province, 712100, China.
*E-mail address:* gb510894@nwafu.edu.cn (B. Gong).
[1] These authors equally contributed to the work.

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1998; Çelikel et al., 2002). Gibberellic acid (GA3) or benzyladenine (BA) significantly delayed the yellowing onset of excised Easter lily leaves (Franco and Han, 1997) and potted hybrid and Oriental lilies (*L. longiflorum*) during cold storage (Ranwala and Miller, 1998, 1999, 2000; Ranwala et al., 2003) and evaluation phases at ambient temperature (Han, 1997; Ranwala et al., 2000). Following these works, promalin, a commercial formulation of mixed 1.8% 6-benzyladenine (6-BA) and 1.8% gibberellic acid ($GA_{4+7}$) was developed in 2001 in USA. Promalin and 1-Methylcyclopropene (1-MCP) were applied on potted oriental lilies and maintained a better flower quality during the cold storage period compared to the control group (Çelikel et al., 2002). The efficacy of Promalin on cold-induced disorder prevention has been demonstrated to act generally in 20 cultivars representing three major groups (Oriental, Asiatic and LA) of hybrid lilies (Ranwala and Miller, 2005).

Flower senescence responded to ethylene in low temperature stored Oriental lilies plant, but this was not observed in freshly harvested plant. Treatment with 1-MCP prior to cold storage prevented all effects of cold storage on bud opening for Oriental lily cv. Star Gazer (Han and Miller, 2003); Bud opening in most Oriental lily cultivars was unaffected by exposure to a high ethylene dosage (100 $\mu L\,L^{-1}$ for 24 h). However, some Asiatic hybrids showed minor responses (Elgar et al.,1999). van Doorn and Han (2011) had sumarized that ethylene induces lack of flower opening in lilies that have been cold-stored. Following the positive effect of 1-MCP and Promalin treatment on cold-induced disorder prevention in potted lilies, their combination effect on cut lily preservation deserves to be investigated. In traditional practice, treatments for the purpose to prolong storage longevity or vase-life were performed usually after harvest. However, pre-harvest treatments have more advantages which include reduced manual damage on the samples, enable easier operation and labor-savings compared to post-harvest treatments (Huang et al., 2017). The pre-harvest application of 1-MCP and Promalin was studied. Moreover, activities of key enzymes in the process of mitochondrial metabolism would affect the energy synthesis and thus, be responsible to the shortage of cellular energy which can accelerate cell senescence (Liu et al., 2011; Pan et al., 2017). Energy charge, activities of these enzymes were presented in this study to report the effect of pre-harvest treatment of promailn and 1-MCP on the quality of cut lilies at different cold storage conditions and the possible energy metabolism related mechanism.

## 2. Materials and methods

### 2.1. Plant material

Lily (*Lilium* Oriental, cv. Sorbonne) bulbs (18–20 cm in circumference) were purchased from a gardening company (Yunnan Longgran Horticulture Co., Ltd. China) and then were grown in the green house of Northwest Agriculture and Forestry University (Yangling, Shaanxi Province, Northwestern China) from early tenth of January throughout middle of April in 2016. All bulbs were stratified at 5 ℃ for about 20 days till a 0.5–1.0 cm sprout of each bulb emerged. The germinated bulbs were planted in slightly acidic formula soil with spacing of 15 × 20 cm, depth of 4–5 cm, watered by a drip irrigation system. The greenhouse was controlled at 20–25 ℃ in the daytime, 7–16 ℃ in the night, 70–80% relative humidity, natural photoperiod scattered sunlight.

### 2.2. Pre-harvest treatments

After a growth period of four months, when the first bottom flower reached coloring stage, the

uniform plants were selected for experiments and divided into four groups of 105 (comprising three replicates of 35). Each group was individually sprayed with one of the following four solutions: (1) deionized water (control); (2) 25 $mg\,L^{-1}$ Promalin, a commercial mixture of 1.8% 6-benzyladenine (6-BA) + 1.8% gibberellic acid ($GA_{4+7}$) was diluted about 1500 times according to the instruction to 12.5 $mg\,L^{-1}$ each of $GA_{4+7}$ and BA, namely 25 $mg\,L^{-1}$ Promalin (Sinopharm Chemical Reagent Beijing Co., Ltd, China); The concentration of 25 $mg\,L^{-1}$ was selected also based on the adequate GA concentration for postharvest treatment to prevent leaf yellowing in study of Ranwala et al.(2003). (3) 30 $\mu L\,L^{-1}$ 1-MCP, a commercial emulsion with 1% active ingredient (Shandong Yingyangyuan Food technology Co., Ltd, China); (4) mixed solution with 25 $mg\,L^{-1}$ Promalin and 30 $\mu L\,L^{-1}$ 1-MCP (1-MCP + Promalin). Each plant (flower, leave and stem) was sprayed to run off. Concentration adopted in this study for 1-MCP treatment was successfully screened in pre-test and no effect of auxiliary additive of liquid 1-MCP on vase life and storage longevity was confirmed (data not shown).

After 24 h, the healthy flowering stem of each plant from four group of lilies were cut at their base and then dipped in deionized water before transporting to the lab within an hour. Upon arrival, flowering stems with 4–6 flower buds were selected from each treatment group and cut to a stem length of 50 cm and placed in the bucket with deionized water, then stored at 3 ± 1 ℃, relative humidity (RH) 80%–90%. A sample of eight stems from each group was randomly collected at the storage time of 0, 1, 3 and 5 weeks. Four stems were held in one vase with deionized water, kept at a free from any ethylene pollution room, at temperature (18 ± 1 ℃), RH 60%–80%., and a 12-h photoperiod with 15 $\mu mol\,m^{-2}\,s^{-1}$ irradiance using cool-white fluorescent lamps for the observation of vase life and flower opening rate each day. The other four stems were used to measure respiration rate and ethylene production rate, then three inner layer petals of the first base flower were removed and frozen with liquid nitrogen, kept at −80 ℃ until used. Three independent replicates were conducted at the same time and the experiments were arranged according to completely randomized block.

### 2.3. Evaluation of vase life and buds opening rate

Vase life of each batch of stored cut lilies was assessed with two indexes: inflorescence life and flower life. According to the method adopted from Han and Miller (2003), inflorescence life is defined as the days since the lowermost (first) flowers on the inflorescence opening until 50% petal of the last opening flower is wilted. Flower life refers to the days counted from 0 d in vase to 50% petals of itself wilted for the lowermost flower (Peng et al., 2007).

The opening rate is the percentage of flowers that opened during vase holding from the total number of floral buds in one inflorescence after a given storage period.

### 2.4. Dry weight of lily petal

To determine the dry weight, fresh petal discs made with hole punch (Φ = 1.5 cm)were cut into pieces and exposed at 70 ℃ till a constant weight was reached. The results were expressed as $mg\,cm^{-2}$.

### 2.5. Respiration rate

Respiration rate of cut lilies were assayed as described in Wang et al. (2016) with some modifications. First base flowers on sample stems from each treatment were removed, weighted and placed with their base in water into a sealable glass jar (4 L) which had been previously equilibrated for 30 min at 3 ± 1 ℃ in the cold room. The respiration rate was determined in the cold room by using a carbon dioxide and temperature monitor (Telaire 7001; Amphenol Corp, Calif., USA).

### 2.6. Ethylene production

After respiration measurement, cut flowers were sealed again in the jar (4 L) for nine hours. Gas samples (5 mL) were collected with a syringe for ethylene determination. Ethylene content was quantitatively