The Effect of Methyle Jasmonate on Anthocyanin Synthesis in Oriental Hybrid Lily cv. Sorbbone.

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ABSTRACT

This study was conducted to investigate the effects of methyl jasmonate spraying on anthocyanin synthesis in florrets of oriental hybrid lily cv Sorbonne and its relationship with other qualified traits of cut flower. Two concentration of methyl jasmonate (0.2 and 0.4 mM) were separately sprayed on plants at three stages of inflorescence growth (flower buds initiation, florrets length of 3 to 5 cm, and 2 to 3 days before harvest). Results showed that there was no significant difference between applied concentrations regarding postharvest characteristics. In case, there was obvious difference in traits among three stages of spraying. Spraying with methyl jasmonate didn't have any significant difference on leaf greenness over postharvest period but it could preserve higher amount of fresh weight for cut flowers in the late storage life. Other traits such as petal anthocyanin content, vase life, total soluble solid and antioxidative activity were also in the highest amount in this treatment. Methyl jasmonate spraying 2 to 3 days before harvest, may enhance flowers vase life by avoiding fresh tissue disruption due to increments in anthocyanin content and antioxidative activity of petals. This may be a good way to improve flowers marketing value.

Keywords: Antioxidant activity, anthocyanin, oriental hybrid lily, postharvest, vase life.

Abbreviations:


INTRODUCTION

Being a major cut flower in global markets, Lilies are predominantly grown under cover. There are huge numbers of hybrid lily varieties which are bred for cut flower market. The genus Lilium is classified into six sections. The Oriental hybrid lily, one of the most important ornamental plants worldwide, is derived from inter-specific crosses of species of the section Sinomartagon (Leslie, 1982). Most Oriental hybrid lily cultivars have pink flowers due to anthocyanin pigmentation. Three basic groups of anthocyanin pigments appear in higher plants: the derivatives of pelargonidin, cyanidin and delphinidin (Davies and Schwinn, 1997). Most Oriental Lilies contains cyanidin 3-O-b-rutinoside as a major anthocyanin (Norbak and Kondo, 1999). Biochemical and enzymatic studies have clarified the anthocyanin biosynthesis pathway, and many genes encoding anthocyanin biosynthetic enzymes have been isolated (Holton and Cornish, 1995; Dooner et al., 1991). Induction of a wide spectrum of secondary metabolites by jasmonates, mainly by methyl jasmonate (JA-Me), was documented not only in many cell suspension cultures (Beerhues and Berger, 1995; Dittrich et al., 1992; Gundlach et al., 1992; Mizukami et al., 1993; Nojiri et al., 1996; Urbanek et al., 1996), but also in differentiated plants (Aerts et al., 1994; Bodnaryk, 1994). JA-Me has already been reported to stimulate anthocyanin accumulation in hypocotyls of light-grown seedlings of soybean (Franceschi and Grimes, 1991), in leaves of seedlings of a wild-type of Arabidopsis (Feys et al., 1994), and in detached corollas of Petunia (Tamari et al., 1994). This signal molecule is involved in some signal transduction systems, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids or pathogenesis-related (PR) proteins (Halbroock and Scheel, 1989; Creelman and Mullet, 1995; Tamari et al., 1995; Van Loon, 1995). This can result in induction of defense responses and
provide protection for plants from pathogen-attack (Kozlowski et al., 1999). Tamari et al. (1995) showed that methyl jasmonate induces expression of chalcone synthase (chs) and dihydroflavonol reductase (dfr) which are the most important genes in anthocyanin synthesis pathway. Saniewski et al. (2003) resulted that using methyl jasmonate in a lanoline paste on the stems of ornamental crassula causes more anthocyanin production. Strawberry (Fragaria ananasa) fruits treated by MJ showed more anthocyanin content and antioxidant activity in fresh tissue (Zavala et al., 2005). Darras et al. (2005) presented that vapor treatment of MJ on Freesia hybrida flowers enhanced vase life and prevented decreasing in fresh weight of cut flowers. It also delayed senescence of petals by suppressing Botrytis cinerea growth.

Anthocyanin pigments show potent antioxidant properties in vitro. In a recent analysis of red and green leaf extracts from Elatostema rugosum it was founded that the anthocynic leaves had the significantly greater antioxidant potential (Neill et al., 2002). Along with other flavonoids, the anthocyanins can directly scavenge molecular species of active oxygen, including hydrogen peroxide, singlet oxygen, and the superoxide, hydroxyl, and peroxyl radicals (Bors et al., 1994; Yamasaki et al., 1996). Thus, anthocyanins have an inherent potential to protect cell membranes from the effects of oxidative damage and prevent plant tissue disruption and senescence.

Our objective with this research was to evaluate the effect of methyl jasmonate spraying on anthocyanin content of petals and its relationship with flower’s vase life and other qualified characteristics and also to find out the most suitable stage of spraying and benefit concentration to enhance marketable value of flowers.

MATERIALS AND METHODS

Plant Material and Treatments:

Oriental hybrid lily (cv. Sorbbone) bulbs (14-16 cm circumference) were obtained from a commercial grower and planted in 1.4 L plastic containers. Plants were spaced 20×20 cm and grown in a glass greenhouse. Soil mixture was a combination of washed sand, leaf mold and garden soil which was disinfect in an autoclave with 120 °C over 15 minutes. Plants were fertigated with water containing 20N-10P-8K soluble fertilizer with trace elements (Melsspray) at 0.2 gL⁻¹ and they occasionally were flushed with water. A set point temperature of 22-16 °C was used during the experiment with day time temperatures occasionally rising to 26 °C. Plants were separately sprayed at three stages of inflorescence growth (flower buds initiation, length of 3 to 5 cm in florets, and 2 to 3 days before cut flower harvest) with growth regulator solutions containing various concentrations of GA₃ (100 and 200 mgl⁻¹) to run off. In all the treatments, spray solutions contained 0.2 % Tween 20 as a surfactant. Plants remained into the greenhouse until harvesting time. Flower stems in all treatments were cut when the first flower was fully colored but not open. After harvest, they were taken to a postharvest evaluation room (20±2°C, 70% relative humidity, 950 Lux light for 12 h) and placed into jars contained distilled water before initial weighing. Postharvest traits such as anthocyanin content of petals, antioxidant activity, Total soluble solids (TSS), fresh weight, and leaf greenness were measured at determined intervals as follow:

**Anthocyanin Extraction of Petals:**

Petals of first, second, and third florets (0.4 g of each treatment) were macerated in 1 ml of acidic methanol solution (1% HCL in MeOH) in a test tube and allowed to equilibrate overnight at 4 °C. These samples were centrifuged at 10,000 g for 10 minutes at 4°C. Two stages of opening (entire opening stage and 7th day after opening) for first and second florets were considered and for the third floret, anthocyanin content was only measured at entire opening stage.

**Determination of Total Anthocyanin Content:**

Total anthocyanin (TA) content of the petal extract was measured using the pH differential method described by Wrolstad and Giusti (2001). A crude petal extract were separately dissolved in potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate (CH₃CO₂Na₂H₂O, 0.4 M, pH 4.5) with a pre-determined dilution factor. Sample measurement absorbencies were read at 541 and 700 nm against a blank cell containing distilled water by a spectrophotometer (6405 UV-Vis, Jenway, England). The absorbance (A) of the diluted sample was then calculated as follow:

\[
A = \frac{(A_{541 pH 1} - A_{700 pH 1}) - (A_{541 pH 4.5} - A_{700 pH 4.5})}{(A_{541 pH 1} - A_{700 pH 1})}
\]

The total anthocyanin (TA) pigment (mgl⁻¹) was calculated as follow:

\[
\text{Total anthocyanin (TA)(mgl}^{-1}\text{)} = (A/a)(10^3)(b)(c)
\]

In which: A: Absorbance; a: molar extinction coefficient of Cyanidin-3-rutinoside (=28800); b: Cyanidin-3-rutinoside molecular weight (=595.2); c: dilution factor (=1); 10³: conversion factor.

**Antioxidant Activity:**

Petal antioxidant activity was measured on 7th day according to Ramandeep et al. (2005). The antioxidant activity of the petal extracts was evaluated by DPPH free radical-scavenging methods. 100 μL of various concentrations of the extracts in methanol was added to 2.900(μL) of a 0.1 mM DPPH methanol solution. The mixture was shaken immediately after adding DPPH solution and allowed to stand at room temperature in the dark. The decrease in absorbance at 517 nm was
measured after 25 min until the reaction reach a plateau. These experiments were run in triplicate at 25°C. The inhibitory percentage of DPPH was calculated as follows:

**Scavenging effect (%)** \[(Ao - (A - Ab))/Ao \times 100\]

In which: \( Ao \) : \( A_{517} \) of DPPH without sample (control); \( A: \) \( A_{517} \) of sample and DPPH; \( Ab: \) \( A_{517} \) of sample without DPPH (blank).

**Vase Life:**

Vase life of cut flowers were recorded (in days) until more than 50% of petals started wilting and became discolored. The laboratory room was illuminated at 950 Lux at flowers level by Sylvania cool white fluorescent lamps (12h on-off cycle).

**Fresh Weight:**

Flower fresh weight was immediately measured with a digital balance after cutting and it considered as an initial weight. This trait was also measured every third day over the storage period. Data are presented as proportional (%) change relative to the initial fresh weight (Han, 2001).

**Total Soluble Solids:**

TSS is presented as percentage and includes a collection of different sugars, proteins, organic acids and, etc. in an extracted tissue. In order to determine TSS content, a refractometer (CETI, Belgium) was used. A small piece of petal in each treatment was squeezed then a drop of obtained extract was used to measure refractive index by the refractometer. This trait was measured on 3\(^{rd}\) and 7\(^{th}\) days of postharvest life.

**Statistical Analysis:**

All treatments were arranged in a factorial complete randomized design with three replicates. Data were subjected to one way analysis of variance to determine treatment effects, and treatment means were compared using Tukey’s multiple range test (General Linear Models Procedure, SAS software).

**RESULTS AND DISCUSSION**

**Antocyanin content:**

Results showed that there was no significant difference between two used MJ concentrations at each stage on anthocyanin content but three stages were significantly different with each other. Third stage of spraying (2 to 3 days before harvest) significantly resulted more anthocyanin synthesis in first and second florets petals \((p < 0.05)\) (Fig. 1). Anthocyanin of third floret wasn’t impressed by MJ spraying at any time.

Some scientific research have showed that MJ is one of the important plant growth regulators that influences anthocyanin synthesis pathway. It induces some of genes expression and enhances enzymes activity like \( chs \) and \( dfr \) (Tamari et al., 1995). Zavala et al. 2005 showed that exogenous application of MJ enhances anthocyanin content and antioxidant activity in fresh tissue of *Fragaria ananassa* fruits. In most plants, anthocyanin synthesis in the flowers is under developmental regulation and its accumulation coincides with petal growth (Mol et al. 1996). Anthocyanin accumulation usually occurs at later stages of petal development (Martin and Gerats, 1993). Third stage of MJ spraying in our work was along with the initial stage of pigmentation process and expression of related genes. That is probably why exogenous application of MJ at mentioned stage has been resulted in intense color of petals.

**Antioxidant activity:**

Spraying stages showed significantly difference in antioxidant activity of petals \((p<0.01)\) (Table 1). Third stage treatment had higher amount of antioxidants. No significant difference was seen between used concentrations. This treatment also caused more anthocyanin content of petals. Many researchers have showed that Anthocyanins can directly scavenge molecular species of active oxygen, including hydrogen peroxide, singlet oxygen, and the superoxide, hydroxyl, and peroxyl radicals (Bors et al., 1994; Yamasaki et al., 1996). Also Neill et al. 2002 stated that anthocyanic leaves of *Elatostema rugosum* had the significantly greater antioxidant potential. Thus higher antioxidant activity in flowers at third stage spraying is probably related to higher anthocyanin content.

**Vase life:**

Vase life of flowers treated at third stage, regardless of used concentrations, was significantly more than two other stages \((p<0.01)\) (Table 1). This may be related to more anthocyanins and their role in delaying plant senescence due to scavenging free radicals and protecting cell membranes from the effects of oxidative damage. Lilies are so susceptible to gray mold due to *Botrytis cinerea* growth on petals at postharvest period (De Hortogh, 1996). Some signaling molecules, like MJ when exogenously applied, have been shown to move systemically through plants, resulting in the expression of a set of defense genes that are activated by pathogen infection, thus inducing
resistance against pathogens (Kozlowski et al., 1999). Darras et al. (2005) presented that vapor treatment of MJ on *Freesia hybrida* flowers enhanced vase life of cut flowers. It delayed senescence of petals by suppressing fungal infections like *Botrytis cinerea*. This can be the other reason for showing more vase life in flowers sprayed by MJ.

**Fresh weight:**

Fresh weights of cut flower stems didn’t show any difference on 3rd, 6th and 9th days but significant variation (p<0.01) was seen among spraying stages in the late storage period on 12th day at the laboratory. Among 3 mentioned stages, flowers sprayed at 3rd stage had the highest fresh weight (Table 1). More fresh weight can be resulted due to more vase life.

**Total soluble solids:**

Results showed that there was no significant difference between concentrations but stages of spraying were significantly different with each other (p<0.05) (Table 1). Total soluble solids in petals of flowers sprayed at third stage were higher than two other stages on 7th day. Several antifungal compounds, such as hexanal and taxol have been found to increase in various plant tissues after MJ application (Darras et al., 2005). This ability of MJ can be the reason of preservation more total soluble solids (sugars) in petals. Injured cells on account of such biotic stresses can produce more ethylene which increases respiration rate and decreases tissue sugar supply (Meir et al., 1998).

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**Table 1.** Effect of MJ spraying stages on qualified traits of cut *Sorbbon* Lily flowers.

<table>
<thead>
<tr>
<th>Spraying stages</th>
<th>Antioxidant activity (%)</th>
<th>Vase life (day)</th>
<th>Fresh weight On 12th day</th>
<th>TSS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.6 b†</td>
<td>16.0 b</td>
<td>77.41 c</td>
<td>3.1 b</td>
</tr>
<tr>
<td>T2</td>
<td>3.3 ab</td>
<td>16.3 b</td>
<td>86.61 b</td>
<td>3.4 ab</td>
</tr>
<tr>
<td>T3</td>
<td>3.9 a</td>
<td>17.8 a</td>
<td>94.91 a</td>
<td>4.3 a</td>
</tr>
</tbody>
</table>

† Means in each column with the same letter are not significantly different based on Duncan’s multiple range test at p < 0.01.

**CONCLUSION**

Taken together, our results suggest that MJ spraying 2 to 3 days before harvest, can enhance flowers vase life and quality. This may be a good way to improve flowers marketable value.
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