# Improving quality indices of Rosa 'Yellow Finesse' using methyl jasmonate and benzyl adenine

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## Abstract

Plant growth regulators (PGRs) play a key role in regulating physiological and morphological development of plant species. Application of these PGRs can improve pre- and post-harvest quality and quantity of ornamental flowers and lessen the harmful effects of injury incurred at harvesting time. The objective of this study was to determine the appropriate spraying solution for improving pre- and postharvest quality indices of *Rosa hybrida* L. cv. 'Yellow Finesse'. So, the effect of methyl jasmonate (MeJA) at 0, 50, 100, or 150 $mg*l^{-1}$ alone or in combination with benzyl adenine (BA) at 0, 10, or 20 $mg*l^{-1}$ was investigated. Plants were sprayed at a 15-day interval for two months and then harvested at a mature bud stage. The results showed that flower head diameter and leaf chlorophyll content were improved by applying 100 $mg*l^{-1}$ MeJA + 20 $mg*l^{-1}$ BA. The application of 100 $mg*l^{-1}$ MeJA + 10 $mg*l^{-1}$ BA or 10 $mg*l^{-1}$ BA resulted in improving its petal carotenoid content. The plants treated with 150 $mg*l^{-1}$ MeJA had the longest shelf life. The findings demonstrated that while 100 $mg*l^{-1}$ MeJA + 20 $mg*l^{-1}$ BA and 100 $mg*l^{-1}$ MeJA + 10 $mg*l^{-1}$ BA were the best preharvest spraying solutions due to the highest carotenoid content and largest flower head diameter. Particularly, 150 $mg*l^{-1}$ MeJA was the best spraying solution for extending cut flower shelf life and had the potential to function as a postharvest treatment.

**Keywords:** carotenoid, plant growth regulators, post-harvest, pre-harvest, rose, vase life, Yellow Finesse

## Introduction

Among all cut flowers, yellow finesse cut rose flowers (*Rosa hybrida* cv. 'Yellow Finesse') are commercially very important and are highly required for interior design and bridal bouquet, which emphasize the importance of improving their quality especially their post-harvest quality. Nowadays, because of the adverse effects of chemical preservatives, researchers are attempting to apply natural substances or

plant growth regulators (PGRs) such as methyl jasmonate (MeJA) and benzyl adenine (BA) to improve the post-harvest quality of crops (Darras, et al., 2007; Danaee, et al., 2011). The exogenous application of MeJA can exert numerous beneficiary effects on crops. It can induce plant defense genes and activate their mechanisms against biotic and abiotic stresses. Application of MeJA seems to improve the plant defense system against physical injury (e.g., by producing antidigestive proteins against insects) (Darras, 2012), wound healing (Srivastava, 2002; Guiné, et al., 2010), and also microbial pathogens like *Botrytis cinerea* (Meir, et al., 2003). Additionally, it stimulates defense mechanisms against osmotic stress and ameliorates abiotic stress, such as the deleterious effects of ozone on plant tissues (Srivastava, 2002). The efficacy of MeJA on promoting post-harvest quality of raspberry (Chanjirakul, et al., 2006; Wang and Zheng, 2005), strawberry, blueberry (Chanjirakul, et al., 2007), and apple (Wang and Zheng, 2005) by increasing their antioxidant activity, free radical-scavenging, and enhancing their volatile compound and soluble content have also been corroborated. Meir, et al. (2003) showed that application of MeJA on cut rose flowers, improved its petal colour, anthocyanin production, and delayed its anthocyanin breakdown. They showed that pulsing treatment with 200 µM MeJA protected cut roses against *B. cinerea* without increasing ethylene ratio in its plant tissues (Meir, et al., 2003). It has been reported that the effectiveness of cytokinin (benzyl adenine) on the post-harvest quality of cut flowers can be influenced by several factors such as plant species, cultivars, flowers or inflorescence types, and also their harvesting time. One study showed that vase life extension of different anthurium cultivars greatly varied from 20% reduction to 2.5 fold increase (Paull and Chantrachit, 2001). In another study, cut gerbera flower held in a preservative solution containing 250 $mg \cdot L^{-1}$ BA had the highest fresh weight (Jafarpour, et al., 2015).

Since many factors may affect the quality of ornamental flowers and still many of them are not fully understood, the present study designed to evaluate the effectiveness of MeJA and BA, both alone and combined at different levels, on the pre- and post-harvest quality indices of yellow finesse roses.

## Materials and Methods

### Plant preparation

The present work was started on Jan. 10, 2014. We examined the effects of different spraying solutions on *Rosa hybrida* cv. 'Yellow Finesse'. Cut roses were planted in four-liter plastic pots containing coco peat and perlite (1:1) and kept in the standard greenhouse condition at an ambient temperature of 17-25°C, 60-70% RH, and a photosynthetically active photon flux of 555-648 $\mu mol*m^{-2}*s^{-1}$ (30000-35000 Lux). After harvesting flowers, their stems were cut below the flowing water, and set in vases containing deionized water. Then, they were transferred to the laboratory room at an ambient of 25±1°C, 30% RH with 12 h illumination each day and a photosynthetically active photon flux of 15 $\mu mol*m^{-2}*s^{-1}$ (Philips TDL 36W/84 cool white fluorescent tubes).

**Plant growth regulator treatments**

In the greenhouse, the one-year old plants were sprayed with PGR treatments comprised of (1) MeJA at 0, 50, 100 and 150 mg*$l^{-1}$, (2) and BA at 0, 10 or 20 mg*$l^{-1}$ (purchased from Merck factory). The plants were resprayed at every 15 days for two months (four times) before being cut. All plant surfaces were thoroughly wet by approximately 50 ml spraying solution per each plant. Afterwards, they were harvested at an optimal commercial harvesting stage or at the mature bud stage (MB) to investigate the longevity of cut flowers.

**Determination of rose qualitative characteristics**

1) Stem growth (cm): Stem growth was estimated based on the differences in final stem length after the 80$^{th}$ day (in greenhouse) compared to the first day (first spray).
2) Number of inflorescence stems: The number of inflorescence stems grown after the fourth spray (two months) was recorded within one month.
3) Number of flowers per plant: After the fourth spray (two months), the number of growing flowers, ready for harvest, were noted within one month.
4) Number of flower petals: After the fourth spray (two months), the number of flower petals (at the mature bud stage) of the inflorescence stems, were recorded within one month.
5) Maximum flower head diameter (MFHD) (cm): After the fourth spray (two months), the flower head diameter was measured using a Vernier-caliper.
6) Leaf area ($cm^2$): After the fourth spray (two months), the leaf area of the inflorescence stems, which had the flower at the mature bud stage, was measured by using the leaf area meter for one month.
7) Stem diameter (mm): After the fourth spray (two months), the stem diameter of the inflorescence stems, which was ready to harvest, was measured using a digital Vernier caliper.
8) Vase life (day after harvest): Flower vase life was recorded as the number of days kept in a vase after harvesting time until the flowers showed the symptoms of the bent neck or advanced signs of fading on all petals (Liao, et al., 2000).
9) Carotenoid pigments of petal (mg*$g^{-1}$ FW): After freezing with liquid nitrogen, petals were used for the carotenoid identification purposes (Arnon, 1949). Initially, 0.5 g petal was placed in a nitrogen tank (model: MVE 900) and then after a few seconds, freeze-dried petals were transferred to the mortar, grounded and thoroughly mixed with 5 ml of 80% acetone. Later, the upper part of the solution was centrifuged (Kokusan, model H-11N) at 4000 rpm for 20 min. After that, the upper part of the limpid solution was filtered using the watman filter paper and extracted. Ultimately, the amount of extract's carotenoid content was determined using two ray spectrophotometer (Uvikon, model 922) within the wavelength of 480-510. The results were put into the following formula:

$$\text{mg carotenoid } g^{-1} \text{ sample} = 7.6\ (A_{480}) - 1.49\ (A_{510}) \times V/1000 \times 10$$

10) Chlorophyll determination (mg*g$^{-1}$ FW): Spectrophotometer at 663 and 645 nm wavelength was used to determine the amount of chlorophyll content (Arnon, 1967). Briefly, after grounding 0.25 g fresh leaves in the porcelain mortar, they were mixed with 10 ml 80% acetone. Then, after pouring into the laboratory tubes, the solution was centrifuged at 3500 rpm for 5 min. Subsequently, it was filtered and read using the spectrophotometer apparatus. Chlorophyll a, chlorophyll b and total chlorophyll were then calculated using the following equation:

$$C\ Chl\ a\ (mg*g^{-1}\ FW) = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times V/100 \times W$$
$$C\ Chl\ b\ (mg*g^{-1}\ FW) = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times V/1000 \times W$$
$$C\ Chls\ a+b\ (mg*g^{-1}\ FW) = 20.21\ (A_{645}) + 8.02\ (A_{663}) \times V/1000 \times W$$

C = concentration
V = 10 (consumed acetone volume) (ml)
W = 0.25 (Fresh weight of the sample) (g)
A = absorption at specific wavelength (λ)

**Statistical Analysis**

Data were analyzed based on 12 treatments, each involving three plants with three replications (108 pot). Each treatment included nine flowers. For three months in the greenhouse and then two months in the laboratory room, the plant responses were noted. Using the general linear model (GLM) procedure of the SAS software (version 9.4), the analysis of variance of the data was calculated. The experiment was laid out using completely randomized design (CRD) with factorial test and its means were compared using Duncan's multiple range test (P = 0.05).

## Results

In general, the influence of MeJA or BA on the number of flowers and main stems was insignificant. However, plants treated with 20 mg*l$^{-1}$ BA and 100 mg*l$^{-1}$ MeJA + 20 mg*l$^{-1}$ BA (similar to 150 mg*l$^{-1}$ MeJA + 10 mg*l$^{-1}$ BA) had the largest stem diameter and flower head diameter, respectively (Table 1.). Compared to the control treatment, leaf dimension and length of inflorescence stem were markedly enhanced by the pre-harvest spraying solution of 20 mg*L$^{-1}$ BA (Table 2.). The quantity indices were not highly affected by PGRs, whereas the head flower diameter as the most important element of the quality index was improved by applying 100 mg*l$^{-1}$ MeJA + 20 mg*l$^{-1}$ BA (Table 1. and 2.). Plants treated with 100 mg*l$^{-1}$ MeJA combined with 10 or 20 mg*l$^{-1}$ BA had the highest amount of chlorophyll content. However, the negative impact of higher doses of MeJA (150 mg*L$^{-1}$) on the chlorophyll concentration was also evident (Table 3.). Spraying solutions containing 100 mg*l$^{-1}$ MeJA + 10 mg*l$^{-1}$ BA, and also 10 mg*l$^{-1}$ BA alone were the most effective treatments for increasing the amount of carotenoid content in petals. Compared to the control treatment, the amount of carotenoid content was significantly enhanced by these treatments (Table 4.). As shown in Table 4., the vase life of yellow finesse roses reached its peak by applying the pre-harvest spraying solution of 150 mg*l$^{-1}$ MeJA, a high dosage, which had a negative influence on chlorophyll content presented in Table 3. Yet, the

findings indicate that the application of 150 mg*l$^{-1}$ MeJA was the best post-harvest treatment, resulting in the highest cut flower longevity (Table 4.).

Table 1. Effect of pre-harvest spraying of solutions of BA and MeJA on the size and number of flowers and stems of roses (cv. Yellow Finesse)

| Treatment MeJA (mg*l$^{-1}$) | Treatment BA (mg*l$^{-1}$) | No. of flowers per plant | No. of inflorescence stems per plant | Stem diameter (mm) | Maximum flower head diameter (cm) |
|---|---|---|---|---|---|
| 0 | 0 | 3.1ab$^z$ | 2.3ab | 6.7b | 10.6abc |
| 0 | 10 | 3.0ab | 3.0a | 6.6b | 9.8bcd |
| 0 | 20 | 2.9ab | 2.4ab | 7.8a | 11.3ab |
| 50 | 0 | 3.7ab | 2.3ab | 6.2b | 10.4abc |
| 50 | 10 | 2.4b | 1.9b | 6.7b | 11.2ab |
| 50 | 20 | 3.3ab | 2.5ab | 7.2ab | 8.6d |
| 100 | 0 | 4.0a | 2.3ab | 6.4b | 10.2bc |
| 100 | 10 | 2.8ab | 2.0ab | 7.2ab | 10.8abc |
| 100 | 20 | 3.2ab | 2.5ab | 6.3b | 11.9a |
| 150 | 0 | 3.1ab | 2.1ab | 7.1ab | 9.4cd |
| 150 | 10 | 2.4b | 2.2ab | 7.2ab | 11.9a |
| 150 | 20 | 2.3b | 2.5ab | 6.3b | 9.3cd |

$^z$All values are means. Mean values in each column followed by the same lower-case letters are not significantly different (P < 0.05) by the Duncan's test.

Table 2. Effect of pre-harvest spraying solutions of BA and MeJA on the leaf area, stem length, stem growth, and petals number of roses (cv. Yellow Finesse)

| Treatment MeJA (mg*l$^{-1}$) | Treatment BA (mg*l$^{-1}$) | Leaf area (cm$^2$) | Length of inflorescence stem (cm) | Inflorescence stem growth (cm) | No. petals per flower |
|---|---|---|---|---|---|
| 0 | 0 | 22.9bcd$^z$ | 45.9ab | 7.4de | 46.2a |
| 0 | 10 | 27.3ab | 47.4ab | 18.3a | 43.0ab |
| 0 | 20 | 31.7a | 56.0a | 13.1bc | 41.8ab |
| 50 | 0 | 23.4bcd | 42.1b | 10.5cde | 44.2a |
| 50 | 10 | 24.1bcd | 47.5ab | 8.9cde | 42.5ab |
| 50 | 20 | 21.8bcd | 49.9ab | 12.7bc | 44.1a |
| 100 | 0 | 25.2bc | 43.5b | 7.7de | 46.2a |
| 100 | 10 | 23.9bcd | 49.3ab | 9.4cde | 43.2ab |
| 100 | 20 | 20.8cd | 50.2ab | 11.7bcd | 38.3b |
| 150 | 0 | 26.9abc | 50.8ab | 10.0cde | 41.0ab |
| 150 | 10 | 18.8g | 47.1ab | 6.8e | 44.3a |
| 150 | 20 | 21.8bcd | 49.9ab | 12.8bc | 44.1a |

$^z$All values are means. Mean values in each column followed by the same lower-case letters are not significantly different (P < 0.05) by the Duncan's test.

Table 3. Effect of pre-harvest spraying solutions of BA and MeJA on the leaf chlorophyll concentration of roses (cv. Yellow Finesse)

| Treatment | | Chl a | Chl b | Chl a+b |
|---|---|---|---|---|
| MeJA ($mg*l^{-1}$) | BA ($mg*l^{-1}$) | ($mg*g^{-1}$ FW) | ($mg*g^{-1}$ FW) | ($mg*g^{-1}$ FW) |
| 0 | 0 | 0.010282abc[z] | 0.003462cd | 0.013675d |
| 0 | 10 | 0.009751bcd | 0.006372bcd | 0.016096bcd |
| 0 | 20 | 0.010373abc | 0.003867cd | 0.014169cd |
| 50 | 0 | 0.012373ab | 0.00952ab | 0.021375ab |
| 50 | 10 | 0.012667ab | 0.004142cd | 0.016725bcd |
| 50 | 20 | 0.008144cd | 0.012393a | 0.019711bc |
| 100 | 0 | 0.009562bcd | 0.008375abc | 0.017824bcd |
| 100 | 10 | 0.013799a | 0.011681a | 0.02548a |
| 100 | 20 | 0.013808a | 0.012405a | 0.02582a |
| 150 | 0 | 0.006233d | 0.00222d | 0.008411e |
| 150 | 10 | 0.011942ab | 0.004123cd | 0.015996bcd |
| 150 | 20 | 0.00668cd | 0.013221a | 0.019796bc |

[z]All values are means. Mean values in each column followed by the same lower-case letters are not significantly different (P < 0.05) by the Duncan's test.

Table 4**.** Effect of pre-harvest spraying solutions of BA and MeJA on the carotenoid content in petals and vase life of roses (cv. Yellow Finesse)

| Treatment | | Carotenoid | Vase life |
|---|---|---|---|
| MeJA ($mg*l^{-1}$) | BA ($mg*l^{-1}$) | ($mg*g^{-1}$ FW) | (day after harvest) |
| 0 | 0 | 0.000505d[z] | 8.0bc |
| 0 | 10 | 0.001077a | 8.7abc |
| 0 | 20 | 0.000715bc | 8.7abc |
| 50 | 0 | 0.000734bc | 9.4ab |
| 50 | 10 | 0.000578cd | 8.2abc |
| 50 | 20 | 0.000802b | 9.1ab |
| 100 | 0 | 0.000775b | 9.1c |
| 100 | 10 | 0.001225a | 9.7ab |
| 100 | 20 | 0.000585cd | 8.3abc |
| 150 | 0 | 0.000725bc | 10.0a |
| 150 | 10 | 0.000585cd | 9.7ab |
| 150 | 20 | 0.000651bcd | 8.7abc |

[z]All values are means. Mean values in each column followed by the same lower-case letters are not significantly different (P < 0.05) by the Duncan's test.

## Discussion

Rose (*Rosa* species) is one of the most popular ornamental flowers cultivated all over the world. It is a versatile plant adapted to disparate climatic conditions. It is also known as one of the most valuable and profitable plants in the floral industry. So far, numerous studies have reported that rose flowers are greatly sensitive to the bent neck, ethylene gas (Pietro, et al., 2012), and *B. cinerea* infection (gray mold) (Jones, 2001; Meir, et al., 2003). Hence, a preservative solution and its dosage that improves the flower quality without increasing the tissue's ethylene level was planned to be examined on roses. Considering the fact that the application of synthetic compounds

such as dicarboximides for *B. cinerea* suppression has resulted in developing strains resistant to fungicides (Banno, et al., 2008), this study designed to apply natural substance (MeJA) or PGRs (BA) in accordance with IDM (integrated disease management) strategy to ward off harmful effects of chemical compounds that threaten both environment and human health. One study showed that application of MeJA did not stimulate ethylene production of rose petals, did not cause any phytotoxic effects, and suppressed artificial gray mold infection in most of the treated cultivars (Meir, et al., 2003). It has also been demonstrated that MeJA application can activate defense-related genes and the synthesis of phytoalexins, and in higher concentration it directly affect defense responses (Wang, et al., 2015). Likewise rose flowers, freesia flowers are also susceptible to *B. cinerea* infection. An investigation showed that MeJA vapor treatment successfully suppressed *B. cinerea* on cut freesia flowers, delayed flower senescence, and improved flower vase life and fresh weight of flowers (Darras, et al., 2005). In another study on freesia, the higher beneficiary effects of spraying MeJA compared to pulse treatments were also corroborated (Darras, et al., 2007). Consistent with their findings, our results presented in Table 4 showed that spraying rose pot flowers with 150 $mg*l^{-1}$ MeJA extended cut flower longevity, which possibly could be attributed to its antimicrobial properties in vase solution and prevention of xylem vessels from blockage (Buta and Moline, 1998). It has also been reported that MeJA can stimulate or inhibit ethylene synthesis. Depending on the fruit development stage, responses to exogenous MeJA may vary by promoting or inhibiting ethylene synthesis (Fan, et al., 1997).

Studies have disclosed that besides plant defense responses, MeJA may affect many other physiological and morphological changes such as leaf expansion, root, stem growth, and photosynthetic pigment contents (Kovač and Ravnikar, 1994). MeJA may also delay the senescence process by delaying chlorophyll and carotenoid degradation (Kovač and Ravnikar, 1994; Hamberg and Gardner, 1992). Likewise, in this survey the pre-harvest spraying treatments of 100 $mg*l^{-1}$ MeJA combined with 10 $mg*l^{-1}$ BA had the highest positive effect on increasing petal carotenoid content and exhibited a high level of leaf chlorophyll content as well (Table 3. and 4.). As a result of hindering carotenoid degradation, petal colour fading was postponed, hence the improvement of rose quality and vase life. The orange colour of rose petals was boosted by applying 100 $mg*l^{-1}$ MeJA + 10 $mg*l^{-1}$ BA, which was evident on the flowers grown even after three months of the last spray and then gradually changed to the yellow colour similar to the untreated plants (data not shown). Interestingly, sprayed petals with 10 $mg*l^{-1}$ BA alone showed virtually the same level of carotenoid concentration as observed in the best treatment (100 $mg*l^{-1}$ MeJA + 10 $mg*l^{-1}$ BA) without substantial changes (Table 4.). In regard with chlorophyll content, the best spraying solutions were comprised of 100 $mg*l^{-1}$ MeJA + 10 or 20 $mg*l^{-1}$ BA (Table 3.). While BA application had a positive effect on enhancing petiole carotenoid content, the leaf chlorophyll concentration exposed to BA alone did not increase (Table 3. and 4.). This point toward the positive interaction effects of these two phytohormones on increasing both carotenoid, chlorophyll contents, and also on improving flower head diameter (Table 1., 3. and 4.). The highest longevity, however, was achieved by spraying 150 $mg*l^{-1}$ MeJA (Table 4.). This results would seem to suggest that MeJA at 150 $mg*l^{-1}$ could function as a postharvest treatment for prolonging the cut flower vase life.

Recent studies have shown the widespread application of BA combined with $GA_3$ in preservative or spraying solutions for promoting flower quality and extending its vase life (Janowska and Stanecka, 2011; Emami, et al., 2011). Previous study showed that application of 50 $mg*l^{-1}$ BA + 50 $mg*l^{-1}$ $GA_3$ exerted a strong positive impact on physio-biochemical processes of gerbera such as improving its longevity, fresh weight, water uptake, membrane stability, and total soluble solids (Danaee, et al., 2011). More recently, another study showed that the positive influence of BA on increasing cut gerbera fresh weight was even stronger than all other potent synthetic compounds such as 8-HQS (Jafarpour, et al., 2015). It has been suggested that acid invertase activity instigate sucrose translocation from leaves to petals and catalysis its conversion to hexose in order to reduce petal water potential, which subsequently lead to petal cell expansion and bud opening. While invertase activity naturally increases before bud opening, its activity level needed for osmotic pressure regulation can be remained for a few days by even postharvest application of MeJA, resulting in improving petal fresh weight and delaying senescence process (Horibe, et al., 2013). In this study, because each solution affected different physiological and morphological aspects of rose flowers, application of specific dosage at specific growth stage is suggested to achieve the highest efficiency. The data showed that the pre-harvest spraying solution containing 100 $mg*l^{-1}$ MeJA + 20 $mg*l^{-1}$ BA was the best treatment for obtaining the largest flower head diameter, while 10 $mg*l^{-1}$ BA or 100 $mg*l^{-1}$ MeJA + 10 $mg*l^{-1}$ BA was the best treatment for obtaining the highest petal's carotenoid content. For the highest longevity, pretharvest treatment of cut flowers at 150 $mg*l^{-1}$ MeJA had the best effect. The findings corroborated that plant's physiological and morphological responses was affected by treatments and plant's growth stages.

In conclusion, application of MeJA and BA can improve the pre- and post-harvest quality and quantity indices of rose flowers. These results demonstrate that spraying treatment with MeJA as a natural substance is an efficient alternative management method to be substituted with chemical preservatives to improve pre- and post-harvest quality and quantity indices of *Rosa* 'Yellow Finesse'.

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