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Research Article

# Physiological characteristics and vase life responses of rose cut flowers (*Rosa hybrida* L. cv. 'Royal Baccara') to benzyl adenine and 1- metylcylcopropene

Mojdeh FARAZMANDI[1], Abbas MIRZAKHANI[2]*, Nour A. SAJEDI[3]*, Mohammad NASRI[4], Masoud GOMARIYAN[3]

[1]*Department of Horticulture, Arak Branch, Islamic Azad University, Arak, Iran; m.farazmandi@yahoo.com*
[2]*Horticulture Crops Research Department, Markazi Agricultural and Natural Resources Research and Education Center, AREEO, Arak, Iran; mirz51@yahoo.com (*corresponding author)*
[3]*Department of Agronomy and Plant Breeding, Research Center of Applied Plant sciences, Arak Branch, Islamic Azad University, Arak, Iran; N-sajedi@iau-arak.ac.ir; M-gomarian@iau-arak.ac.ir*
[4]*Department of Agronomy, Varamin Branch, Islamic Azad University, Varamin - Pishva, Varamin,Tehran, Iran; nasri@iauvarami.ac.ir*

## Abstract

+prolonging vase life and decreasing post-harvest losses are considered a basic strategy towards sustainable development of agriculture, and the application of growth regulators, e.g. benzyl adenine(BA) and 1-metylcylcopropane(1-MCP), is one of the practical methods for them. The effects of benzyl adenine(BA) and 1-metylcylcopropene(1-MCP) on some biochemical characteristics and vase life of rose cv. 'Royal Baccara' (*Rosa hybrida* L. cv. 'Royal Baccara'), were explored in a factorial experiment based on a randomized complete block design with three replications in 2017. The factors included BA at three levels of 0 (control), 75 and 150 ppm and 1-MCP in three levels of 0 (control), 1 and 2 µl/$L^{-1}$. The results showed that the maximum soluble protein content was observed in 150 (ppm) BA +2 (µl.$L^{-1}$) 1-MCP treatments on the second, fourth, sixth, and eighth days. The results of this research revealed that BA at the rates of 75 and 150 ppm and 1-MCP at the rates of 1 and 2 (µl.$L^{-1}$) enhanced soluble protein content by 10.49, 13.37, 15.18, and 18.18 percent as compared to the control, respectively. The results showed that the maximum proline content was related to the control and the minimum content was observed in the plants treated with 150 (ppm) BA+ 2 (µl.$L^{-1}$) 1-MCP. The endoproteases enzyme content was slightly decreased on the eighth day, indicating the efficacy of BA and 1-MCP in inhibiting the senescence process and prolonging vase life of rose flowers. The results showed that the application of 1 or 2 µl.$L^{-1}$ 1-MCP and 150 mg $L^{-1}$ BA can improve the vase life of cut roses

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***Keywords:*** endoproteases enzyme; growth regulator; proline content; rose flower

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## Introduction

Rose,( *Rosa hybrid*a L.), belongs to the important family of Rosaceae and is one of the most important cut flowers in the world so that, it presently has the first rank in terms of cultivation and trade in most countries of the world (Anonymous, 2016). In spite of their high economic value, cut flowers are highly prone to putrefaction. Due to their high rate of respiration and vulnerability, cut flowers need more attention in the postharvest stage (Kader, 2013). Flower senescence symptoms include wilting, abscission and discoloration of petals. These symptoms vary in different flowers. In rose cut flowers, petal wilting is accompanied by a decrease in water absorption, flower senescence, abscission and discoloration of petals. These symptoms vary in different flowers. These consequents indicate the inability of petals to absorb water, which results in flower senescence (Jin *et al.*, 2016). Low water absorption and transpiration give rise to an increase in cut flower wastes (Hahn *et al.*, 2011). Hence, the control of production conditions and the use of growth regulators and anti-stress compounds are very important for the quantitative and qualitative improvement of flowers (Yang and Kao, 2002). Nowadays the short vase life of cut flowers and leaves is one of the most important problems of their production (Van Meeteren *et al.*, 2015). It is important to use methods that can prolong flowers vase life (Wagstaff *et al.,* 2002).

Benzyl adenine (BA) and1-metylcylcopropene(1-MCP) are compounds that influence rose flowers postharvest life longevity (Huang *et al.*, 2009). BA is considered a synthetic cytokinin. Cytokinins mainly affect cell division, thereby delaying senescence and shoot branching in ornamental plants (Iqbal *et al.*, 2012). Due to its considerable effects on increasing crop quality, 1-MCP has recently been used to protect crops from the destructive effects of ethylene in different countries all around the world (Mutu, 2011). 1-MCP seemingly prevents ethylene bonding to its certain receptors in the plant and inhibits the expression of some regulator genes in maturation stages such as tACS2, tACS4 and tAco (Sisler *et al.*, 2009). Researchers have attributed cytokinin effectiveness to the stimulation of calcium ion absorption in cell walls and demonstrated that certain cytokinins impact the induction of phosphoenolpyruvate carboxylase (the key enzyme in crassulacean acid metabolism, accumulation of proline, and PEPCase and carbonic dehydrogenase in plants) (Ranwala and Miller, 2000). Applying these compounds to plants decreases stress damage, improves solution absorption and maintains petals turgor pressure. Likewise BA reduces respiration rate, sensitivity to ethylene and hinders ethylene synthesis (Serek *et al.*, 2014). The presence of cytokinins also delays protein degradation and cell death by inhibiting ribonuclease activity (Rao *et al.*, 2012). Researchers have asserted that mechanisms by which BA growth regulator delays senescence in lily's fallen leaves are still unknown, but as the respiration rate was low in the leaves treated with BA, it can be said that it results in a rapid decrease in protein degradation. Therefore, BA supplies the deficiency in the required adenine for ribonucleic acid as such, it results in the molecule regeneration and consequently delays protein degradation (Ozden and Karaaslan, 2011). Proline accumulation is another biochemical index in flower senescence. In plants exposed to stress, proline content increases faster than other amino acids (Bates et al., 1973). Proline accumulation during leaf senescence has previously been reported in calla lily (Sahi, 2009) and rice (Yang and Kao, 2002) leaves. In a research conducted on the vase life of some cattleya orchid flowers, a combination of BA treatment with 1-MCP had some kind of exacerbating effect on the shelf life of Pink Villa cultivar. Therefore, 1-MCP, sucrose, and BA treatment were reported as the most effective ways for improving the viability of cattleya flowers (Ranwala and Miller, 2000). In a research, 1-MCP at a rate of 10 $nl.L^{-1}$ resulted in an increase in vase life and a decrease in respiratory quotient in the stock cut flower(Darras *et al.*, 2010). Similarly a positive effect of 1-MCP was reported on the postharvest quality of tulip cut flowers by lowering ethylene efficacy (Chutichudet *et al.*, 2010). In flowers treated with 1-MCP, water absorption has always been more than the control as well; therefore, this factor can have a great impact on extending vase life, and it can be concluded that a decrease in water up take in rose flowers is associated with their vase life loss(Paull and Goo, 2005). This experiment was conducted to assess the effects of BA and 1-MCP on some physiological traits and vase life of roses cv. 'Baccara'

## Materials and Methods

*Experimental design*

To study the effects of BA and 1-MCP on the physiological characteristics, persistence, and vaselife of *Rosa hybrida* L. cv. 'Royal Bacara', a factorial experiment was conducted based on a randomized complete block design in 2017. The research was carried out with two factors including BA, the first factor at three levels of 0, 75, and 150 ppm, and 1-MCP at three rates of 0, 1, and 2 ($\mu l.L^{-1}$), in three replications on cut roses(*Rosa hyblida* L.) cv. 'Royal Bacara'. The cut flower postharvest treatments were accomplished as short-term treatments (pulsing). The flowers were procured from a commercial greenhouse in Tehran province. One gram 1-MCP pills used in this research were made in the USA[1], which were 0.127 percent pure according to claims of the manufacturing company. The cut flowers were put in the desired concentrations of 1-MCP for about 12 hours to assess the interaction of this compound with BA that was procured at Merck Germany Company. Distilled water (solution with no chemicals) was used as the control and all cut flowers were soaked in it. Then the cut flowers were placed in a standard control room at 22 ±1 °C, under a light intensity of 15 $\mu mol\ m^{-2}\ s^{-1}$, with a relative humidity of 60 to 70% and lightness/darkness cycle of 12:12 hours. It should be mentioned that nine cut flowers were used in each treatment. The following traits were eventually measured.

*Vase life*

In all the treatments, the number of days after harvest up to the appearance of petals senescence symptoms (welting of two-thirds of the petals) were considered as the vase life (Liao *et al.*, 2009).

*Solution uptake*

Preservative solution uptake was calculated by the following equation (Humaid, 2004):

$$FW_{\text{mL day}^{-1}\text{g}^{-1}} = \frac{S_{t-1} - S_t}{W_t}$$

where St is the weight of vase solution (g) at t = day 1, 2, 3, etc., $St_{-1}$ is the weight of vase solution (g) on previous day and $W_t$ is the initial fresh weight.

*Biochemical traits*

The soluble protein content was measured as follows (Eason *et al.*, 2002):

$$(\mu g\ mg\ FW^{-1}) = (X\ \mu g\ 50\ \mu l^{-1}) \times (1000\ \mu l\ 100\ mg\ FW^{-1})$$

To measure the activity of endoproteases, the following optimized method was applied (Eason *et al.* 2002; Wagstaff *et al.*, 2002):

$$(Endoproteases\ activity)\Delta A440\ (units\ mg\ FW_{h-1}) = \{A440\ (sample) - A440\ (blank)\} \times 0.0347$$

The soluble carbohydrates concentration was measured by using the high performance liquid chromatography (Humaid, 2004; Ichimura *et al.,* 2015). The proline content of petals was measured according to the method of Irigoyen *et al.* (1993).

*Ethylene measurement*

Ethylene was measured by the Gas chromatography method. For this purpose, each branch of the cut flowers was placed in a 2-liter impermeable bottle at 20 ◦C and after 1 h, it was sampled with a special syringe (about 1ml of bottle gases) and injected to the gas chromatography (GC) apparatus and the amount of ethylene was recorded.

1- Ethylbloc (Rohm–Haas, Spring House, PA), USA

*Measurement of soluble carbohydrate concentration*

To measure the concentration of soluble carbohydrates, 1g of petals and stems were harvested and their carbohydrates content in them were determined by high performance liquid chromatography (Humaid, 2004).

*Proline measurement*

Free proline content was determined according to the method described by Irigoyen *et al.*, (1993) with slight modifications. A 2% homogenate of the fresh leaf was prepared with 3% aq. sulfosalicylic acid and centrifuged at 3500 rpm at 4°c for 15 min. Two milliliter of supernatant was taken and 2 ml of glacial acetic acid and acid ninhydrin reagent was added. The reaction mixture was boiled in water bath for 60 min and then cooled on ice. Then 4 ml of toluene was added and incubated at room temperature for 30 min. Tubes were then shaken for 15 sec and allowed to stand for 10 min for phase separation. The upper phase was separated and absorbance was measured using spectrophotometer and the concentration of free proline was calculated using proline standard.

*Statistical analysis*

At the end of the experiment, the results of each treatment were analyzed in variance by the SAS software and the data means were compared at the levels of 1% and 5% using Duncan's multiple range test.

## Results and Discussion

The effects of BA and 1-MCP on the physiological traits, persistence and viability of cut roses (*Rosa hybrida* L.) cv. 'Royal Baccara' were assessed on different days. The variance analysis table suggested that the soluble protein content, solution uptake, proline content, endoprotease enzymes, stem and petal carbohydrates and vase life were affected by the simple and interactive effects of BA and 1-MCP on different days, and the observed differences were statistically significant at one and five percent levels (Tables 1, 3, 5, 7 and 9).

*Soluble protein*

The results of the means comparison of the interactive effects demonstrated that the maximum soluble protein content was observed in the plants treated with 150 (ppm) BA + 2 ($\mu l.L^{-1}$) 1-MCP on the second, fourth, sixth, and eighth days, which statistically had no significant difference with those treated with 150 (ppm) BA + 1 ($\mu l.L^{-1}$) 1-MCP, 75 (ppm) BA +2 ($\mu l.L^{-1}$) 1-MCP, and 150 (ppm) BA. The minimum soluble protein was observed in the control treatment (Table 2). Cytokinins delay senescence by preventing protein and chlorophyll degradation and BA supplies the deficiency of adenine required for ribonucleic acid, thereby regenerating molecules and delaying degradation of proteins (Mutu, 2011). Cytokinins are found as one of the alkalis adjacent to anticodon in some transfer RNAs, so they might be responsible for binding transfer RNA to ribosomes during protein generation (Yamada *et al.*, 2012). The results revealed that BA at the rates of 75 and 150 ppm and 1-MCP at the rates of 1 and 2 ($\mu l.L^{-1}$) enhanced soluble protein content by 10.49, 13.37, 15.18, and 18.18 percent, respectively, as compared to the control. The interaction of anti-aging substances could generate the maximum soluble protein content, increase the intracellular macromolecules, prevent ion leakage and cell wall degradation, reduce water absorption, and result in senescence. From the eighth day onwards, the water absorption rate and soluble protein content started to diminish in all treatments compared to the sixth, fourth days and it seems that with the onset of the senescence process, the main metabolites are transferred from the aged organs into the plant and consumed for the growth of other organs. This is consistent with the results of other studies (Buchanan-Wollaston, 2015).

**Table 1**. Analysis of variance of soluble protein affected by BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day2 | Day4 | Day6 | Day8 |
| BA | 2 | 0.045$^{*}$ | 0.42$^{*}$ | 0.071$^{*}$ | 0.082$^{*}$ |
| 1-MCP | 2 | 0.039$^{*}$ | 20.81$^{**}$ | 0.065$^{*}$ | 0.031$^{ns}$ |
| 1-MCP×BA | 4 | 29.42$^{**}$ | 51.43$^{**}$ | 42.4$^{**}$ | 125.401$^{*}$ |
| Error | 27 | 0.005 | 0.061 | 0.009 | 0.015 |
| CV | - | 5.81 | 6.28 | 7.21 | 7.14 |

$^{ns}$, $^{**}$ and $^{*}$ represent non-significant and significant at the 1% and 5% probability levels, respectively.

**Table 2**. Comparison of the interactive effects of BA and 1- MCP on soluble protein

| | (Soluble protein mg/fw) | | | |
|---|---|---|---|---|
| Treatment | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 1.73e | 1.72d | 1.69d | 1.62c |
| 1 µl/L 1-MCP | 1.93d | 1.86cd | 1.83c | 1.74bc |
| 2 µl/L 1-MCP | 2.14bc | 2.05bc | 1.92abc | 1.82b |
| 75ppm BA | 2.07cd | 1.92bcd | 1.84c | 1.78b |
| 1 µl/L 1-MCP×75ppm BA | 2.12c | 1.99bc | 1.88bc | 1.81b |
| 2 µl/L 1-MCP×75ppm BA | 2.28ab | 2.12ab | 1.96ab | 1.87ab |
| 150 ppm BA | 2.15bc | 1.96c | 1.95ab | 1.84ab |
| 1 µl/L 1-MCP×150ppm BA | 2.23abc | 2.08b | 1.97ab | 1.91$^{a}$ |
| 2 µl/L 1-MCP×150ppm BA | $^{a}$2.34a | 2.24a | 2.09a | 1.98a |

Numbers followed by the same letters are not significantly different (P<0.05).

*Solution uptake*

The results showed that the minimum amount of the absorbed solution was in the control treatment and the maximum was in the 150 (ppm) BA+ 2 (µl.L$^{-1}$)1-MCP treatment on the second, fourth, sixth, and eighth days, which had no significant difference with the treatment of 150 (ppm) BA + 2 (µl.L$^{-1}$)1-MCP and so that all were placed in the same statistical group (Table 4). Although BA and 1-MCP reduced the petal aging trend to a great extent, the amount of solution uptake was remarkably decreased on the eighth day, which is the onset of the cell aging process. This amount was even lower than the absorbed solution on the first day (Table 4). The flowers treated with 1-MCP took up more water than the control (Paull and Goo, 2005). The results suggested that the value of 1.35 (ml gr$^{-1}$.FW$^{-1}$) in the control rose to 2.09 (ml gr$^{-1}$.FW$^{-1}$) which is a 35 percent increase. This value rose to 3.13 (ml gr$^{-1}$.FW$^{-1}$) on average in the flowers treated with 75(ppm) BA+ 2 (µl.L$^{-1}$)1-MCP, which revealed an increase of 57 percent. The increased water absorption induced by these compounds increases the growth of the cell wall, improves the structure of macromolecules, and inhibits the dimerization of proteins. By protecting the structure and increasing sugar content by respiratory and osmotic pressure regulations, the aging process delays, so this factor might have a significant influence on the vase life prolongation. The results revealed that the decline in water uptake in the rose flowers was associated with the decreased vase life (Ichimura *et al.*, 2015), which is quite evident in this experiment.

**Table 3**. Analysis of variance of solution uptake as affected by BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day 2 | Day 4 | Day 6 | Day 8 |
| BA | 2 | 6257.4$^{*}$ | 12463.6$^{*}$ | 7159.2$^{*}$ | 14591.3$^{*}$ |
| 1-MCP | 2 | 2810.3 $^{ns}$ | 14093.2$^{*}$ | 7024.9$^{*}$ | 72985.4$^{**}$ |
| 1-MCP×BA | 4 | 7659.1$^{*}$ | 13802.7$^{*}$ | 6526.4$^{*}$ | 58482.1$^{**}$ |
| Error | 27 | 1482.2 | 1987.4 | 998.1 | 2218.3 |
| CV | - | 8.43 | 7.12 | 8.01 | 8.27 |

$^{ns}$, $^{**}$ and $^{*}$ represent non-significant and significant at the 1% and 5% probability levels, respectively.

**Table 4**. Comparison of effects of interactions between AB and 1- MCP on absorbed solution

| | Absorbed solution (ml/gr.FW) | | | |
|---|---|---|---|---|
| Treatments | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 1.36a | 1.41d | 1.47c | 1.35d |
| 1 µl/L 1-MCP | 1.68cd | 1.74d | 1.81c | 1.62cd |
| 2 µl/L 1-MCP | 1.91cd | 2.16cd | 2.27bc | 2.09c |
| 75ppm BA | 2.08c | 2.23cd | 2.38bc | 2.11c |
| 1 µl/L 1-MCP×75ppm BA | 2.46bc | 2.78bc | 2.91b | 2.64bc |
| 2 µl/L 1-MCP×75ppm BA | 2.89b | 3.26abc | 3.47ab | 3.13abc |
| 150 ppm BA | 2.57bc | 2.71c | 2.83ab | 2.67bc |
| 1 µl/L 1-MCP×150ppm BA | 3.06ab | 3.38ab | 3.56a | 3.28ab |
| 2 µl/L 1-MCP×150ppm BA | 3.64a | 3.94a | 4.07a | 3.86a |
| | | | | |

Numbers followed by the same letter(s) are not significantly different ($P < 0.05$).

*Proline content*

The results of the means comparison for the interactive effects suggested that the maximum proline content was in the control treatment and the minimum content was observed in 150 (ppm) BA+ 2 ($\mu l.L^{-1}$)1-MCP treatment, on the second, fourth, sixth, and eighth days (Table 6). Although BA and 1-MCP reduced the petal aging trend to a great extent, proline content was considerably increased on the eighth day by 11 percent versus the second day, which indicates an improvement in cell resistance to environmental stimulants (Table 6). Plants enhance their resistance against senescence through the accumulation of osmotic regulator materials, e.g. proline, amino acids and soluble carbohydrates (Ashraf and Foolad, 2007). Researchers have attributed cytokinin effectiveness to the stimulation of calcium ion absorption in cell walls and demonstrated that certain cytokinins are effective in inducing phosphoenolpyruvate carboxylase [the key enzyme in crassulacean acid metabolism (CAM)] metabolism, proline accumulation, and PEPCase and carbonic dehydrogenase in plants (Rao *et al.*, 2012). Recent studies have revealed that the treatment of iris flowers with1-MCP restrained petal proteins from degradation and delayed senescence. Proline accumulation in leaves at the senescence stage has been reported in calla lily while in rose flowers, increase in proline has been only reported at aging petals (Kumar *et al.*, 2008). The results of this experiment suggested that proline content was increased by 10 percent in the control treatment from the second to eighth experimental days from 1.186 (µmol/g.FW) to 1.324 (µmol/g.FW), but with the application of BA and 1-MCP, it was increased from 0.887 (µmol/g.FW) on the second day to 0.958 (µmol/g.FW) on the eighth day, implying only 7 percent increase. Therefore, the simultaneous application of BA and 1-MCP reduced ethylene production and increased $O^{-2}$ and $H_2O_2$ breakdown and inhibited the synthesis of cellular free radicals (Macnish *et al.*, 2010). By changing ethylene receptor bands (Sisler *et al.*, 2009) induced a competitive increase in the freshness duration of the petal. In fact, by declining protein degradation(Darras *et al.*, 2010) the proline content was decreased and BA brought about molecule regeneration by supplying the required adenine for ribonucleic acid (Mutu, 2011), so the proline increase rate was lessened which is evident in the present research.

**Table 5**. Analysis of variance of proline content under BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day2 | Day4 | Day6 | Day8 |
| BA | 2 | $0.0211^{*}$ | $0.082^{*}$ | $6.138^{*}$ | $125.012^{**}$ |
| 1-MCP | 2 | $0.0193^{*}$ | $0.078^{*}$ | $6.457^{*}$ | $78.429^{**}$ |
| 1-MCP×BA | 4 | $0.285^{*}$ | $22.49^{**}$ | $33.452^{**}$ | $491.387^{**}$ |
| Error | 27 | 0.0031 | 0.012 | 0.98 | 0.0105 |
| CV | - | 4.51 | 5.28 | 3.92 | 4.23 |

$^{ns}$, ** and * represent non-significant and significant at the 1% and 5% probability levels, respectively.

**Table 6**. Comparison of the interactive effects of AB and 1- MCP on proline

| | Proline (µmol /g.FW) | | | |
|---|---|---|---|---|
| Treatments | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 1.104b | 1.116ab | 1.127c | 1.216abc |
| 1 µl/L 1-MCP | 0.979cd | 1.025b | 1.041bc | 1.096bc |
| 2 µl/L 1-MCP | 1.027c | 1.064ab | 1.081bc | 1.122ab |
| 75ppm BA | 0.937de | 0.982bc | 0.993c | 1.036c |
| 1 µl/L 1-MCP×75ppm BA | 0.902de | 0.968bc | 0.976cd | 0.983cd |
| 2 µl/L 1-MCP×75ppm BA | 0.949d | 0.974bc | 0.986cd | 1.072bc |
| 150 ppm BA | 0.916de | 0.953bc | 0.968cd | 0.986cd |
| 1 µl/L 1-MCP×150ppm BA | 0.887e | 0.926c | 0.937d | 0.958d |
| 2 µl/L 1-MCP×150ppm BA | 1.104b | 1.116ab | 1.127c | 1.216abc |

Numbers followed by the same letter(s) are not significantly different (P<0.05).

*Endoproteases enzyme*

The results suggested that the maximum endoproteases enzyme content was observed in the control treatment on the second, fourth, sixth, and eighth days, which statistically had no significant difference with all applied levels of 150 (ppm) BA and 1-MCP. The minimum endoproteases enzyme content was seen in 150 (ppm) BA+ 2 (µl.L)1-MCP treatment (Table 8). The endoproteases enzyme content was slightly decreased on the eighth day, indicating the efficacy of BA and 1-MCP in inhibiting the senescence process and increasing rose flower viability and vase life. However, the rise of endoproteases enzyme content was not remarkable on the eighth day. BA increased the postharvest longevity of rose flower by reducing endoproteases activity along with water absorption and following that, through inhibiting protein degradation. The endoproteases activity increase during the postharvest period was reported in alstroemeria and sandersonia flowers (Wagstaff *et al.*, 2002) and it was shown that BA concentration in sandersonia flower resulted in increase of its postharvest life time and 4 days after treatment with 1-MCP, the endoproteases activity was delayed (Eason *et al.*, 2002). The study of the observed biochemical changes by the BA treatment demonstrated that the activity of protease associated with petals gain, delayed, and therefore, the vase life enhanced. It seems that in this research BA contributed to the prolongation of flower longevity by lessening protease activity, which is consistent with other researchers' results (Eason *et al.*, 2002). The results indicated that the simultaneous utilization of BA and1-MCP lowered the endoproteases activity by 35 percent on the eighth day, but the endoproteases activity of the control was increased by about 11 percent on the eighth day compared to the second day, which indicates the impact of BA and1-MCP on the senescence decline and the rise of vase life in this experiment.

**Table 7**. Analysis of variance of endoproteases under BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day2 | Day4 | Day6 | Day8 |
| BA | 2 | $28.42^{ns}$ | $684.61^{*}$ | $403.42^{*}$ | $142.98^{*}$ |
| 1-MCP | 2 | $32.37^{ns}$ | $371.27^{ns}$ | $84.02^{ns}$ | $31.29^{ns}$ |
| 1-MCP×BA | 4 | $219.42^{*}$ | $769.43^{*}$ | $351.22^{*}$ | $1985.27^{**}$ |
| Error | 27 | 29.27 | 102.48 | 49.54 | 20.81 |
| CV | - | 8.45 | 7.12 | 7.46 | 7.02 |

$^{ns}$, ** and * represent non-significant and significant at the 1% and 5% probability levels, respectively.

**Table 8**. Comparison of interactions between AB and 1- MCP for endoproteases

| | Endoproteases (u/h.mg.FW) | | | |
|---|---|---|---|---|
| Treatments | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 2.67a | 2.75a | 2.84a | 2.97a |
| 1 µl/L 1-MCP | 2.41a | 2.61a | 2.63a | 2.71ab |
| 2 µl/L 1-MCP | 2.35ab | 2.41ab | 2.51ab | 2.58b |
| 75ppm BA | 2.31ab | 2.37bc | 2.39abc | 2.53b |
| 1 µl/L 1-MCP×75ppm BA | 2.16ab | 2.22c | 2.26bc | 2.41bc |
| 2 µl/L 1-MCP×75ppm BA | 2.08ab | 2.16cd | 2.19bc | 2.22bc |
| 150 ppm BA | 2.20ab | 2.21c | 2.24bc | 2.32bc |
| 1 µl/L 1-MCP×150ppm BA | 1.93b | 1.93cd | 2.04bc | 2.07c |
| 2 µl/L 1-MCP×150ppm BA | 1.82b | 1.82d | 1.89c | 1.93c |

Numbers followed by the same letter(s) are not significantly different (P<0.05).

*Petal carbohydrate content*

The results of the means comparison for the interactive effects (Table 10) indicated that the maximum content of stem and petal soluble carbohydrates was obtained from 150 (ppm) BA+ 2 (µl.L)1-MCP treatment, on the second, fourth, sixth, and eighth days, which had no significant difference with the 150 (ppm) BA+ 1 (µl.L) 1-MCP treatment, so that both treatments were statistically the same. The minimum content of stem and petal soluble carbohydrates was seen in the control (Table 10). It seems that the respiration rate was low in the leaves treated with BA and therefore, it prevented the reduction of carbohydrates which is consistent with the results of Ranwala and Miller (2000). Carbohydrate content had an ascending trend from the second to the eighth day but with regard to the abovementioned points, it descended on the eighth day. This reduction was 7 percent in the control treatment and 3 percent in 150 (ppm) BA+ 2 (µl.L) 1-MCP treatment. BA application resulted in the accumulation of structural carbohydrates in the flowers in normal conditions (Wang, 2009). BA application resulted in increased dissolved solids in petals and chlorophyll content, and by producing assimilates, enhanced the carbohydrate content. It should be mentioned that the clogging of stem xylems by unwanted microorganisms is one of the factors preventing its remobilization from the stem into the petals. 1-MCP prevented the growth of bacteria in the postharvest circumstance (Sood *et al.*, 2006) and enhanced the stem water uptake ability, and the stem sugar content and intracellular water were increased by sucrose degradation (Mutu, 2011) which increased the rose flower vase life.

**Table 9**. Analysis of variance of soluble carbohydrate under BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day2 | Day4 | Day6 | Day8 |
| BA | 2 | 687201.2$^{*}$ | 287457.2$^{*}$ | 151806.2$^{**}$ | 138217.2$^{**}$ |
| 1-MCP | 2 | 701452.6$^{*}$ | 332185.7$^{*}$ | 69517.5$^{*}$ | 13521.4$^{*}$ |
| 1-MCP×BA | 4 | 1745321.8$^{**}$ | 2983001.6$^{**}$ | 852432.3$^{**}$ | 983297.7$^{**}$ |
| Error | 27 | 98453.8 | 49413.7 | 10821.7 | 21502.6 |
| CV | - | 7.82 | 8.29 | 8.48 | 8.96 |

$^{ns}$, ** and * represent non-significant and significant at the 1% and 5% probability levels, respectively.

**Table 10**. Comparison of interactions between BA and 1- MCP on soluble carbohydrate

| | Soluble carbohydrate (mg /g) | | | |
|---|---|---|---|---|
| Treatments | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 12.24d[d] | 12.07d | 12.38d | 11.87d |
| 1 µl/L 1-MCP | 13.07cd | 12.63cd | 13.17cd | 12.04d |
| 2 µl/L 1-MCP | 13.51cd | 13.24cd | 13.88bc | 12.96c |
| 75ppm BA | 13.64c | 13.52c | 13.71c | 13.25bc |
| 1 µl/L 1-MCP×75ppm BA | 13.98bc | 13.81bc | 14.13bc | 13.67bc |
| 2 µl/L 1-MCP×75ppm BA | 14.46bc | 14.49ab | 14.86bc | 14.37b |
| 150 ppm BA | 14.21bc | 14.13b | 14.28bc | 14.09b |
| 1 µl/L 1-MCP×150ppm BA | 14.67b | 14.56ab | 14.94b | 14.41b |
| 2 µl/L 1-MCP×150ppm BA | 15.86a | 15.18a | 16.13a | 15.36a |

Numbers followed by the same letter(s) are not significantly different (P<0.05).

*Vase life*

The mean comparison table of BA and 1-MCP interactive effects on the vase life suggested that the longest vase life was obtained from the application of 150 (ppm) BA+ 2 (µl.L) 1-MCP with an average of 12.81 days and shortest from the control treatment with an average of 8.04 days. The results indicated that BA was more effective than 1-MCP on vase life. Ethylene content was diminished by the increase in BA concentration and consequently, the vase life was enhanced. But the best result was obtained when BA and 1-MCP were used together at the highest concentrations. Both of these anti-aging substances disturb ethylene functioning by the competitive inhibition method, which resulted in the vase life prolongation. The increased viability and vase life of the cut flowers might be due to the increase in carbohydrate content in the treatment (Argueso *et al.*, 2009). 1-MCP maintained the plants' leaves (cut flowers) in a good condition and prevented the reduction of their dry weight by lowering the percentage of weight loss and inhibition of chlorophyll and carbohydrates damage (Serek *et al.*, 2014) which extended their vase life. The experiments results demonstrated that in the control treatment, the vase life was decreased by 37 percent compared to the 150 (ppm) BA+ 2 (µl.L) 1-MCP treatment (Figure 1). Water uptake was higher in the flowers treated with 1-MCP compared to the control. Hence this factor might have played an important role in vase life improvement (Paull and Goo, 2005).

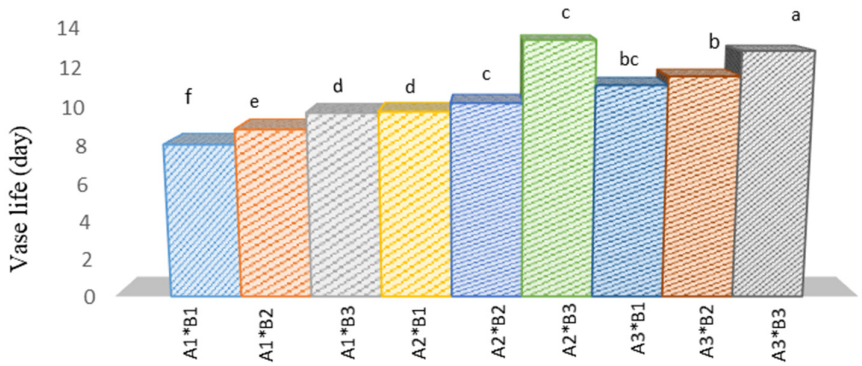


**Figure 1**. The interaction of BA and 1-MCP on vase life; A1 (0 ppm BA), A2 (75 ppm BA), A3 (150 ppm BA), B1 (0 µl.L$^{-1}$ 1-MCP), B2 (1 µl.L$^{-1}$ 1-MCP), B3 (2 µl.L$^{-1}$ 1-MCP)

*Ethylene*

The results of the analysis of variance showed that ethylene was affected by simple and interactive effects of BA and 1-MCP on different days (Table 11). The results of the means comparison showed that on

the second day, the highest amount of ethylene was observed in the control treatment with an average of 2.431 nl/gFW.h and the lowest was related to 150 ppm BA + 2 µl.L$^{-1}$ 1-MCP with an average of 1.602 nl/gFW.h (Table 12). According to the results in Table 12, the highest ethylene content was observed in the control treatment with an average of 2.628 nl/g FW.h on the fourth day and the lowest was obtained from the treatment of 150 ppm BA + 2 µl.l$^{-1}$ 1-MCP (an average of 1.871 nl/g FW.h.) (Table 12). Although the concentration of BA and 1-MCP significantly reduced the petal aging process, the amount of ethylene was increased significantly by day 8, which was the beginning of the cell aging process. The results showed that the lowest ethylene level was observed on day 8, from 150 ppm BA + 2 µl.L$^{-1}$ 1-MCP with an average of 2.021 gl/g FW.h. and the highest ethylene content was observed in the control treatment with an average of 2.963 nl/g FW.h.).

**Table 11**. Analysis of variance of ethylene under BA and 1- MCP

| | | | M.s | | |
|---|---|---|---|---|---|
| S.O.V | df | Day2 | Day4 | Day6 | Day8 |
| BA | 2 | 0.0041$^{*}$ | 0.742$^{*}$ | 0.051$^{*}$ | 0.281$^{*}$ |
| 1-MCP | 2 | 0.0037$^{*}$ | 0.829$^{*}$ | 0.045$^{*}$ | 0.315$^{*}$ |
| 1-MCP×BA | 4 | 2.215$^{**}$ | 125.412$^{**}$ | 0.048$^{*}$ | 0.287$^{*}$ |
| Error | 27 | 0.0005 | 0/12 | 0.007 | 0. 41 |
| CV | - | 6.04 | 5.86 | 7. 41 | 6.94 |

$^{ns}$, ** and * represent non-significant and significant at the 1% and 5% probability levels, respectively.

The aging of flowers is accompanied by many physiological and biochemical changes. In rose flowers, when the flowers are in the bud stage, they produce little ethylene, but gradually ethylene production increases as the petals open, and in fully open flowers, the amount of ethylene reaches its maximum (Sood *et al.*, 2006).

The effect of cytokines has been shown, is delay aging due to changes in the ethylene signaling pathway. Exogenous cytokinin concentrations in the petals of carnation changed their sensitivity to ethylene as well as their endogenous ethylene production (Chang *et al.*, 2005). Other studies are well characterized by ethylene and cytokinin during the aging process in petals, which shows that ethylene accelerates the process of cytokinin degradation, thereby accelerating the aging process (Taverner *et al.*, 1999).

**Table 12**. Comparison of interactions between BA and 1- MCP for ethylene

| | Soluble carbohydrate (mg /g) | | | |
|---|---|---|---|---|
| Treatments | Day 2 | Day 4 | Day 6 | Day 8 |
| Control | 2.431a | 2.628a | 2.785a | 2.963a |
| 1 µl/L 1-MCP | 2.284b | 2.481b | 2.604a | 2.741a |
| 2 µl/L 1-MCP | 2.197bc | 2.372bc | 2.532ab | 2.611ab |
| 75ppm BA | 2.253bc | 2.486b | 2.584ab | 2.658ab |
| 1 µl/L 1-MCP×75ppm BA | 2.218bc | 2.041cd | 2.192bc | 2.394bc |
| 2 µl/L 1-MCP×75ppm BA | 2.145c | 1.928de | 2.011c | 2.186c |
| 150 ppm BA | 2.143c | 2.186c | 2.304abc | 2.438b |
| 1 µl/L 1-MCP×150ppm BA | 1.741d | 1.819cde | 2.054bc | 2.241bc |
| 2 µl/L 1-MCP×150ppm BA | 1.602d | 1.871e | 1.987c | 2.021c |

Numbers followed by the same letter(s) are not significantly different (P<0.05).

Although ethylene was increased in the present study from the second day, due to the effect of 1-MCP, the vase life of the cut flowers was increased. The positive effects of 1-MCP on reducing the harmful effects of ethylene on rose, gerbera, and orchid plants were also reported (Song *et al.*, 2014). It was also found that the

effect of cytokinins in delaying aging is due to alterations in the ethylene signaling pathway (Chang *et al.*, 2005).

## Conclusions

In this study, it was found that BA preserved the structure of proteins by delaying the degradation of degraded nitrogenous compounds that play an important role in protein structure. The aging of flowers is accompanied by many physiological and biochemical changes. The interaction of BA and 1-MCP on the 8th day showed that without anti-aging substances, the treatments produced the highest amount of ethylene. Flowers treated with 1-MCP had higher water uptake than the control. By protecting proteins by reacting with phospholipids, proline stabilizes cell membranes and acts as a purifier of active species of energy storage and nitrogen source. BA, together with water retention, reduced endoprotease activity and inhibited protein degradation, thus prolonging postharvest longevity in roses. Our results showed that ethylene decreased as the concentration of BA was increased, and the vase life of cut roses was increased. But the best results were obtained when BA and 1-MCP were used in combination with the highest concentration, both of which inhibit ethylene work by competitive inhibition and increase flower vase life.

## Authors' Contributions

All authors read and approved the final manuscript

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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