RESEARCH ARTICLE

Increasing plant longevity and associated metabolic events in potted carnation *(Dianthus caryophyllus* L. Clove Pink)

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ABSTRACT

The effects of aminooxyacetic acid, benzyladenine, and 1-methylcyclopropene treatments on the post-production flower quality of potted carnation plants (*Dianthus caryophyllus* L. Clove Pink) were investigated considering ethylene production and antioxidant metabolism. Maximum plant longevity (17 days) was obtained using 70 ppb of 1-methylcyclopropene. As compared to control plants, ethylene production was significantly decreased by aminooxyacetic acid at concentrations over 100 mg L^{-1}, benzyladenine at 20 or 30 mg L^{-1}, and 1-methylcyclopropene at 70 and 140 ppb. A significant increase in 1-aminocyclopropane-1-carboxylic-acid concentration was observed in 1-methylcyclopropene treated plants compared with the control ones. On the other hand, decline in 1-aminocyclopropane-1-carboxylic-acid concentration was observed after using 100 or 150 mg L^{-1} of aminooxyacetic acid. Use of 1-methylcyclopropene (70 or 140 ppb), aminooxyacetic acid (100 or 150 mg L^{-1}), and benzyladenine (20 or 30 mg L^{-1}) significantly decreased H_2O_2 concentration and superoxide radical when compared with the untreated control. Significant increases in activities of superoxide dismutase, catalase, and peroxidase were noticed when plants were treated with 70 ppb 1-methylcyclopropene. In conclusion, aminooxyacetic acid, benzyladenine (at high concentrations), and 1-methylcyclopropene treatments can be suitable candidates for extending plant longevity, maintaining the visual quality, and reducing the loss of flower anthocyanin.

Keywords: aminooxyacetic acid, benzyladenine, ethylene production, flower, 1-methylcyclopropene.

INTRODUCTION

Carnations (*Dianthus caryophyllus* L.) have long been grown as a cut flower, while their presentation as a potted plant is more recent and follows the development of dwarf species (Banon et al., 2002). The quality of potted carnations is often lowered during transportation and by indoor environmental conditions. It has been shown that ethylene can reduce postharvest quality of potted plants (Reid and Wu, 1992). It is a gaseous plant hormone synthesized by the oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC).

Cytokinins (such as benzyladenine, BA) have been particularly effective in delaying senescence of carnation flowers by inhibiting ethylene biosynthesis (Cook et al., 1985). Different *Anthurium* cultivars, dipped into 200 mg L^{-1} BA, presented a variable effect on vase life (Paull and Chantrachit, 2001). The efficient ethylene action inhibitor (1-MCP) competitively blocks the hormonal action of ethylene through its irreversible binding to the ethylene receptor (Sisler and Serek, 1997). The ACC content of senescing miniature rose flowers pre-treated with 1-MCP was clearly higher than in the untreated

control (Muller et al., 2001). The aminooxyacetic acid (AOA) is also used for extending the vase life of ethylene sensitive cut flowers (Rattanawisalanona et al., 2003). It inhibited senescence and delayed flower abscission in *Salvia splendens* (Ferrante et al., 2006).

During senescence there is an overproduction of free radicals such as superoxide anion (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2), which may cause damage and cell death. Superoxide dismutase (SOD) is the only enzyme capable of scavenging O_2^-, where as H_2O_2 it can be directly degraded by catalase (CAT) or peroxidase (POD) in the presence of a reductant (Mates, 2000; Djanaguiraman et al., 2010). The decrease in O_2^- and H_2O_2 contents in 1-MCP-sprayed plants may be due to the lower levels of ethylene production and scavenging of O_2^- and H_2O_2 by such enzymes (Larrigaudiere et al., 2004).

The present study was carried out to investigate the effect of BA, AOA, and 1-MCP on ethylene production and antioxidant metabolism in potted carnation, in order to provide basic information for future strategies with the aim of increasing post-production of plant longevity in this species.

MATERIAL AND METHODS

Plant material: Potted carnation (*Dianthus caryophyllus* L. Clove Pink) cuttings were received from a commercial grower in Pakdasht, Varamin, Iran. The cuttings with 60–70 mm height were placed in boxes filled with perlite. Root formation at 18 to 20°C took about four to five weeks. The rooted cuttings were transplanted into plastic pots (1.5 L) that were filled with a mixture of peat and perlite (3:1, v/v), and placed under greenhouse condition: from 20 to 25/10 to 15°C (day/night), and 50 to 60% relative humidity. Flowering occurred after five or six months.

Treatments with benzyladenine, aminooxyacetic acid, and 1-methylcyclopropene: Pots containing uniform and healthy plants were selected at flower bud stage. Treatment with BA, AOA, and 1-MCP was done immediately after the first flower buds were almost fully opened in each pot. Plants were sprayed with solutions containing 10, 20, or 30 mg L^{-1} of BA (Sigma-Aldrich, Tehran, Iran) and 50, 100, or 150 mg L^{-1} of AOA (Sigma-Aldrich, Tehran, Iran), with a fine mist to cover all surfaces of the flowers and foliage. After such technique, the plants were held in a greenhouse overnight to allow leaves to dry. For 1-MCP treatment, the plants were placed in 60-liters-plastic containers and sealed with polyethylene bags. Water was added to the powder of EthylBloc™ (Rohm and Hass Philadelphia, PA, USA) to evolve 1-MCP at a concentration of 70 or 140 ppb. After application of all treatments, the plants were placed in an evaluation room where the environmental conditions were: 20±2°C, Relative Humidity (RH) >60%, and 12 hours under photosynthetic photon flux density of 15 µmol m^{-2} s^{-1}, using cool-white fluorescent lamps. Such measurements were made after opening three flowers per plant.

Evaluation of plant longevity: Flower senescence was evaluated daily and defined when at least 50% of the flowers per pot were senesced.

Measurement of ethylene production: In all treatments, flowers (one for each replication) were sealed in a 250 mL glass vessel for the measurement of ethylene production. After two hours, 1 mL of the gaseous mixture of each glass was injected in a gas chromatograph (Shimadzu Gas Chromatograph) equipped with an activated alumina column fitted in a flame ionization detector. Nitrogen was used as a carrier gas. The amount of ethylene was presented as nL g^{-1} FW h^{-1}.

Extraction and analysis of 1-aminocyclopropane-1-carboxylic-acid: For the ACC extraction, 2 g of crushed, frozen petal tissue was homogenized in 4 mL of 5% sulfosalicylic acid solution, and centrifuged for 10 minutes at 3,090 g_n in a pre-cooled centrifuge at 4ºC. ACC was assayed as described by Bulens et al. (2011). Briefly, 0.4 mL of 10 mM $HgCl_2$ was added to 1.4 mL of the extract in a 9 mL vial and was immediately sealed with a serum cap. Approximately 0.2 mL of the NaOH-NaOCl mixture was injected into the vial through the serum cap. The mixture was mixed for five seconds and allowed to react during four minutes on ice. The sample was mixed again for five seconds in order to release all ethylene content into the vial headspace. Following the second mixing, a 1 mL gas sample was removed for ethylene determination by gas chromatography.

Antioxidant enzymes: POD (EC 1.11.1.7): for peroxidase assay, petals (100 mg FW) were crushed in a phosphate buffer (0.1 M, pH=7.0) containing 15% (w/w) PVPP, 2 mM EDTA, and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 10,000 g_n for 20 minutes and the supernatant was assayed for POD. Peroxidase activity was determined following oxidation of odianisidine in the presence of H_2O_2 at 470 nm (Aebi, 1983). The enzyme extract was determined according to Bradford (1976). All enzyme activities and protein concentration were quantified spectrophotometrically (6405 UV/Vis, Jenway, England).

SOD (EC 1.15.1.1): The activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture was prepared by mixing 0.1 mM nitroblue tetrazolium,

0.1 mM EDTA, and 50 µM xanthine and xanthine oxidase in 50 mM potassium phosphate buffer (pH=7.8). One unit of SOD was defined as the amount of enzyme that inhibits by 50% the control rate (0.025 units of absorbance at 550 nm min^{-1}) (McCord and Fridovich, 1969).

Catalase - CAT (EC 1. 11.1.6): The reaction mixture had 15 mM H_2O_2, up to 100 µL of homogenate (7 mg mL^{-1} protein) with 0.2% (v/v) Triton X-100 in 50 mM potassium phosphate buffer, pH=7.0 (Aebi 1983).

Oxidants: Hydrogen peroxide (H_2O_2) levels in petals were measured by following the method described by Patterson et al. (1984). One mL of cold-acetone-extracted supernatant was added to 0.1 mL 20% titanium reagent (20% (w/v) $TiCl_4$ in 12.1 M HCl and 0.2 mL 17 M ammonia solution. The solution was centrifuged at 3,000 g_n at 4ºC for ten minutes, and the supernatant was discarded. The pellet was dissolved in 3 mL of 1 M sulfuric acid. Absorbance of the solution was measured at 410 nm with a spectrophotometer. Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2, which were expressed in nmol g^{-1} FW.

For superoxide anion (O_2^-), petals were homogenized in ice cold sodium phosphate buffer (0.2 M, pH=7.2) containing diethyl dithiocarbamate. The homogenate was immediately centrifuged for one minute at 3,000 g_n. In the supernatant, superoxide anion was measured by its capacity to reduce nitro blue tetrazolium (2.5×10^{-4} M). Absorbance of the end product was measured at 540 nm with a spectrophotometer. Superoxide anion was expressed as a change in optical density (OD) in min^{-1} g^{-1} FW (Chaitanya and Naithani, 1994).

Anthocyanin determination: Petal tissues were extracted using methanol containing 1% HCL for 24 hours, and the absorbance was determined by a spectrophotometer at 520 to 700 nm (Paliyath et al., 2008).

Statistical analysis: The experiment was carried out in a completely randomized design with four replications. Data were statistically analyzed using SAS software (Version 6.12). Mean comparisons to identify significant differences among treatments were performed using the least significant difference (LSD) at a 0.05 probability level.

RESULTS

Plant longevity: The longevity of potted plant was improved after using 1-MCP (at all concentrations), AOA (100 or 150 mg L^{-1}), and BA (20 or 30 mg L^{-1}). As compared to control, such variance was increased 9, 4.8, and 2.5 days by 70 ppb 1-MCP, 150 mg L^{-1} AOA, and 30 mg L^{-1} BA, respectively. The plants treated with 10 mg L^{-1} BA and 50 mg L^{-1} AOA showed the lowest plant longevity (Figure 1).

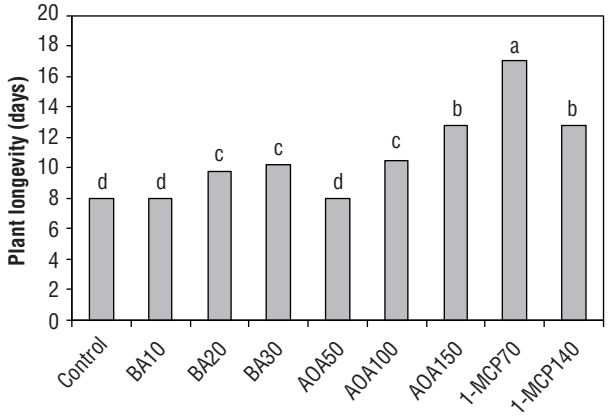

Figure 1. Plant longevity of potted carnation in response to pre-treatment with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 70 and 140 ppb. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

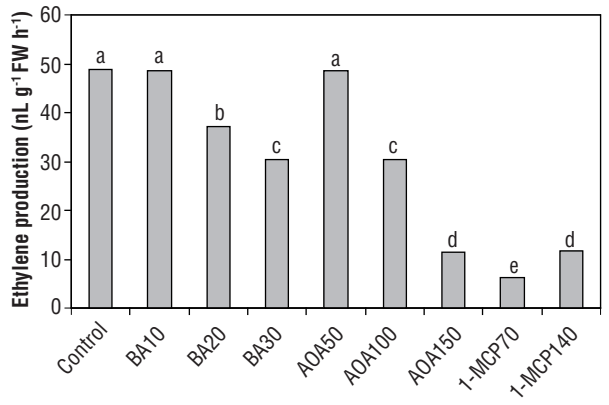

Figure 2. Flower ethylene production of potted carnation plants (on day 8) in response to pre-treatment with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 70 and 140 ppb. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

Effect of 1-methylcyclopropene, aminooxyacetic acid, and benzyladenine on ethylene production: The treatments with AOA (100 or 150 mg L^{-1}), BA (20 or 30 mg L^{-1}), and 1-MCP (70 or 140 ppb) significantly inhibited the flower ethylene production (Figure 2). Ethylene production increased sharply in the untreated flowers until the eighth day and decreased thereafter (Figure 3). Evaluation of the regression relationship between plant longevity and ethylene production showed a significant negative association between ethylene

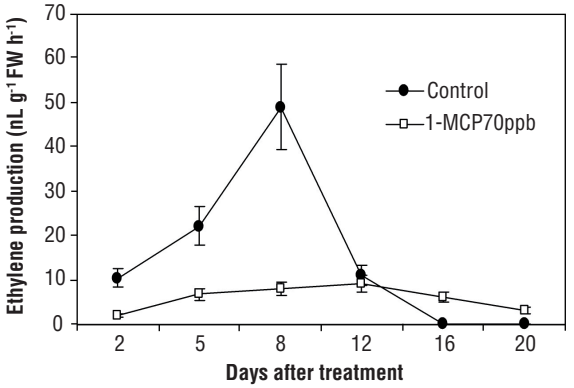

Figure 3. Flower ethylene production by control plants and ones treated with 70 ppb 1-MCP, this is the best treatment for increasing longevity of potted carnation plants. Symbols are the mean values (n=4)±standard error.

production and plant longevity, i.e., plant longevity decreases as ethylene production increases (Figure 4).

Changes in 1-aminocyclopropane-1-carboxylic-acid content of potted carnation: The effect of different treatments on ACC content of flowers was significant ($p<0.001$). The highest one was observed with 70 ppb 1-MCP. In contrast, the application of AOA in high concentrations significantly decreased the ACC content in the flowers compared with 1-MCP and BA, and the untreated control (Figure 5).

Anthocyanin determination: Anthocyanin concentration of color parts in the petals increased significantly in 1-MCP (70 or 140 ppb), AOA (100 or 150 mg L^{-1}), and BA (20 or 30 mg L^{-1}) treatments (Figure 6).

Effect of 1-methylcyclopropene, aminooxyacetic acid, and benzyladenine on antoxidant metabolism: The application of 1-MCP (70 or 140 ppb), AOA (100 or 150 mg L^{-1}) and BA (20 or 30 mg L^{-1}) significantly decreased H_2O_2 content and superoxide radical (O_2^-) compared with the untreated control (Table 1). The lowest H_2O_2 and superoxide radical contents were obtained in the treatment with 70 ppb 1-MCP. The highest SOD, CAT, and POD activities were found in the treatment with 1-MCP. Moreover, SOD, CAT, and POD activities were significantly higher in the AOA (100 or 150 mg L^{-1}) and BA (20 or 30 mg L^{-1}) treatments if compared with the untreated plants (Table 1).

DISCUSSION

The present study clearly indicated that treatment with 1-MCP (70 or 140 ppb), AOA (100 or 150 mg L^{-1}) and BA (20 or 30 mg L^{-1}) decreased ethylene production in flowers of potted carnation Clove Pink (Figure 2). They delayed the onset of wilting in the flowers, which agrees with the findings of Lerslerwong and Ketsa (2008) for *Dendrobium flowers* and Seglie et al. (2011) for *Dianthus caryophyllus* cut ones.

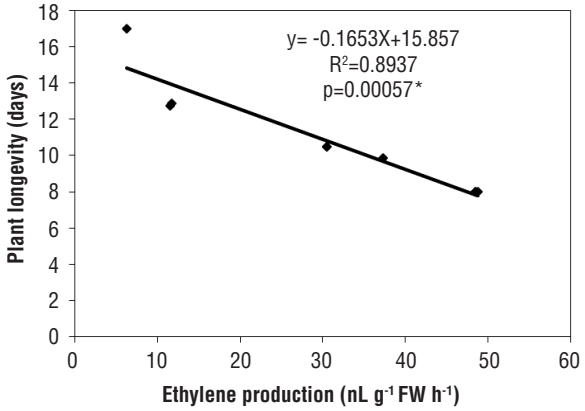

Figure 4. Relationship between ethylene production and longevity of potted carnation plants.*Significant negative correlation.

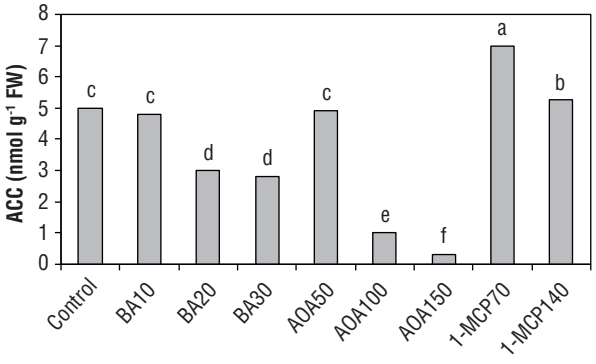

Figure 5. 1-Aminocyclopropane-1-carboxylic-acid content in petals (on day 8) as response to pre-treatment with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 70 and 140 ppb. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

The success in extending the plant longevity (Figure 1) using BA could be attributed to its role in inhibiting ethylene biosynthesis. The present results are in agreement with those of Cook et al. (1985) and Han and Miller (2003). Hassanpour Asil and Karimi (2010) reported that spraying cut *Lisianthus* flowers with 25 or 50 mg L^{-1} BA delayed ethylene production and extended its vase life. AOA is a well-known ethylene biosynthesis inhibitor and blocks the ACC synthase activity (Mensuali-Sodi et al., 2005). AOA is used for preserving

cut flowers sensitive to ethylene (Rattanawisalanona et al., 2003). These results showed that the application of 1-MCP, an ethylene perception inhibitor, significantly decreased ethylene production rate in flowers (Figures 2 and 3). The decreased ethylene production in 1-MCP-treated 'pink' pots may have also been due to the inhibition of the autocatalytic ethylene production Pathak et al., 2003).

The increase in the ACC content of the petals coincided closely with that in the ethylene production by the flowers. The ACC content of the 'pink' plants pre-treated with 1-MCP (especially at the concentration of 70 ppb) was clearly higher than in control. The accumulation of ACC in 1-MCP treated flowers may indicate that the treatment reduces ACC oxidase activity and to a lesser extent ACC synthase. However, no accumulation of ACC was observed after the AOA retreatment (especially at the concentration of 150 mg L^{-1}), which suggests that ACC synthase was inhibited by AOA. Muller et al. (2001) observed that treatment with 1-MCP resulted in increased accumulation of ACC and reduced ethylene production during senescence in miniature rose flowers.

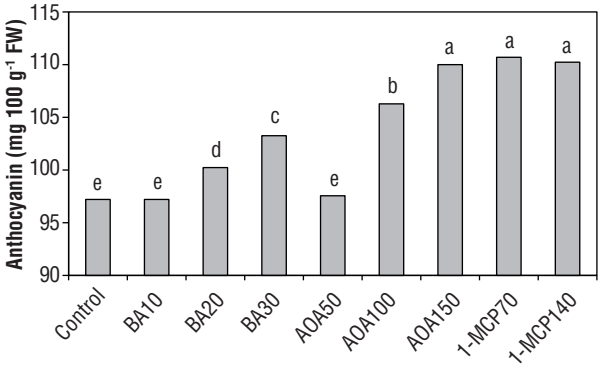

Figure 6. Anthocyanin content in petals (on day 8) as response to pre-treatment with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 70 and 140 ppb. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

Plants possess a well-defined enzymatic antioxidant defense system to protect them against the reactive oxygen species (ROS), such as H_2O_2, OH^- and O_2^- (Mates, 2000). Larrigaudiere et al. (2004) analyzed that ethylene was involved in ROS production. During senescence, there is an overproduction of free radicals that may cause damage and consequently cell death. In our study a low level of O_2^- and H_2O_2 was recorded in 0.5 mg L^{-1} (Table 1). The decreases in O_2^- and H_2O_2 contents in 1-MCP sprayed plants may be due to lower levels of ethylene production and scavenging of O_2^- and H_2O_2 by SOD and POD enzymes (Larrigaudiere et al., 2004). This study also showed that the 1-MCP-treated flowers had significantly higher SOD, CAT, and POD activities compared with the control, AOA, and BA treatments (Table 1), which is in accordance with the findings of Djanaguiraman et al. (2011) and Wang et al. (2009). Application of 1-MCP could inhibit probable loss of membrane integrity (Yuan et al., 2010), therefore lipid peroxidation could be regulated by ethylene.

Color fading and discoloration are important factors in determining visual quality of flowers and in many cases they are the main reasons for determination of post-production quality (Amarjit, 2000). The major types of pigments contributing to the color of the flowers are carotenoids and anthocyanins (Amarjit, 2000). The improvement of petal color expression is at least partially due to the increase in anthocyanin contents. Ethylene has been known to cause petal color fading. In the present study, treatments with 20 or 30 mg L^{-1} BA, 100 or 150 mg L^{-1} AOA and 70 or 140 ppb 1-MCP reduced ethylene production in flowers. In some cases, anthocyanin degradation happens due to changes in the vacuoles that decrease the stability of the pigments and cause the chemical degradation of the anthocyanin, which results in senescence process (Hershkovits et al., 2005). Pre-treatment with 1-MCP could

Table 1. Activities of superoxide dismutase, peroxidase, catalase, and H_2O_2 and O_2^- concentrations in petals of potted carnation plants treated with benzyladenine at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene at 70 and 140 ppb.

Treatments	SOD (U mg Pro^{-1})	POD (nM min^{-1} mg Pro^{-1})	CAT (nM min^{-1} mg Pro^{-1})	H_2O_2 (nmol g^{-1} FW)	O_2^- (ΔOD min^{-1} g^{-1} FW)
Control	9.66	3.92	0.76	14.07	1.15
BA 10	9.00	3.85	0.80	14.05	1.15
BA 20	14.00	4.12	0.81	13.81	1.13
BA 30	17.00	11.00	0.89	13.51	1.08
AOA 50	10.00	3.06	0.79	14.07	1.16
AOA 100	14.00	6.80	0.89	13.50	1.08
AOA 150	20.00	9.38	0.93	13.21	1.05
1-MCP 70	29.25	13.75	1.22	12.82	1.00
1-MCP 140	24.00	11.51	0.96	13.11	1.04
LSD (0.05)	1.71	1.49	0.13	0.08	0.01

SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; BA: benzyadenine; AOA: aminooxyacetic acid; 1-MCP: 1-methylcyclopropene; LSD: least significant difference.

reduce the damage of membrane in fresh product, which is an important factor involved in retaining bract discoloration (Hershkovits et al., 2005).

This study on antioxidant metabolism of potted carnation petals can be understood not only as experimental evidence confirming the hypothesis of a link between ethylene and free radicals generation in senescence, but also as a key to the development of adequate methods to prevent or delay deterioration in potted flowers.

Therefore, it could be concluded that AOA, BA (at high concentration), and 1-MCP treatments may be good candidates for extending plant longevity, maintaining the visual quality of flowers in potted carnation plants. The treatment with AOA (100 and 150 mg L^{-1}), BA (20 or 30 mg L^{-1}), and 1-MCP prevented the increase in the ethylene, O_2^-, and H_2O_2 production and increased the antioxidant enzyme activity measured in petals.

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