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Change in ethylene production and ACC content of potted carnation in response to anti-ethylene treatments

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Abstract

The quality of potted carnations (*Dianthus caryophyllus* L.) is often lowered during transportation and by indoor environmental conditions. We tested the ability of aminooxyacetic acid (AOA), benzyladenine (BA), and 1-methylcyclopropene (1-MCP) to improve post-production flower quality of potted carnation plants. Maximum plant longevity (15 days) was obtained using 0.6 µl $L^{-1}$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 30 mg L^{-1}, and 1-methylcyclopropene at 0.6 or 1.2 µl L^{-1}**.** A significant increase in ACC content was observed in 1-MCP treated potted plants compared with the control. However, the decline in 1-aminocyclopropane-1-carboxylic acid (ACC) content was observed after using 100 or 150 mg L^{-1} AOA. A significant increase in Superoxide dismutase, Catalase and peroxidase enzyme activities were noticed when plants were treated 0.6 mg L^{-1} 1-MCP. Use of 1-methylcyclopropene (0.6 or 1.2 µl L^{-1}), aminooxyacetic acid (100 or 150 mg L^{-1}), and benzyladenine (30 mg L^{-1}) significantly decreased H_2O_2 concentration and superoxide radical.

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Introduction

Carnations (*Dianthus caryophyllus* L.) are used as ornamental plants in the form of potted plants as well as in the form of cut flowers. Potted carnations usually grow in a dwarf form. Potted carnations as a house plant are fairly new to the market. The quality of this plant 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). One ethylene action inhibitor commercialized is 1-Methylcyclopropene, which has been extensively evaluated for protecting ornamentals from ethylene damage (Serek *et al.,* 1994). 1-MCP decreased ethylene production in the flowers of cut spray carnation (Karimi *et al.*, 2012). AOA is a well-known ethylene biosynthesis inhibitor and blocks the ACC synthase activity (Mensuali-Sodi *et al.*, 2005). AOA is used for preserving flowers sensitive to ethylene (Rattanawisalanona *et al.*, 2003). Cytokinins (such as benzyladenine, BA) have been particularly effective in delaying senescence of carnation flowers by inhibiting ethylene biosynthesis (Cook *et al.*, 1985). Karimi and Hassanpour Asil (2010) reported that treating *Eustoma* cut flowers with 50 mg L^{-1} BA in combination with 3% sucrose delayed flower senescence and ethylene production. (Hassanpour Asil and Karimi, 2010). Plants use a diverse array of antioxidative enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT), in addition to low molecular weight antioxidants, such as reduced glutathione and ascorbic acid. These agents scavenge different types of reactive oxygen intermediates, thereby protecting cells against injury and potential damage (Mittler, 2002). The objective of this study was to investigate the effect of 1-MCP, AOA and BA on ethylene production and antioxidant metabolism in potted carnation 'Lilac on purple', 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. cv. Lilac on purple) 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 soil: peat: perlite (1:1:1, 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 0.6 or 1.2 µl L^{-1}. 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 ACC

2 g of crushed, frozen tissue (petal) was homogenized in 4 ml of 5% sulfosalicylic acid (SSA) solution, and centrifuged for 10 min at 3,090 × g in a pre-cooled centrifuge at 4 °C. ACC was assayed essentially as described by Bulens *et al.* (2011). Briefly, 0.4 ml of 10 mM $HgCl_2$ was added to 1.4 ml of extract in a 9 ml vial, which 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 vortexed for 5 seconds and allowed to react for 4 min. on melting ice. The sample was vortexed again for 5 seconds to release all ethylene into the vial headspace. Following the second vortexing, 1 ml gas sample was removed for ethylene determination by gas chromatography.

Measurement of Chlorophyll Content

For chlorophyll measurement, six leaf disks, each 6.25 mm in diameter, were punched from the same general area of the leaf. The disks were placed immediately into 8 mL of 100% methanol, and pigments were allowed to be extracted in the dark at 30°C for 24 h. Absorbance of the extract was measured using spectrophotometer (6405 UV/ Vis, JENWAY, ENGLAND).at 652 and 665 nm (Porra *et al.*, 1989).

Enzyme assays

Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase 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 is defined as the amount of enzyme that inhibits the control rate by 50% (0.025 units of absorbance at 550 nm min^{-1}) (McCord and Fridovich, 1969).

Peroxidase (POX; 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 rpm for 20 min. 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).

Catalase (CAT; EC 1. 11.1.6)

For catalase assay, the reaction mixture contained 15 mM H_2O_2, up to 100 µl of homogenate (7 mg protein mL^{-1}) with 0.2% (v/v) Triton X-100 in 50 mM potassium phosphate buffer (pH 7.0) (Aebi, 1983). All enzyme activities and protein concentration were quantified spectrophotometrically.

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) TiCl4 in 12.1 M HCl and 0.2 mL 17 M ammonia solution. The solution was centrifuged at 3,000 *g* at 4°C 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*. 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).

Experimental design and statistical analysis

This experiment was conducted in a completely randomized design with four replications. Results were analyzed using SAS software. Mean comparisons to identify significant difference between treatments were performed using least significant difference (LSD).

Results

Flower longevity

The longevity of potted plant is presented in Table 1. Treatment with 1-MCP (at all concentrations), AOA (100 or 150 mg L^{-1}) and BA (30 mg L^{-1}) significantly extended flower longevity. The use of 0.6 µl L^{-1} 1-MCP resulted in a greater extension in flower longevity (15 days) than the other treatments.

Chlorophyll Content

Both treatments 1-MCP and 150 mg L^{-1} AOA delayed the chlorophyll degradation of leaves as compared with the other treatments. The plants treated with 10 or 20 mg L^{-1} BA and 50 mg L^{-1} AOA showed the lowest chlorophyll content (Tab. 1).

Table 1. Plant longevity, Chlorophyll content, Ethylene production and ACC content 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 0.6 and 1.2 µl L^{-1}.

Treatments	Flower longevity (Days)	Chlorophyll content (µg ml^{-1})	Ethylene production (nl g^{-1}FW h^{-1})	ACC (nM g^{-1}FW)
Control	9.00^{d*}	8.00^{d}	48.92^{a}	5.76^{c}
BA 10	9.00^{d}	8.11^{d}	52.85^{a}	5.80^{c}
BA 20	9.00^{d}	9.50^{d}	47.12^{a}	5.81^{c}
BA 30	11.50^{c}	13.10^{bc}	35.00^{b}	5.00^{c}
AOA 50	8.50^{de}	8.17^{d}	48.06^{a}	4.98^{d}
AOA 100	10.50^{cd}	14.25^{bc}	33.80^{b}	1.89^{e}
AOA 150	13.00^{b}	19.43^{a}	13.38^{c}	0.50^{f}
1-MCP 0.6	15.00^{a}	20.25^{a}	8.75^{d}	8.22^{a}
1-MCP 1.2	13.00^{b}	19.00^{a}	12.51^{c}	6.67^{b}

*Means within each column followed by different letters are significantly different (p = 0.05).

Effect of 1-methylcyclopropene, aminooxyacetic acid, and benzyladenine on ethylene production

The treatments with AOA (100 or 150 mg L^{-1}), BA (30 mg L^{-1}), and 1-MCP (0.6 or 1.2 µl L^{-1}) significantly inhibited the flower ethylene production (Tab. 1). Evaluation of the regression relationship between plant longevity and ethylene production showed a significant negative association between ethylene production and flower longevity, i.e., plant longevity decreases as ethylene production increases (Fig. 1).

Changes in 1-aminocyclopropane-1-carboxylic-acid (ACC) content of potted carnation

The maximum ACC content was observed in the treatment with 0.6 µl L^{-1}1-MCP (8.22 nmol g^{-1} FW). In contrast, application of AOA in high concentrations significantly decreased the ACC content in the flowers compared with 1-MCP and BA, and the untreated control (Tab.1).

Effect of 1-methylcyclopropene, aminooxyacetic acid, and benzyladenine on antoxidant metabolism

Significantly higher activities of SOD, CAT and POD were observed in petals when potted carnations were treated with 1-MCP. Moreover, SOD, CAT and POD activities were significantly higher in the AOA (100 or 150 mg l-1) and BA (30 mg L^{-1}) treatments compared with the untreated plants (Fig. 2). The application of 1-MCP (0.6 or 1.2 µl L^{-1}), AOA (100 or 150 mg L^{-1}) and BA (30 mg L^{-1}) significantly decreased H_2O_2 content and superoxide radical ($O_2^{\bullet-}$) compared with the untreated control (Figs. 3, 4). The lowest H_2O_2 and

$O_2^{\bullet-}$ contents were obtained in the treatment with 0.6 µl L^{-1}1-MCP.

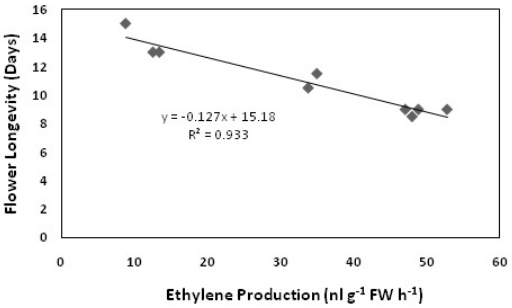

Fig. 1. Regression relationship between ethylene production and longevity of potted carnation plants.

Discussion

Treatment of flowers with 1-MCP, AOA and BA is found to be beneficial in delaying senescence processes but the response to these applications varies depending on flower type, cultivar, and stage of flower development and concentration of treatment.

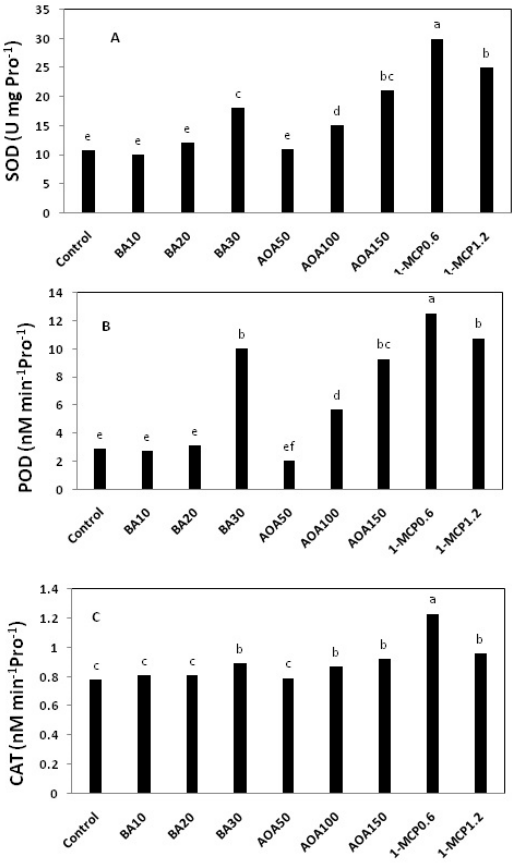

Fig. 2. Changes in the level of SOD (A), POD (B) and CAT (C) activity in the petals of potted carnation plants treated with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 0.6 and 1.2 µl L^{-1}. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

The quality of potted carnations is often lowered during transportation and by indoor environmental conditions. Ethylene is the major coordinator of senescence in many flowers. Ethylene causes premature wilting, color fading, abscission of flower petals and leaf yellowing (Cameron and Reid, 2001; Celikel *et al.*, 2002). The present study clearly indicated that treatment with 1-MCP (0.6 or 1.2 µl L^{-1}), AOA (100 or 150 mg L^{-1}) and BA (30 mg L-1) decreased ethylene production in flowers of potted carnation cv. Lilac on purple (Tab. 1). 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.

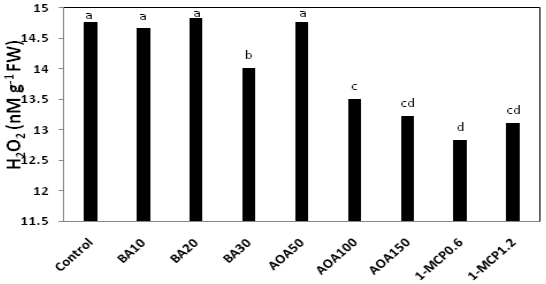

Fig. 3. Changes in H_2O_2 content activity in the petals of potted carnation plants treated with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 0.6 and 1.2 µl L^{-1}. Diversified letters indicate significant differences ($p<0.05$) among means (n=4).

Extending the flower longevity of the potted carnation using 1-MCP could be attributed to the role of 1-MCP as an inhibitor of ethylene biosynthesis as well as ethylene binding (Serek and Sisler, 2001; Serek *et al.*, 1994). Although 1-MCP molecules bind irreversibly to most ethylene receptors after pretreatment, new ethylene receptors can be synthesized during further plant development (Serek and Sisler, 2001). 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 'Lilac on purple' plants pre-treated with 1-MCP (especially at the concentration of 0.6 µl L^{-1}) 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. AOA is a well-known inhibitor of ACC synthase (Mensuali-Sodi *et al.*, 2005). Yu *et al.* (1979) reported that AOA inhibits the activity of ACC synthase by complexing with the essential co-factor, pyridoxal phosphate.

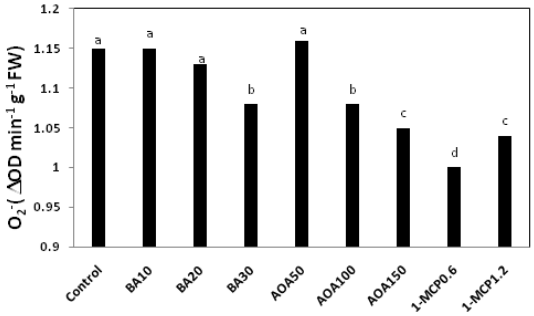

Fig. 4. Changes in $O_2^{\cdot-}$ content activity in the petals of potted carnation plants treated with different concentrations of benzyladenine (BA) at 10, 20, and 30 mg L^{-1}, aminooxyacetic acid (AOA) at 50, 100, and 150 mg L^{-1}, and 1-methylcyclopropene (1-MCP) at 0.6 and 1.2 µl L^{-1}. Diversified 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, $OH^{\cdot}$ and $O_2^{\cdot-}$ (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 $O_2^{\cdot-}$ and H_2O_2 was recorded in 0.6 µl L^{-1} 1-MCP (Figs. 1, 2). The decreases in $O_2^{\cdot-}$ and H_2O_2 contents in 1-MCP sprayed plants may be due to lower levels of ethylene production and scavenging of $O_2^{\cdot-}$ 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 (Fig. 2), which is in accordance with the findings of Karimi *et al.* 2012, 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.

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 (30 mg L^{-1}), and 1-MCP prevented the increase in the ethylene, $O_2^{\cdot-}$, and H_2O_2 production and increased the antioxidant enzyme activity measured in petals.

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