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Effects of 1-MCP and Ethylene on Antioxidant Enzymes Activity and Postharvest Physio-Biochemical Characteristics of Cut Carnation Flower cv. 'Fortune'

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Abstract

Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers in the world. The majority of the carnation cultivars are sensitive to ethylene which affected the physiological and biochemical postharvest characteristics of these flowers. Applying inhibitors of biosynthesis and action of ethylene is important factor to protect the display quality and extend postharvest life. In order to evaluate the effects of 1-methylcyclopropene (1-MCP) and ethylene on antioxidant enzymes activity of cut carnation cv. Fortune and subsequently on extending the vase life, this experiment was designed in Completely Randomized Design (CRD) with 3 replications. Carnation cut flowers were firstly treated with 1-MCP at concentrations of 0, 0.5, 1 and 1.5 µl/l for 24 h and subsequently exposed to ethylene (1 µl/l) for 16 h. Data were analyzed using MSTAT-C statistical software and means were compared based on Least Significant Differences (LSD) test ($p < 0.01$). Our results showed that 1-MCP treatment had significant effects on vase life and biochemical characteristics like contents of leaf chlorophyll, petal anthocyanin, petal cell membrane stability and antioxidant enzymes activity such as catalase, peroxidase, and superoxide dismutase. The highest vase life and cell membrane stability were appeared in 1.5 µl/l 1-MCP which was significantly higher than 0 and 0.5 µl/l, although there was no significant difference with 1 µl. The highest chlorophyll and anthocyanin contents were also measured under 1.5 µl/l 1-MCP which was significantly higher than other treatments. The highest and lowest catalase and peroxidase activity were related to 1 and 0 µl/l 1-MCP, respectively. The highest and lowest superoxide dismutase activity was observed in 1.5 and 0 µl/l 1-MCP. In conclusion, application of 1-MCP improved and delayed the onset of senescence symptoms resulted in extending the vase life of cut carnation cv. Fortune.

Keywords: Ethylene, Peroxidase enzyme, Postharvest longevity, Senescence, Superoxide dismutase.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) flowers as one of the most important cut flowers are commercially cultivated in the world and they are also used as ornamental plant for decorative purposes in orchards and landscape (Singh *et al.,* 2005). Vase life of cut flowers is of the most important characteristics in crop quality evaluation, which depends on genetic and environmental factors (Seglie *et al.*, 2011).

Ethylene as a gaseous plant hormone plays prominent role in acceleration of senescence phenomena of most plant organs such as fruits, flowers and floral buds which, concomitantly with increasing in endogenous ethylene production (Yang and Hoffman, 1984). When plants produce ethylene or are exposed to external source of ethylene, receptors perceive ethylene and then signal is transferred by downstream active genes. So, ethylene induces gene expression and physiological characteristics affecting vegetative and reproductive organs are appeared (Ahmadi *et al.*, 2008). It has been detected that exogenous ethylene in some plants like miniature roses, geranium and begonia has undesirable effect on flower quality and results in accelerating senescence and decreasing flower life (Ahmadi *et al.,* 2009; Seglie *et al.*, 2011). Application of external ethylene in rose petals causes considerable increase in ethylene production and activity of ACC-synthase and ACC-oxidase genes (Ma *et al*., 2006; Ahmadi *et al.,* 2009), although ethylene receptor genes was not affected by external ethylene (Ahmadi *et al.*, 2009).

Senescence is an oxidative process in which reactive oxygen species and antioxidants play role. In general, reactive oxygen species accelerate flower senescence by increasing cell membrane permeability, due to decreasing proteins and nucleic acids in result of different protease and nuclease enzymes activities (Barth *et al.,* 2006). Decreasing antioxidant enzymes activity and increasing peroxidation of cell membrane lipids have been indicated as possible reasons of senescence in different plant species (Buchanan-Wollaston, 1997). In addition, cell death is accelerated in this phase because of boosting ethylene production during senescence period (Ebeles *et al.,* 1992). Plants gain from antioxidant mechanisms to alleviate the effects of free radicals. These mechanisms include changes in content of defense-related enzymes such as peroxidase, catalase, polyphenol oxidase and other compounds like phenols (Staskawicz *et al.*, 1995).

Since post-harvest senescence is an important restricting factor in crop presentation and marketability of many cut-flowers, using high confidence methods to delay crop senescence is of great importance. 1-MCP as an anti-ethylene compound has been proved to be effective on inhibiting ethylene response by competing with ethylene for bonding with ethylene receptors (Seglie *et al.*, 2011; Daneshi Nergi and Ahmadi, 2014.). Studies have shown that 1-MCP has inhibited the phenomena of petal falling in geranium, considering that its effectiveness depends on transport conditions, storage temperature and application times (Cameron and Reid, 2001). Study on cut carnation showed that all concentrations of 1-MCP decreased ethylene production and chlorophyll destruction delayed in comparison with control plants (Asil *et al*., 2013). Black tulip flowers treated with 1-MCP for 8 hours showed maximum anthocyanin till 12th day of vase life (Chutichudet *et al.*, 2010 b). Application of 1-MCP on soybean plants decreased hydrogen peroxide in comparison with untreated plants as well as production of ethylene and free radicals but increased the activity of antioxidant enzymes (Djanaguiraman *et al*., 2011). Considering the role of 1-MCP as an ethylene inhibitor, the purpose of current study was the evaluation of 1-MCP in extending display quality 'Fortune' cut carnation. To gain a deep understanding of biochemical characteristics of cut-flowers, enzyme assays have been performed.

MATERIALS AND METHODS

Carnation cut-flowers of 'Fortune' cultivar were harvested from commercial greenhouses in Pakdasht town of Iran, according to standard indexes. Flowers were immediately transferred to laboratory of post harvest physiology of Horticulture Department, Faculty of Agriculture, Tarbiat Modares University. Then, healthy and uniform flowers were selected for considered treatments.

Cut-flowers placed in the vase solution were treated with 1-MCP (0, 0.5, 1 and 1.5 µl/l) for 24 h in 200 L glass aquarium chambers. After an hour, the aquariums lids were re-sealed and ethylene was injected inside each chambers using Hamilton syringe, to expose cut flowers to 1 µl/l ethylene for 16 h (Daneshi Nergi and Ahmadi, 2014). After finishing ethylene treatment, the lids of the glass chambers were opened and the vases were placed on the bench lab. Vase life room conditions: temperature of 20 ± 2 °C, relative humidity of 60-65%, light intensity of 15 µmol/m^2s^{-1} and 12 h light and 12 h darkness (Daneshi Nergi and Ahmadi, 2014). This experiment was conducted in a completely randomized design with 3 replications and 4 treatments. Sampling was carried out for evaluation of physiological and biochemical characteristics at desired time and data were analysed using MSTAT-C statistical software and means were compared according to the Least Significant Differences (LSD) test ($P < 0.01$) and graphs were designed using Excel software.

Vase life

In this study, vase life was considered as the time during which cut-flower can keep its marketability quality and before senescence symptoms including bending of petal margins and wilting are appeared (Singh, 1994). Cut-flower durability was evaluated from cut flower treatment till their ornamental value has disappeared.

Leaf chlorophyll analysis

For measuring chlorophyll, 0.5 g leaf samples were ground using a mortar and pestle with liquid nitrogen, dipped in 15 ml 80% acetone in test tubes and were centrifuged in 6000 rpm and 4 °C. After that the absorbance of the solutions were read against blank (solvent) at 663 and 646 nm using spectrophotometer (BIO-RAD) (Richardson *et al.*, 2002) and chlorophyll content was calculated as follows:

Chlorophyll a (µg ml) = $12/5A_{663}$ -$2/79A_{646}$

Chlorophyll b (µg ml) = $21/51A_{646}$ -$5/1A_{663}$

Total Chlorophyll (µg ml) = Chlorophyll a + Chlorophyll b

Petal anthocyanin

200 mg petal samples were pulverized in 3 ml 99:1 (v/v) methanol and hydrochloric acid and obtained extracts were centrifuged at 12000 rpm for 20 min at 4 °C. Supernatants were kept in 4 °C and under darkness condition for 24 h. After that, light absorption was estimated by spectrophotometer in 550 nm wavelength and using silence coefficient (ε =33000 mol^2 cm^{-1}) (Krizek *et al.,* 1993).

Petal membrane stability index

For determining petal membrane stability, two samples of petals each including 200 mg of each replication were weighted and dipped in 10 ml double distilled water. One of them was placed in 40 °C Benmary for 30 min and second one at 100 °C Benmary for 15 min. After reaching to the room temperature, electrical conductivity of the solutions was measured with a EC meter and the stability percent of the membrane was determined according Ezhilmathi *et al.*, 2007, as follow:

Membrane stability index (percent) = [1-(C1/C2)] × 100

Enzymes assays

A. Peroxidase (POD) enzyme

Peroxidase (POD) was extracted from 200 mg homogenized samples in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 28 mM Guaiacol, 5 mM hydrogen peroxide and crude extract was prepared and its absorbance at 470 nm was detected during 1 min,

using spectrophotometer (BIO-RAD). Enzyme activity was expressed as absorption delta of 470 nm per mg protein (Chance and Maehly, 1955).

B. Catalase (CAT) enzyme

Catalase (CAT) was extracted from 200 mg samples homogenized in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to enzyme extract. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 10 mM hydrogen peroxide and crude extract was prepared and its absorbance at 240 nm was detected using a spectrophotometer (BIO-RAD). Enzyme activity was described by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, as the decrease of absorbance per time per mg of protein (Cakmak and Horst, 1991). Enzyme activity was expressed as absorption delta of 240 nm per mg protein. All steps of enzyme extraction were performed on ice.

C. Superoxide dismutase (SOD) enzyme

200 mg plant tissues were extracted in 50 mM HEPES-KOH buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was transferred to centrifuge tubes and was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to enzyme extract. For assay, a mixture consisting of 50 mM HEPES-KOH (pH 7.8) containing 0.1 mM Na-EDTA, 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 µM Nitro Blue Tetrazolium (NBT), 1µM Riboflavin and crude extract was prepared and enzymatic extract as a unit of SOD activity was considered as enzymatic amount which resulted in 50 % inhibition of NBT in 560 nm (Chance and Maehly, 1955). Reaction mix absorption was measured by spectrophotometer.

Total soluble protein was measured using Bradford (1976) method. Absorption of 1 ml Bradford reagent along with 100 µl enzymatic extract were mixed completely and registered in 595 nm. Protein content was estimated using calibration curve of cow albumin serum (BSA) (Bradford, 1976).

RESULTS AND DISCUSSION

Vase life

Vase life is one of the important postharvest characteristics of ornamental plants especially for cut carnation, which is highly affected by ethylene. Reduction of unfavorable effects of ethylene is an appropriate method for enhancing postharvest durability of plants or plant organs and compounds like 1-MCP are used extensively in order to alleviate ethylene undesired effects in horticultural crops. Results showed that the effect of 1- MCP on characteristics cut carnation cv. Fortune was significant ($P < 0.01$). Increasing 1-MCP concentration enhanced vase life of cut flowers so that the highest vase life (11.8 days) was related to 1.5 µl/l 1-MCP which was significantly ($P < 0.01$) higher than control and 0.5 µl/l 1-MCP, while there was no significant ($P < 0.01$) difference with 1 µl/l 1-MCP. Treatment with 1-MCP by preventing external ethylene action increased vase life. In accordance with our results, Yamane *et al.*, (2004) showed that 1-MCP increased vase life of cattleya flowers. It seems that 1-MCP prevents from ACC-oxidase and ACC synthase expression, subsequently decreasing ethylene production in primary days of treatment with 1-MCP and so resulted in increasing vase life (Yamane *et al.*, 2004; In *et al*., 2013; Yang *et al*. 2013;). This is also in agreement with the results of Chutichudet *et al*., (2010 b) who reported that 1-MCP protects of tulip cut-flower quality by preventing of ethylene production (Chutichudet *et*

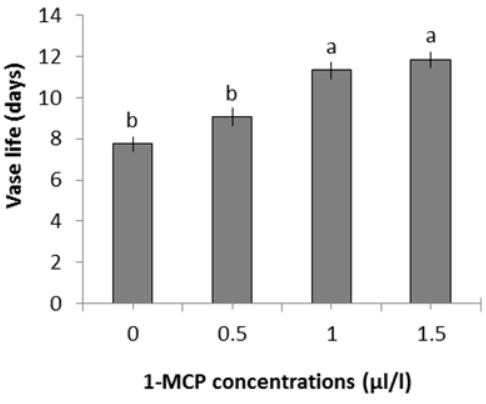

Fig. 1. Effect of 1-MCP on vase life of carnation cv. Fortune.

al., 2010b). Hence, increasing vase life of cut-flowers treated with 1-MCP is related to the inhibiting ethylene action and also ethylene biosynthesis (Serek *et al.*, 1994; Serek and Sisler, 2001).

Membrain stability index

The 1-MCP treatment preserved membrane stability in cut carnation, so that the highest membrane stability in seventh day after treatment was related to 1.5 µl/l 1-MCP with no significant ($P < 0.01$) difference with 1 µl/l but it was significantly ($P < 0.01$) higher than control and 0.5 µl/l 1-MCP. 1-MCP can prevent membrane degradation by decreasing the lipid peroxidation which is regulated by ethylene (Yuan *et al*., 2010). Since ethylene is the main factor of increasing respiration rate in climacteric crops and causes accelerating physical characteristics changes and cell membrane phospholipids degradation, it seems that 1-MCP protects membrane stability by preventing of ethylene action.

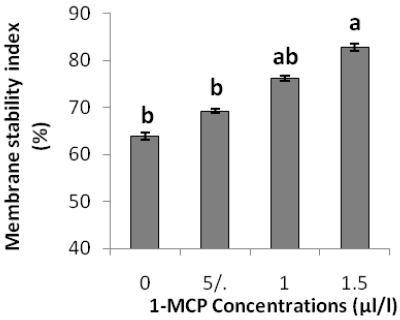

Fig. 2. Effect of 1-MCP on petal membrane stability of carnation cv. Fortune.

Chlorophyll content

Based on the results, the highest chlorophyll content was observed in seventh day after treatment measured in 1.5 µl/l 1-MCP ($P < 0.01$). The effect of 1-MCP on protection of chlorophyll content is a result of ethylene action and consequently inhibition of ethylene biosynthesis, which is considered as the most important factor of leaf chlorosis in ornamental plants. In accordance of the present results, 1-MCP treatment in all concentrations decreased ethylene biosynthesis which followed by reduction of chlorophyll destruction compared with control plants (Asil *et al.*, 2013). According to Serek *et al*. (1998), 1-MCP inhibited of leaf chlorosis in chrysanthemum and geranium cv. Isable (Serek *et al.,* 1998). In this case, the effect of 1-MCP was attributed to binding ethylene receptors. Recent studies show that 1- MCP can increased shoot capacity for carbon assimilation by the inhibition of acid Tri-carboxylic cycle and stimulates the biosynthesis of gibberellin (Wang *et al.,* 2014).

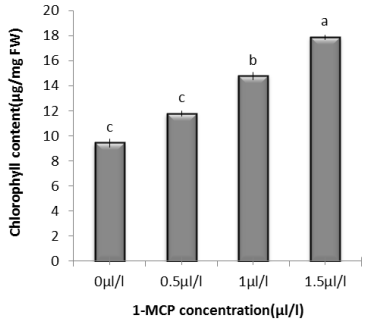

Fig.3. Effect of 1-MCP on petal chlorophyll content of carnation cv. Fortune

Anthocyanin content

Our results showed that the highest anthocyanin content in seventh day after treatment was related to 1.5 µl/l 1-MCP with significant ($P < 0.01$) difference compared to other treatments. Positive effects of 1-MCP on inhibition of external ethylene action, delaying senescence and accordingly protection of suitable cell pH were the factor of anthocyanin photosynthesis pigments. Chutichudet *et al*. (2010 b) found that black tulip cut flowers treated with 300 ppb 1-MCP for 8 h showed the highest anthocyanin till 12th days of vase life (Chutichudet *et al.*, 2010 b). Usually, postharvest destruction of anthocyanin pigments is as a result of bracteoles, membrane function destruction (Jiang and Chen 1995; Jiang *et al.,* 2004). Stability of anthocyanin may be owing to the role of 1-MCP, which can decrease membrane destruction of fresh crops (Hershkovitz *et al.,* 2005). Vacuole pH enhances during senescence and anthocyanin gets free of color

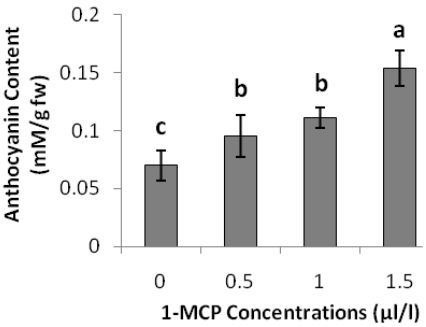

Fig. 4. Effect of 1-MCP on petal anthocyanin content of carnation cv. Fortune.

before destruction (Zhang *et al.*, 2001). As well, anthocyanin destruction occurs by polyphenol oxidase activity (Francis, 1989). Ethylene significantly prevent the accumulation of anthocyanins in tissues. Research on Arabidopsis thaliana showed that among ethylene, carbohydrates and anthocyanins, there is a negative self-regulation system. Ethylene stress by reducing the absorption of carbohydrates, reduces the accumulation of pigments (Das *et al.*, 2011).

Antioxidant enzymes

The highest activity of catalase was found in the flower treated with 1 µl/l 1-MCP, which was not significantly ($P < 0.01$) higher than 1.5 µl/l while it was significantly ($P < 0.01$). higher than control and 0.5 µl/l 1-MCP treatments. The highest activity of peroxidase detected in 1 µl/l 1-MCP with significant ($P < 0.01$) difference compared to other treatments. The highest activity of superoxide dismutase was found in samples treated with 1.5 µl/l 1-MCP which was significantly higher than 0 and 0.5 treatments, although there was no significant difference with 1 µl treatment. 1-MCP protected antioxidant enzymes activity by inhibition of ethylene action and biosynthesis and finally prevention of respiration and in result temperature increase made favorable temperature conditions for plant enzyme activity. Petal senescence process causes metabolic and physiological changes, which result to the petal death. Senescence begins with the expression of a set of genes related to senescence and it is emerged in metabolic level as oxidative processes and often catabolic processes in senescence increase as irreversible (Buchanan-Wollaston, 1997).

In microsomal membrane of carnation, lots of superoxides are generated during senescence (Mayak *et al.,* 1983). Here also, increasing free radicals from ethylene stress in plants untreated with (0 µl/1) 1-MCP damaged to immunity system of antioxidant and reduction of immunity enzymes activity. While in treatment with different concentrations of 1-MCP, there was more antioxidant enzyme's activity because of decreasing effects of external ethylene. Peroxidase has different biological functions such as detoxification of hydrogen peroxide, lignin biosynthesis, hormonal signaling and response to stress (Gao *et al.*, 2010).

Catalase is considered as an important biological factor that its major function is in the process of superoxide metabolism and plays an important role in releasing oxygen and hydrogen peroxide free radicals and preventing creation of hydroxyl radicals (Spanou *et al.*, 2012). Superoxide dismutase like Cu-Zn superoxide dismutase, Mn superoxide dismutase and outside cell superoxide dismutase play a critical role in inhibition of superoxide (Miao and Clair, 2009). In fact, peroxidase, catalase and superoxide dismutase play role in protection of metabolism balance of oxygen in plant tissue (Xie *et al*., 2003). Superoxide causes lipid peroxidation, cell membrane damage and finally senescence; 1-MCP can affect enzyme activities, which remove superoxide (Li *et al.,* 2007). In accordance with our obtained results, increasing antioxidant enzymes (catalase, superoxide dismutase and peroxidase) activity of gladiolus, florets treated with 1-MCP has been reported. It seems that this treatment decreases oxidative stresses in cut-flowers (Hassan and Ali, 2014). In other words, activity of these enzymes is a factor for the protection of cells against oxidative stresses (Zhou *et al*., 2014).

It should be mentioned that even if ethylene decreases in response to 1-MCP, the activity of antioxidant enzymes will increase. As well, considerable increase in peroxidase, catalase and superoxide dismutase activity was observed in petals of carnation flower cv. Lilacon Purple treated with 0.5 µl/l 1-MCP. The 1-MCP decreased hydrogen peroxide and superoxide (peroxide anion) compared to control plants (Karimi, 2014), this decreasing may be in result of low ethylene biosynthesis and inhibition of hydrogen peroxide and peroxide anion by peroxide and superoxide dismutase enzymes (Larrigaudiere *et al*., 2004). In asparagus, 1-MCP hindered the ethylene signal transduction and resulted in delaying by affecting on ethylene biosynthesis, superoxide dismutase and catalase activities (Zhang *et al.*, 2012).

CONCLUSION

In conclusion, 1-MCP prevented the effects of exogenous ethylene and alleviates the stress conditions induced by ethylene in cut carnation flowers. It seems that 1-MCP treatment increased capacity of tissue/organ by boosting the activity of antioxidant enzymes resulted in favorable physiological and biochemical organ activities.

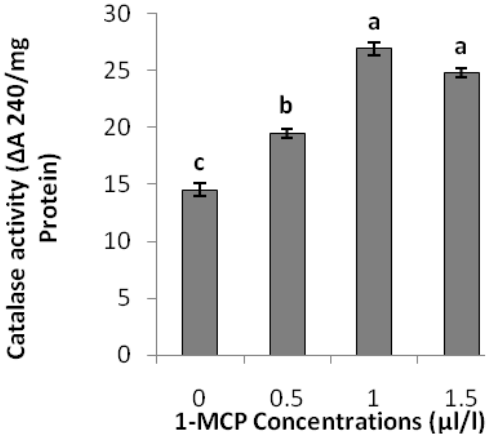

Fig. 5. Effect of 1-MCP on petal catalase enzyme activity of carnation cv. Fortune.

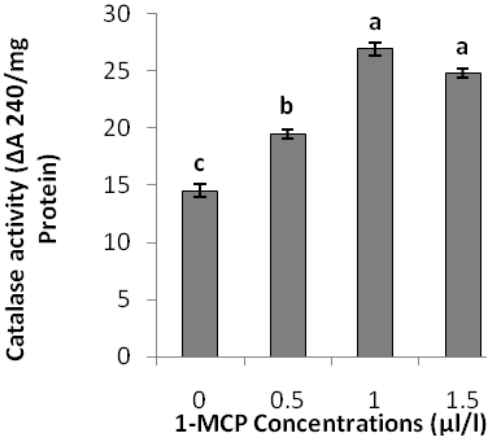

Fig. 6. Effect of 1-MCP on petal peroxidase enzyme of carnation cv. Fortune.

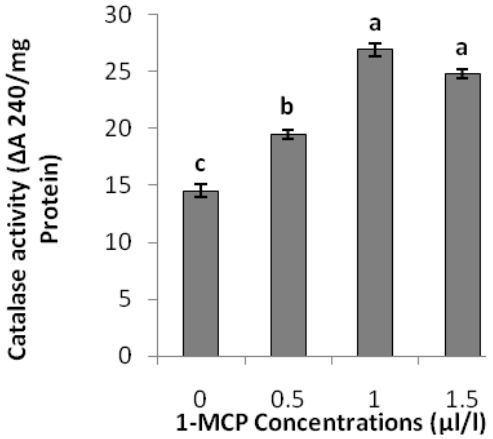

Fig. 7. Effect of 1-MCP on petal superoxide dismutase enzyme of carnation cv. Fortune.

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