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Effect of Salicylic Acid, Malic Acid, Citric Acid and Sucrose on Antioxidant Activity, Membrane Stability and ACC-oxidase Activity in Relation to Vase Life of Carnation Cut Flowers

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ABSTRACT

Short postharvest vase life is one of the most important problems on the cut flowers. Combinations of malic acid, salicylic acid, citric acid and sucrose were used as preservative mixture for cut carnations and their effect on regulation of senescence was examined. The study was conducted in a factorial arrangement, carried out in a complete randomized design. The factors were malic acid (0,100 and 150 mg L^{-1}), salicylic acid (0, 1.5 and 3 mM), citric acid (0 and 150 mg L^{-1}) and sucrose (0 and 3% w/v). The effects of treatments and their interaction on the total chlorophyll content, ACC-oxidase activity, anthocyanin leakage, membrane stability and malondialdehyde content of cut flowers of carnations (*Dianthus caryophyllus* L. cv. White) were investigated. 150 mg L^{-1} MA and 1.5 mM SA both caused significant decrease in anthocyanin leakage, ACO activity and MDA content compared to other levels ($p \leq 0.05$). Flower stems that were kept in water containing either 150 mg L^{-1} MA or 1.5 mM SA or their combinations; all had significantly increased vase life relative to the control treatment. MA application increased water uptake and decreased microbial growth as well.

Key words: Carnation, membrane stability, vase life, malondialdehyde, ACC-oxidase activity

INTRODUCTION

Carnation is a climacteric flower that is highly sensitive to ethylene (Reid and Wu, 1992; Pun *et al*., 1999; Da Silva, 2003). Ethylene is produced autocatalytically during carnation petal senescence. During the climacteric respiration, there is a coordinated increase in the activities of ACS and ACO, which convert S-adenosylmethionine (SAM) to 1-aminocyclopropane-carboxylic acid (ACC) and ACC to ethylene, respectively (Yang and Hoffman, 1984). Expression of the ACS and ACO genes in carnation petals depends on the presence of ethylene (Savin *et al*., 1995). Senescence of flower petals is characterized by nonreversible cellular processes leading to death. Ethylene production increases sharply with senescence. Ethylene increased flower senescence and degradation of membrane lipids and production causes ROS (oxygen free radicals) and MDA (Liu *et al*., 1987; Allen, 1995; Mittler, 2002; Da Silva, 2003; Shakirova, 2007; Reezi *et al*., 2009; Kazemi *et al*., 2010). During senescence, MDA accumulates rapidly as the product of peroxidation of membrane lipids, (Hernandez *et al*., 1993; Lutts *et al*., 1996; Fadzilla *et al*., 1997), which results in an increase in permeability of plasma membranes. The peroxidation process may be retarded by using ethylene inhibitors, which could delay flower

senescence in many sensitive cut flowers (Serek and Sisler, 2001). Inhibitors of ethylene biosynthesis such SA reduced cut flowers senescence, ACO activity and ROS with increasing antioxidant enzyme activity (Li *et al*., 1992; Srivastava and Dwivedi, 2000; Khan *et al*., 2003; El-Tayeb *et al*., 2006; Ansari and Misra, 2007; Mba *et al*., 2007; Mahdavian *et al*., 2007; Canakci, 2008; Shi and Zhu, 2008; Karlidag *et al*., 2009; Joseph *et al*., 2010). Fan *et al*. (2008) reported that SA decreasing ACO activity and ROS with increasing antioxidant enzyme activity. CA with reducing the vase solution pH reduced the bacterial growth (Nowak and Rudniki, 1990). SUC is another organic molecule known to delay senescence and prevents up-regulation of senescence-associated genes in carnation petals (Hoeberichts *et al*., 2007). Darandeh *et al*. (2010) reported that MA sprays during the growth period increased chlorophyll content of cut flowers while, CA spray caused extended post harvest vase life. Based on these results and use of CA in many floral preservative formulas as a water conductance aid, we considered testing MA in floral preservative mixture to investigate any potential positive effect (s). As, MA is readily metabolized by plants but not by many microorganisms, so we considered as using it as a possible substitute for SUC. Use of SUC necessitates the addition of biocidal agents, which is not considered an environment friendly method due to the side effects like facilitating the emergence of resistant strains of microorganisms to frequently used biocides. Therefore, In this study, the preservative effects of MA, SA, CA, SUC and their interaction on the vase life of cut carnation flowers were studied.

MATERIALS AND METHODS

Plant material and storage conditions: The experiment was started on February 15, 2010 and chlorophyll content, membrane stability, MDA content and ACC Oxidase activity were measured on the last day of vase life for each flower. Cut flowers (*Dianthus caryophyllus*) L. cv. White were harvested in open stage in the morning from a local commercial greenhouse (Pakdasht, Tehran, Iran) and transported with appropriate covers immediately to Laboratory (horticulture laboratory of agriculture faculty of Islamic Azad university, Karaj Branch). Stems were recut to 40 cm length. In this study, three levels of MA (0,100 and 150 mg L^{-1}), two levels of SUC (0 and 3% w/v), three levels of SA (0, 1.5 and 3 mM) and two levels of CA (0,150 mg L^{-1}) were applied on 144 carnation cut flowers cv. White. After recording the fresh weight, each flower was placed in a 250 mL bottle containing preservative solutions. The flowers were held at ambient temperature (19±5°C).

Vase life: Vase life was determined as the number of days to wilting of flowers. The flowers were checked once a day for signs of deterioration.

Chlorophyll index: Chlorophyll index was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan), which is presented by SPAD value. Average of 3 measurements from different spots of a single leave was considered.

Determination of anthocyanin leakage: Anthocyanin leakage was measured based on the method of Poovaiah (1979). Petal samples were cut into 1*1 cm segments and placed in individual tubes containing 25 mL of deionized water. After two washes with distilled water to remove surface contamination, 10 mL of distilled water was added to samples. After 12 h incubation at 25°C the anthocyanin leakage to liquid was determined at 525 nm using spectrophotometer (Perkin-Elmer-EZ-201).

Determination of ACC-oxidase (ACO) activity: The extraction and quantification was conducted based on the method described by of Moya-Leon *et al.* (2004) with slight modifications. One gram petal tissue was homogenized by a mortar and pestle with 3 mL extraction buffer consisting of 1% (w/v) polyvinyl polypyrrolidone, 0.1 mM tricine with (pH adjusted to 7.5), 10% glycerol, 5 mM DTT and 30 mM sodium ascorbate for 2 min. The homogenate was centrifuged at 20000 xg for 20 min and the supernatant was collected for enzyme assays. All procedures were conducted at 4°C.

After a 20 min incubation of the enzyme extract with the ACC containing enzyme activation complex [0.1 M tricine (pH 7.5), 30 mM sodium ascorbate, 0.1 mM ferrous sulfate, 10% (v/v) glycerol, 1 mM ACC, 2.5 mM DTT and 30 mM sodium bicarbonate], the ACO activity was assayed as the amount of evolved ethylene, which was quantified on a GC apparatus.

Assays of MDA content (lipid peroxidation): Malondialdehyde content was measured based on the method of Heath and Packer (1968) with some changes. Fresh petals tissue was homogenized with a mortar and pestle in 5 mL solution of 1% trichloroacetic acid. The homogenate was centrifuged at 10000 xg for 5 min. 4.5 mL TCA 20% solution containing 0.5% TBA acid was added to 1 mL of the supernatant and incubated for 30 min at 95°C water bath. The mixture was cooled immediately in ice and again centrifuged at 10000 xg for 10 the absorption was measured with a spectrophotometer at 532 nm.

Microbe population: In day 11, samples were isolated from vase solutions of carnations in sterile containers. Aliquots of the vase solutions were diluted 100-times and 25 μL aliquots of the diluted solution were spread on sterile nutrient agar in sterile petri plates. The plates were allowed to incubate for 48 h at room temperature and individual colonies of bacteria were counted.

Water absorption by cut flowers: The water uptake was calculated by subtracting the mean volume of water evaporated from three control bottle without cut flowers, from the amount of water decreased in bottles containing flowers in experimental course.

Experimental design and statistical analysis: Experiment was arranged in a factorial test with complete randomized design with four replications. Analysis of variance was performed on the data collected using the General Linear Model (GLM) procedure of the SPSS software) Version 16, IBM Inc.). The mean separation was conducted by Tukey analysis in the same software ($p \leq 0.05$).

RESULTS AND DISCUSSION

Anthocyanin leakage, ACO activity and MDA content: The results indicate that 150 mg L^{-1} MA and 1.5 mM SA both caused a significant decrease in anthocyanin leakage, ACO activity and MDA content compared to other levels ($p \leq 0.05$). CA and SUC caused no positive effect or had a negative effect as increasing anthocyanin leakage and ACO activity (Table 1). On the other side, highest means of ACO activity were found in cut flowers treated with 100 mg L^{-1} MA+3 mM SA and 3 mM SA (Table 1) ($p \leq 0.05$). The interaction between MA and SA on ACO activity was significant, as well. Kazemi *et al.* (2011a-b) reported that treatment with SA acid significantly extends the vase life. The results indicate that 150 mg L^{-1} MA and 1.5 mM SA and their combination caused a significant increase in SPAD value ($p \leq 0.05$). Application of SUC caused

Table 1: Effect of malic acid, salicylic acid, citric acid and sucrose combinations in preservative mixture on cut carnations

MA (mg L^{-1})	SA (mg L^{-1})	SUC (% w/v)	Vase life (day)	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h^{-1} mL^{-1})	Anthocyanin leakage (absorption at 525 nm)	MDA (µmol mg^{-1} protein)	Water uptake (mL $flower^{-1}$)	Colony count (CFU mL^{-1})
0	0	0	6.5	2.2	19.5	234.0	159.9	105.0	42.5
		30	5.0	0.7	35.2	334.5	199.4	75.0	68.5
	1.5	0	9.0	5.5	10.6	128.0	131.3	95.0	17.5
		30	8.5	4.3	12.7	173.0	143.6	97.5	32.0
	3	0	3.5	0.8	39.1	475.5	234.3	77.5	22.5
		30	4.5	1.1	35.2	407.5	197.4	75.0	32.5
100	0	0	6.0	1.4	24.4	252.5	191.0	102.5	18.5
		30	7.0	1.6	20.0	199.0	159.6	97.5	31.5
	1.5	0	7.0	2.1	15.9	191.0	140.6	97.5	17.0
		30	7.0	2.0	15.9	216.5	142.4	95.0	30.5
	3	0	4.5	0.9	35.6	456.0	221.2	82.5	14.5
		30	5.5	1.0	31.9	432.5	189.7	77.5	28.0
150	0	0	9.0	6.4	9.5	146.0	121.3	127.5	13.0
		30	8.5	3.9	12.1	179.0	117.6	115.0	20.5
	1.5	0	10.1	6.0	8.5	115.5	117.0	145.0	10.5
		30	10.5	5.5	8.8	116.5	116.2	127.5	19.0
	3	0	5.5	1.3	24.1	344.5	200.7	97.5	13.0
		30	6.0	0.8	27.1	290.5	180.9	95.0	20.0
F-test probabilities									
		MA	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		SA	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		SUC	0.414	0.040	0.354	0.970	0.183	0.036	0.000
		CA	0.053	0.282	0.424	0.078	0.242	0.007	0.064

Data were recorded in the last day of vase life, The citric acid, which was not effective on most variables, was omitted from first part to avoid an unnecessary complicated table, Means in each column followed by similar letters are not significantly different at 5% level, Bolded row is the control combination

a significant decrease in chlorophyll index while CA had no significant effect (Table 1). Kazemi *et al*. (2010) and Kazemi *et al*. (2011a-b) reported that treatment with Si, SA and MA significantly extends the vase life with reduced the MDA, Proline Content A and increasing Chlorophyll content and superoxide dismutase activity. Holding carnation cut flowers in vase solutions containing 150 mg L^{-1} MA significantly increased their vase life and delayed flower senescence compared to flowers either held in 100 mg L^{-1} MA or distilled water (Table 1). MA was found to be significantly and positively correlated with vase life of the carnation cut flowers as well. Flower stems which were kept in water containing either 150 mg L^{-1} MA or 1.5 mM SA or their combinations all had significantly increased vase life compared to the control treatment ($p \leq 0.05$, Table 1). Some factor-levels containing CA and SUC were among those with significantly higher vase life than control. Kazemi *et al*. (2011a-b) reported that treatment with salicylic acid significantly extends the vase life with reduced the Anthocyanin leakage and ACO activity. Kazemi *et al*. (2010) also reported that treatment with malic acid significantly extends the vase life with decrease ACC-oxidase activity. The 150 mg L^{-1} MA caused an increase in water uptake by flowers while 1.5 mM SA was not effective significantly. MA caused a linear reduction in the colony count, from 36 in 0 levels to 23 and 16 CFU mL^{-1} in 100 and 150 mg L^{-1} MA, respectively. Both 1.5 and 3 mM SA decreased the colony count significantly from 32 to around 21 CFU mL^{-1}. The 150 mg L^{-1} CA significantly increased water uptake by individual flowers 8 mL per flower but had no effect on colony count. SUC application caused decreased water uptake by

mean of 11 mL per flower and increased the colony count 12 CFU mL^{-1} ($p \leq 0.05$, Table 1). The effect of SA on senescence and vase life extension of cut flowers was reported earlier which is confirmed here was anticipated but the effect of MA on senescence indices, which is reported here for the first time, could be promising. 150 mg L^{-1} MA not only enhanced the senescence related variables like anthocyanin leakage, ACO activity, MDA content and chlorophyll index in par with SA but also reduced the bacterial load of vessel like SA. In addition, MA increased water uptake by cut flowers that was not noted by applied SA levels. MA did the well-known duty of CA as a water uptake-increasing agent in a better manner together with a large effect on senescence related factors, which makes it a suitable substitute for CA in preservative mixtures with a broad-spectrum effect. Presumably, the mode of action for these broad effects by MA may be due to its role as both a carbon source, which could fuel many carbohydrate dependent metabolic pathways and an important anion in cell vacuoles, which could help to sustain the turgor pressure and water uptake and balance. Being unusable for many of ordinary microorganisms growing in vase solutions makes it a possible candidate for substitution of SUC, as well.

CONCLUSION

Result of the present study, Result our showed that treatment with SA and MA extends the vase life of cut carnation flowers. Also, SA and MA reduced chlorophyll total degradation and preserved chlorophyll total content.

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