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Response of Carnation (*Dianthus caryophyllus* L.) to Salicylic Acid and Glutamine

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ABSTRACT

Short postharvest vase life is one of the most important problems on the cut flowers. The study was conducted to investigate the effect of salicylic acid at different concentrations on carnation flower longevity, Aminocyclopropanecarboxylate oxidase (ACC-oxidase, ACO) activity and SOD activity vase solution of cut flowers carnation in laboratory condition. The treatments were distilled water, salicylic acid (0,1.5, 2.5, 3.5 mM) and glutamine (0, 2 and 4 mM). Results show that salicylic acid 2.5 mM followed by glutamine 4 mM is more effective than salicylic acid 1.5, 3.5 and 4.5 mM alone in improving the vase life of cut carnation flowers. The percent of wilting was minimized as a result of using this combined treatment. However, the percent of wilting increased with the increase in concentrations of salicylic acid. 2.5 mM salicylic acid+4 mM glutamine retarded the chlorophyll as well as carbohydrate degradation during the postharvest life. The results showed that salicylic acid and glutamine in preservative mixture increased the SOD activity of cut flowers and reduced ACC-xidase activity, free proline accumulation, number of bacterial on vase solution, as compared to control.

Key words: Carnations, salicylic acid, glutamine, vase life, cut flower

INTRODUCTION

Carnation is very desirable for cut flower because of its wide range of colors and exceptional fragrance. senescence of the carnation petals increase with ethylene production during postharvest life (Da Silva, 2003; Kader, 2003). During the climacteric, there is a coordinate increase in the activities of ACC synthase and ACC oxidase (Have and Woltering, 1997; Yang and Hoffman, 1984). Expression of the ACC synthase and ACC oxidase genes in carnation petals depends on the presence of ethylene (Savin *et al*., 1995). Ethylene promoted flower senescence, increased respiratory activity and loss of cell membrane fluidity (Kazemi *et al*., 2011a-d). The effects of ethylene can be reduced by inhibitors of ethylene biosynthesis or action. It has been found that treatment with salicylic acid and Sucrose prolongs vase life of cut carnation flowers (Burdett, 1970; Dansercau and Vines, 1975; Meeteren, 1979; Fan *et al*., 2008; Yuping, 2009). Zagory and Reid (1986) reported that bacteria in vase solution produced ethylene. VanderMolen *et al*. (1983) reported that ethylene production increased vascular blockage in Ricinus communis. The bactericide

such as SA decrease of bacterial population which block the xylem vessels in the cut flowers (Halevy and Mayak, 1981; Nowak and Rudnicki, 1990; Khan *et al*., 2003; El-Tayeb *et al*., 2006; Ansari and Misra, 2007; Mahdavian *et al*., 2007; Mba *et al*., 2007; Canakci, 2008; Shi and Zhu, 2008; Joseph *et al*., 2010; Kazemi *et al*., 2011a-d).

Application of salicylic acid significantly increased the vase life of rose cut flowers (Nowak and Rudnicki, 1990). Kazemi *et al*. (2011a-d) showed that the treatment of salicylic acid reduced microbial population in vase solution of carnation cut flowers and increased water uptake in carnation cut flowers. Glutamine is readily metabolized by plants and cells can metabolize glutamine for energy, so we considered using it as a possible substitute for sucrose (Kazemi *et al*., 2011b). This study has examined the effects of salicylic acid concentrations and salicylic acid followed by glutamine treatment on carnation flower longevity, ACC-oxidase activity, antioxidant activity, free proline accumulation and bacterial population vase solution of carnation in laboratory condition.

MATERIALS AND METHODS

Plant material: The experiment was started on January 1 2011 and chlorophyll content, membrane stability, MDA content and ACC-oxidase activity were measured on the last day of vase life for each flower. The factors were three levels of salicylic Acid (0, 1.5, 2.5, 3.5 mM) and three levels of glutamine (0, 2 and 4 mM). The experiment was arranged in a factorial test with complete randomized design with 4 replications. Carnations (*Dianthus caryophyllus* L. pink) were grown in the greenhouse standard production methods (Pakdasht, Tehran, Iran). Cut flowers were selected to avoid malformations or damage. Flower stems were cut to 40 cm in length and after recording the fresh weight, each flower was placed in a 250 mL, bottle containing distill water or chemical solutions. All experiments were performed in a postharvest room with a controlled environment at 19°C, 70% relative humidity.

Vase life: The end of vase-life was defined as the time that flowers showed symptoms of wilting, loss and discoloration of the petals, whereas the marketability of cut flowers were determined by salability and consumer acceptance.

Fresh weight and solution uptake: Fresh weight and solution uptake changes were expressed as relative fresh weight (g g^{-1} initial fresh weight day^{-1}) and relative solution uptake (mL g^{-1} initial fresh weight day^{-1}) over the first five days of the experiments.

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 ACC-oxidase activity: The extraction and quantification was conducted based on the method described by 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 and 5 mM DTT and 30 mM sodium ascorbate for 2 min. The homogenate was centrifuged at 20000×g for 20 min and the supernatant was collected for enzyme assays. All procedures were conducted at 4°C.

Determination of ACC oxidase activity: 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 (MDA) 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×g 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×g for 10 min. The absorption was measured with a spectrophotometer at 532 nm.

Microbe population: In the last day of vase life, 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.

Enzyme extractions and assays superoxide dismutase (SOD): Floret (1.0 g) were homogenized with a mortar and pestle at 4°C in 5 mL 50 mM phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid, 1% polyvinylpyrrolidone. The homogenate was centrifuged at 12000×g for 30 min at 4°C and the supernatant was collected for enzyme assays. The activity of SOD was measured by nitroblue tetrazolium Method of Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reduction of nitroblue tetrazolium as monitored at 560 nm.

Determination of proline: The proline content was determined using the method of Bates *et al.* (1973). Fresh material (300 mg each sample) was homogenized in 10 mL of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged at 9000 xg for 15 min. A 2 mL aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin and incubated for 1 h at 100°C. The reaction was terminated in an ice bath and extracted with 4 mL of toluene. The extract was vortexed for 20 sec. The chromatophore-containing toluene was then aspirated from the aqueous phase and its absorbance determined spectrophotometerically at 520 nm (Perkin-Elmer-EZ-201) using toluene for a blank.

Experimental design and statistical analysis: Experiment was arranged in a factorial test with complete randomized design with 4 replications. Analysis of variance was performed on the data collected using the general linear model (Proc GLM) procedure of the SPSS ver 16 software (IBM Corp).Where a significant F-test was observed, treatment means were separated using the Tukey at $p<0.05$.

RESULTS

Cut flowers showed different reaction to chemical treatments. The vase life varied among cut flowers and ranged between 4 and 15.5 days (Table 1). The vase life of carnation cut flowers was

Table 1: Mean comparisons of chlorophyll content, vase life, MDA, SOD activity, membrane stability and ACC oxidase activity in SA and glutamine and their combination treatment

SA (mM)	Glutamine (mM)	Vase life (day)	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h^{-1} mL^{-1})	Proline (μmol g^{-1} FW)	MDA (μmol mg^{-1} protein)	Relative solution uptake[a]	Relative fresh weight[b]	SOD (U g^{-1} protein)	Colony count (CFU mL^{-1})
0	0	4.000	1.000	112.54	91.120	325.14	1.096	1.090	66.18	846.00
	2	6.000	1.360	100.03	82.540	187.36	1.067	1.070	100.08	526.00
	4	7.500	2.140	86.35	79.690	154.23	1.068	1.040	124.50	520.00
1.5	0	9.600	3.020	60.45	60.150	132.11	1.050	1.050	130.45	312.00
	2	9.800	3.150	58.69	58.320	130.75	1.050	10.200	129.87	315.00
	4	10.000	3.230	58.00	59.780	130.00	1.050	1.060	132.45	300.00
2.5	0	11.300	4.080	40.15	40.560	100.74	1.001	1.060	180.12	87.00
	2	11.700	5.360	40.08	40.080	97.35	1.020	1.003	182.40	100.00
	4	15.500	5.870	31.18	32.890	85.12	1.010	1.000	203.59	90.00
3.5	0	6.000	1.010	70.18	70.450	134.12	1.040	1.090	65.18	73.00
	2	7.300	1.250	68.92	66.390	129.87	1.080	1.060	70.89	93.00
	4	7.000	1.650	65.45	67.890	130.17	1.040	1.060	89.15	101.00
F-test probabilities										
Si		0.001	0.000	0.00	0.000	0.00		0.000	0.00	0.00
Glutamine		0.020	0.003	0.04	0.004	0.04		0.010	0.04	0.04

Values are the mean of four replication, Mean separation among treatments was done by Tukey's test at $p \leq 0.05$, [a]mL g^{-1} initial fresh weight day^{-1} during first five days of vase-life, [b]g g^{-1} initial fresh weight day^{-1} during first five days of vase-life

extended by the concentration of salicylic acid 2.5 mM used (Table 1) ($p \leq 0.05$). The two compounds (salicylic acid 2.5 mM+glutamine 4 mM) used significantly extended the vase life of carnation cut flowers compared to control (Table 1) ($p \leq 0.05$). The percent wilting increased with the increase in concentrations of salicylic acid (Table 1). The vase life was terminated on day 9.6 and 6, when cut flowers were treated with 1.5 and 3.5 mM salicylic acid, respectively, compared to 4 days in control ($p \leq 0.05$). Results of Table 1 showed that treatment by 2.5 mM salicylic acid increased the vase life of carnation cut flowers with or without glutamine compared to control ($p \leq 0.05$). When glutamine was added to 2.5 mM salicylic acid, the vase life was extended compared to treatment without glutamine. Fresh weight of cut flowers in all treatments increased initially and declined later. In solution containing 3.5 mM salicylic acid, water uptake decreased to 6 day after treatment. Uptake rate decreased rapidly in distill water and solutions containing 3.5 mM salicylic acid while flowers in the solutions containing 2.5 mM salicylic acid showed the minimum decrease in water uptake rate from day 11.3 (Table 1). Results showed adding SA was found to be negatively correlated with water uptake of the carnation cut flower (Table 1) ($p \leq 0.05$). This indicates that with SA concentration increased, the Water uptake was decreased. The treatment by 2.0 mM salicylic acid+ 4 mM glutamine lead to a considerable delay in degradation of chlorophyll total compared to other concentrations and control (Table 1) ($p \leq 0.05$). Chlorophyll Content decreased rapidly in present cut flower in solutions containing 3.5 mM salicylic acid while flowers in the solutions containing 2.5 mM salicylic acid showed the minimum decrease in chlorophyll content from day 11.3 (Table 1). Means of chlorophyll content of cut flowers in various salicylic acid+glutamine containing vase solutions was significant than control ($p \leq 0.05$). El-Tayeb *et al.* (2006) found that chl a, b and carotenoids increased significantly in SA treated plants in comparison to controls of barley plants. Similarly, Kazemi *et al.* (2011a-d), observed that chlorophyll biosynthesis increased treatment with SA in the cut flower ($p<0.05$). Canakci (2008) found that chl a, b increased significantly in SA treated plants

in comparison to controls of *R. sativus* plants. Treatment with 2.5 mM salicylic acid+4 mM glutamine higher delayed the ACC-oxidase activity and extended vase life of the carnation (Table 1) ($p \leq 0.05$), While, treatment with mM salicylic acid 3.5 increased ACC-oxidase activity and senescence. Liu *et al.* (2006) and Kazemi *et al.* (2011a-c) showed that the treatment of salicylic acid reduced anthocyanin leakage and ACO activity in cut flowers. SA affected on the microbial population in vase solution of carnation cut flowers significantly, the microbial population decreased with the increase in concentrations of SA and the lowest microbial concentration was evident when cut flowers were treated with 2.5 and 3.5 and 4.0 mM SA (Table 1). The effects of SA on the SOD activity, malondialdehyde (MDA) accumulation and proline content are presented in Table 1. Table 1 showed that under the effect of 2.5 mM salicylic acid increase SOD activity and decreased accumulation proline and MDA significantly in compared to control($p \leq 0.05$). Salicylic acid 2.5 mM treatment improved membrane permeability by increasing SOD activity and decrease accumulation proline and MDA in compared to control($p \leq 0.05$). Results showed that addition of salicylic acid maintained SOD activity and decrease accumulation proline and MDA but 3.5 mM salicylic acid could not alleviate or decrease accumulation proline and MDA. The results of the present experiment were similar with the findings of Fan *et al.* (2008) and Capdeville *et al.* (2003) showed that added salicylic acid decreased the accumulation proline and increase enzyme antioxidant activity of petal cells carnation cut flower. In the present study, we found that use of disinfectants improve water conductance by preventing bacterial growth and producing occlusions. Result showed that variation in water uptake, ACC oxidase activity, SOD activity and microbe population on vase solution between cut flowers were main cause of differences in vase life. Salicylic acid is an ethylene biosynthesis inhibitor that blocks the induction effect of ethylene on ACC-oxidase activity, inhibition reduced the senescence of the flowers and consequently, the advance in increase vase life. Fan *et al.* (2008) and Karlidag *et al.* (2009) reported that treatment with salicylic acid+sucrose significantly extends the vase life. Cut flowers treatment with salicylic acid increases the enzyme antioxidant activity, delay the onset of hydrolysis of structural cell components, decrease ethylene production, ACC-oxidase activity and sensitivity. Our result showed that treatment with salicylic acid in combination with glutamine extends the vase life of cut carnation flowers. Also, salicylic acid reduced chlorophyll total degradation and preserved chlorophyll total content. This might be inhibiting ethylene action and as a result, the vase life could be increased. These findings are similar to previous results (Kazemi *et al.*, 2011a-d). In our study, after treatment with salicylic acid solution alone, the cut carnation flowers increase the vase life and decrease climacteric ethylene production of cut flowers. These findings also indicated that salicylic acid alone has high effect on increase the vase life, decrease climacteric ethylene production, ACC-oxidase activity and senescence of cut flowers.

CONCLUSION

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

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