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Effects of Succinic Acid and Glutamin on Acc-Oxidase Activity, Microbe Population and Senescence of Carnation Cut Flowers

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Abstract: This study was conducted to investigate the effect of succinic acid and Glutamin at different concentrations on carnation flower longevity, ACC-Oxidase (ACO) activity, anthocyanin leakage, membrane stability, malondialdehyde (MDA) content of cut flowers of carnations and Microbe population vase solution of carnation in laboratory condition. Cut flowers of *Dianthus caryophyllus L. cv. Pink* were treated with Factors succinic acid (0, 2, 4 and 6 mM) with or without Glutamin (0, 1.5, 3 and 5 mM). The results showed that succinic acid and glutamin treatments increased the vase life and decrease the percentage of wilting compared to the control. The vase solution containing 4mM succinic acid with 5mM glutamin significantly increased vase life compared to the control, in addition, the MDA accumulation, the microbial populations on vase solution of cut flower and ACO activity reduced in the same solution while membrane stability was improved. Results suggest that succinic acid with glutamin increases vase life by affecting many of the age-related changes associated with carnation petal senescence.

Abbreviations: MDA · malondialdehyde · ROS · Reactive oxygene species · ACO · ACC-Oxidase · ROS · Reactive oxygen species

Key words: Carnations · Succinic acid · Membrane stability · Glutamin · Vase life

INTRODUCTION

The length of vase life is one of the most important factors for quality of cut flowers. Short postharvest vase life is one of the most important problems on the cut flowers. Senescence of cut flowers is induced by several factors e.g. water stress [1], carbohydrate depletion, microorganisms [2] and ethylene effects. In carnations, senescence of the petals is associated with a climacteric-like increase in ethylene production during the final stages. Ethylene enhanced flower senescence and wilting [3], increased permeability of petal cells and accelerated the decrease in cell membrane fluidity [4]. The other consequences include increase in cell membrane permeability and solute uptake capacity [5], degradation of membrane lipids and MDA production [6]. Ethylene production causes a sharp increase in production of oxygen free radicals which is responsible for stress dependent peroxidation of membrane lipids [7]. One effect of ROS accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage [8]. There is ample evidence indicating that changes in the activity of several enzymes functioning during plant development play an important role during organ senescence. The effects of Senescence can be reduced by inhibitors of ethylene biosynthesis and increase enzym antioxidant activity. Succinic acid is found in all plant and animal materials as a result of the central metabolic role played by this dicarboxylic acid in the Citric acid Cycle. Succinate can solely as a catalytic system in carbohydrate respiration. The rapid oxidation of succinate was leading to the accumulation of malic acid. Malic acid can reduction Acc-oxidase activity and the microbial populations on vase solution of cut flower [9]. Benedict and Beevers [10] described the reactions involved in the synthesis of sucrose from malate in the same tissue. Glutamine, a multifaceted amino acid used as an energy substrate for most cells. It is important as a constituent of proteins and as a central metabolite for amino acid transamination via á-ketoglutarate and glutamate. It provides nitrogen for a number of biosynthetic pathways,

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serving as a precursor of the purine and pyrimidine rings of nucleic and nucleotides such as adenosine triphosphate (ATP) [11]. Glutamine plays an important role in the nitrogen and carbonskeleton exchange among different tissues, where this amino acid fulfils many different physiological functions [12]. When glucose levels are low and energy demands are high, cells can metabolize amino acids for energy. Glutamine is one of the most readily available amino acids for use as an energy source and it is a major source of energy for many rapidly dividing cell types. Therefore In this study, the preservative effects of succinic acid and Glutamine on the vase life of cut carnation flowers was compared with emphasis on the possibility of succinic acid and Glutamine effect on antioxidative indicators of cut flower.

MATERIALS AND METHODS

Plant Material: This study was on the effect of succinic acid and Glutamine treatments on vase life of carnation cut flowers, in a factorial test with complete randomized design with four replications. Cut flower stems of carnation (*Dianthus caryophyllus* L. cv. pink) (40cm in length) were placed in solution containing succinic acid 0, 2, 4 and 6 mM and Glutamine 0, 1.5, 3 and 5 after cutting. Six cut flowers were placed in a 300mL flask with 250mL of solution. Distilled water was used for the controls and placed in chambers at 25°C. The relative humidity was about 70% while 14h photoperiod was maintained using fluorescent lamps with a light intensity of 15 μmol m^{-2} s^{-1} at the top of the corolla. Data were statistically analyzed using SPSS software. Where a significant F-test was observed, treatment means were separated using the tukey at p= 0.05. The experiment was started on February 1 2010 and chlorophyll content, Membrane stability, MDA content and ACC Oxidase activity were measured at 12th day of vase life.

Vase Life: Vase life was determined as the number of days to wilting of flowers.

Chlorophyll Content Measurement: Total chlorophyll (a+b) content 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 leaf was considered.

Determination of Anthocyanin Leakage: Anthocyanin leakage was measured based on the method of Poovaiah [13].

Determination of Proline: Proline was extracted and its concentration determined following the method of Bates *et al.* [14].

Determination of Acc-oxidase Activity: ACC oxidase activity was assayed by measuring to the method described by Moya-Leòn and John [15].

Assays of Mda Content (Lipid Peroxidation): Oxidative damage to lipids was estimated by measuring the MDA content in floret segment homogenates which were prepared in 10% trichloroacetic acid containing 0.65% 2-thiobarbituric acid (TBA) and heated at 95 ? for 25 min, as described by Heath and Packer [16]. In order to correct for compounds other than MDA, the absorbance at 532 nm of a solution containing plant extract incubated without TBA was subtracted from the absorbance of TBA treated solution at the same wavelength.

Superoxide Dismutase: The activity of superoxide dismutase was assayed by measuring its ability to inhibit to the photochemical reduction of nitroblue tetrazolium as described by Beauchamp and Fridovich [17].

Microbe Population: Test Microbe population were isolated from vase solutions of carnations. When the flowers had senesced (about 11 days), aliquots of the vase solutions were diluted 100-times and 25 u.1 aliquots of the diluted solution were spread on sterile Nutrient Agar, in sterile Petri plates. The plates were allowed to incubate for 48 hr at room temperature and individual colonies of microorganisms, representing the most common colony morphology types, then were picked off the agar media with a sterile loop and streaked on EMB medium for purification. Purified Microbe population were maintained axenically on EMB medium and transferred daily to fresh medium.

RESULTS

Anthocyanin Leakage and ACO Activity: The results indicate that 4mM succinic acid caused significant decrease in anthocyanin leakage and ACO activity compared to control (Table 1). Addition of glutamin to 4mM succinic acid significant decrease in anthocyanin leakage and ACO activity compared to control (Table 1). Highest means of ACO activity was found in cut flowers treated with 6 mM succinic acid and control (Table 1).

Table 1: Mean comparisons of chlorophyll content, Vase life, MDA, SOD activity, Microbe population, Membrane stability and ACC Oxidase activity in succinic acid*glutamin treatments

Treatment			Chlorophyll total (a+b)	ACC Oxidase	Membrane stability				Microbe
Succinic acid	Glutamin	Vase life (day)	content (spad reading)	Activity (nmol/gFW/h)	(Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Proline (µmol.g^{-1} FW)	SOD (U g-1Protein)	population (cfu)
0mM	0 Mm	6 AC	0.55 D	43.14 AD	101.39 D	70.14 AC	71.4 AC	61 AC	84AB
	1.5 Mm	8 C	1.02 AC	30.47 AC	70 AC	55 AB	63.11 AB	76.14 AB	22C
	3 Mm	8.7 C	1.65 AC	30.14 AC	64.54 AB	56.36 AB	60 AB	77 AB	25C
	5 Mm	9.5 B	1.89 AC	30.74 AC	65 AB	54 AB	60.87 AB	80 C	20C
2 mM	0 Mm	8 C	1.8 AC	29.14 AC	68.74 AB	50 AB	60.36 AB	69.74	15B
	1.5 Mm	7.5 AB	2 AB	30.17 AC	60 C	51 AB	55.41 C	80.12 C	12B
	3 Mm	8 C	2.01 AB	30.74 AC	61 C	51.78 AB	45.17 B	80 C	12B
	5 Mm	8.45 C	2.22 AB	26.78 AB	61.32 C	53.35 AB	46 B	86 C	12B
4 mM	0 Mm	11.5 A	6.95 A	11.01 A	32.14 A	17 A	33.17 A	110 A	8.1 A
	1.5 Mm	9.6 B	4.33 C	20.11 C	51 B	28.11 C	40.12 B	101 B	15B
	3 Mm	9.6 B	5.67 B	14 B	49.78 B	20.14 B	38.7 B	105 B	16B
	5 Mm	12A	7.19 A	10.04 A	30.14 A	15 A	30 A	114.8 A	9.04 A
6 mM	0 Mm	6 AC	1 AC	55.87 D	102.09 D	83.39 D	77 AC	63 AC	8A
	1.5 Mm	7 AB	2.41 AB	30.74 AC	80 AC	57 AB	61.01 AB	70 AB	12B
	3 Mm	7 AB	2 AB	30 AC	76.69 AC	55.08 AB	55.13 C	74.54 AB	15B
	5 Mm	7 AB	1.87 AC	31.74 AC	75 AC	57 AB	52.45 C	75 AB	17B
F-test probabilities									
	Succinic acid	0.021	0.001	0.03	0.021	0.01	0.02	0.001	0.011
	Glutamin	0.4	0.03	0.02	0.04	0.041	0.03	0.04	0.01

Means in each column followed by similar letters are not significantly different at 5% level

* Data recorded on day 12 of experiment

MDA and Total Chlorophyll Content: MDA content was reduced by succinic acid and was significantly lower in preservative mixtures containing just succinic acid compared to the control (Table1). A significant negative correlation was observed between succinic acid concentration and the MDA content in carnation cut flowers (Table1). Total chlorophyll content increased along with succinic acid and glutamin concentration in carnation cut flower (Table 1). Result showed that 4 mM succinic acid + 5 mM glutamin leaded to a considerable delay in degradation of chlorophyll compared to other concentrations (6 mM succinic acid and control) and the difference between 6 mM succinic acid and control was significant as well (Table 1).

Proline Accumulation and Microbe Population: The results indicate that 4mM succinic acid caused significant decrease in proline accumulation and the microbial populations on vase solution of cut flower compared to control (Table 1). Addition of glutamin to 4mM succinic acid significant decrease in proline accumulation and the microbial populations on vase solution of cut flower compared to control (Table 1). Highest means of proline accumulation was found in cut flowers treated with control (Table 1).

Superoxide Dismutase Activity: Carnation flowers treated by succinic acid alone or together with glutamin had more Superoxide dismutase activity. The maximum Superoxide dismutase activity was recorded in 4 mM succinic acid + 5 mM glutamin compare other treatments and control (Table 1). Statistically significant differences existed among 4 mM succinic acid + 5 mM glutamin compared to other treatments and control. The minimum Superoxide dismutase activity was noted in 6 mM succinic acid and control (Table 1). A significant negative correlation was observed between succinic acid concentration and the Superoxide dismutase activity in carnation cut flowers (Table1).

Vase Life: Holding carnation cut flowers in vase solutions containing 4 mM succinic acid + 3 mM glutamin significantly increased their vase life and delayed flower senescence compared to flowers either held in 2 and 6 mM succinic acid or distilled water (Table 1). succinic acid was found to be significantly and positively correlated with vase life of the carnation cut flowers as well. In our experiment adding glutamin to vase solutions containing succinic acid could increase the vase life of cut flowers compared to control (Table 1).

DISCUSSION

During senescence, the oxidative stress increases the peroxidative reactions in membrane lipids which damages the membrane function and causes ions and anthocyanin to leak outward which could be considered as an index for lipid peroxidation and senescence progress [18]. Production of MDA as a known biomarker for oxidative stress is another consequence. In our study use of succinic acid and glutamin as a preservative mixture ingredient increased vase life of cut flowers significantly. The observed reduction in MDA content and anthocyanin leakages by succinic acid and glutamin application supports our conclusion of considering it as a practical agent for retarding of carnation cut flower senescence. Presumably, the relatively low level of MDA in succinic acid and glutamin treated flowers is result of alleviated oxidative injuries through raising antioxidant enzymes activity to scavenge newly-produced ROS. The observed decrease in ACO activity by relatively application could be at least one mechanism through which relatively has affected on the senescence process. As succinic acid and glutamin are readily metabolized by plants, but not by many microorganisms, so we considered using it as a possible substitute for sucrose. Sucrose application necessitates addition of biocidal agents, which is not an environment friendly method, considering the side effects like helping in emergence of resistant strains of microorganisms to frequently used biocides. The effect of relatively on retaining of chlorophyll content, which is another factor affected adversely by senescence related processes, supports our assumption further. Based on results we could consider relatively as a new potent agent to be applied in preservative mixtures used for cut flowers.

CONCLUSION

This study shows that succinic acid and Glutamin treatment did show significant effect on quality parameters and carnation flower longevity. succinic acid and Glutamin proved more effective in delaying petal senescence and/or flower wilting. However, our result showed that succinic acid and Glutamin treatments maintained the vase life of flowers for a longer period.

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