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Role of Salicylic Acid in Decreases of Membrane Senescence in Cut Lisianthus Flowers

[1]M. Kazemi and [2]K. Shokri

[1]Department of Horticulture Sciences, Faculty of Agriculture and Natural Resources,
Islamic Azad University Karaj branch, Young Researchers Club, Karaj, Iran
[2]Department of Horticulture Sciences,
Faculty of Agriculture and Natural Resources, University of kurdestan, Iran

Abstract: The Effect of salicylic acid combined with sucrose on lisianthus (*Eustoma grandiflorum* Mariachii. cv. blue) cut flowers was studied. Cut flowers were kept in vases containing 0 and 3% sucrose solutions supplemented with salicylic acid (0,1.5, 3 mM) and water (no chemical treatment). Determinations were made for vase life, bacteria populations in vase flower preservative solution, ACC-Oxidase activity, SOD activity, proline accumulationand lipid peroxidation rates. Results revealed that the vase solution containing 3% sucrose with 1.5 mM salicylic acid significantly Decreased bacteria populations, Lipid peroxidation rates, ACC-oxidase activity and proline accumulation in vase flower preservative solution But increased the vase life and SOD activity of lisianthus cut flower compared to the control. Results suggest that 3% sucrose with 1.5 mM salicylic acid increases vase life by decreasing Lipid peroxidation rates and ACC-oxidase activity and increasing enzyme antioxidant activity.

Key words: Lisianthus · Vase life · Salicylic acid · Lipid peroxidation rates · ACC-oxidase activity

INTRODUCTION

lisianthus (*Eustoma grandiflorum*) (Raf.) Shinn is native to the southern US and mainly inhabits the moist prairies from Nebraska to Colorado and Texas. Eustoma was introduced into Japan more than 60 years ago [1]. Lisianthus (*Eustoma grandiflorum*) hybrids have continued to gain acceptance as new cut flowers, bedding plants and potted flowering plant since their introduction promoted the floriculture trade in the early 1980s [2]. Prolonged vase life is one of the most important factors for quality of cut flowers. Senescence of cut flowers is induced by several factors e.g., water stress [3], carbohydrate depletion [4], microorganisms [5] and ethylene effects [6].In general, the senescence of ethylene-sensitive flowers, such as carnations, is associated with a loss of membrane integrity, climacteric rise of respiration and enchanced ethylene synthesis [7]. Ethylene production of cut Gerbera flowers increased with flower senescence but treatment with salicylic acid (SA), an ethylene inhibitor, extended flower longevity [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 increases in enzyme antioxidant activity. Salicylic acid is a well known phenol that can prevent ACC-oxidase activity that is the direct precursor of ethylene production and decrease ROS and Lipid peroxidation rates with increase enzyme antioxidant activity. Salicylic acid seems to act as a germicide to decrease bacteria that can block the xylem vessels in the cut region and interfere with the normal flux of water through the stem. The postharvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amount of metabolic sugars are factors that affect the rate of senescence. Keeping the flower in vase solutions containing sucrose has been shown to extend their vase-life. Han [9] found that addition of sugar to vase solution improved the intensity of petal color but did not improve bud opening, longevity, or size of non-cold-stored Oriental Lily cv. Stargazer cut flower harvested at

Corresponding Author: M. Kazemi, Department of Horticulture Sciences, Faculty of Agriculture and Natural Resources, Islamic Azad University, Karaj branch, Rajaee St., Eivan, Ilam, Iran, Postal code 69417-47611. Tel: +98 842 3230192, E-mail: kazemimohsen85@gmail.com.

the commercial marketing stage. However, addition of sugar to the vase solution of defoliated stems not only restored the color on the petals but increased the size of the open flowers [9].The purpose of this work was to find responses of the cut Eustoma flowers to salicylic acid application and its effect on vase life, ACC-oxidase activity, proline accumulation and enzyme antioxidant activity.

MATERIALS AND METHODS

Plant Material: 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. This study on the effect of salisylic acid treatments on vase life of lisianthus cut flowers, in a factorial test with complete randomized design with six replications. Cut flower stems of lisianthus (*Eustoma grandiflorum Mriachii cv. Blue*) (40cm in length) were placed in solution containing salicylic acid 0,1.5 and 3 mM and sucrose 0 and 3% after cutting. Six cut flowers (each cut stem contained 2 flowers) were placed in a 300mL flask with 250mL of solution. Distilled water was used for the controls all flowers were 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.

Determination of Vase Life: The vase life of cut flowers was completed when the petals or stem below the flower head lost turgidity.

Determination of Proline: Proline was extracted and its concentration determined following the method of Bates *et al.* [10].

Membrane Permeability Measurement: Anthocyanin leakage was used to assess membrane permeability and measured using Spectrophotometer. The procedure used was based on the method of Poovaiah [11].

Lipid Peroxidation: Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according to Hodges *et al.* [12].

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 [13].

Determination of ACC Oxidase Activity: ACC oxidase activity was assayed as described by Moya-Leòn and John [14].

Chlorophyll (A+b)content Measurement: Chlorophyll total(a+b) content was measured by Chlorophyll meter SPAD-502, Minolta Co. Japan which represented by SPAD value. The petal was inserted into the meter and measured SPAD value 3 times from different spot of a single petal.

Microbe Population: When the flowers had senesced (about 14 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, 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 AND DISCUSSION

Vase Life: Flower stems kept in water containing salicylic acid at 1.5 mM had significantly increased vase life relative to the water control but the 3 mM did not have greater vase life than the water control. (Table 1). The use of 1.5 mM salicylic acid along with 3% sucrose resulted in a greater extension in vase life than other treatments. Mei-hua *et al.* [8] showed that the treatment of salicylic acid extended the vase life and improved flower quality with reduced respiration rate delay senescence and decrease Lipid per oxidation, MDA content. Ichimura and Hiraya [15] reported that treatment with sucrose extends the vase life of florets harvested at a bud stage. In addition, sucrose promotes pigmentation of petal colors in some cut flowers including Eustoma. Keeping the flowers in vase solutions containing sucrose has been shown to extend their vase-life [9,16]. our results showed that adding SA was found to be positivly correlated with vase life of the lisianthus cut flower (Table 3).

Table 1: Mean comparisons of chlorophyll content, Vase life, MDA, SOD activity,Microbe population, Membrane stability and ACC Oxidase Activity in SA treatment

treatment	Vase life(day)	Chlorophyll total (a+b) content(spad reading)	ACC Oxidase Activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Proline (µmol.g^{-1} FW)	SOD (U g-1Protein)	Microbe population(cfu)
control	6.37b	2.13b	24b	204.1b	171.9b	41.59b	87.73b	58.50a
SA1.5Mmol	12.75a	4.89a	12.81c	168.5c	143.8c	20.08c	135.4a	28.50b
SA3Mmol	5.75c	0.833c	39.54a	506a	226.4a	118.2a	67.30c	23.75c

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

Table 2: Simple correlation lines between the SA treatment with other variables

treatment	Vase life(day)	Chlorophyll total (a+b) content(spad reading)	ACC Oxidase Activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Microbe population(cfu)	Proline (µmol.g^{-1} FW)	SOD (Ug-1Protein)
SA	-0.408**	-0.367*	0.439**	0.526**	0.478**	-0.207	0.86**	-0.268

* and **: Significant different at 5% and 1% level, repectively.

Table3: Mean comparisons of chlorophyll content, Vase life, Antocyanin leakage, MDA content, bacteria populations in vase flower preservative solution and ACC Oxidase Activity in SA*SU treatment

SA	SU	vase life(day)	Chlorophyll total (a+b)content (spad reading)	ACC Oxidase Activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Proline (µmol.g-1 FW)	SOD (U g-1Protein)	Microbe population(cfu)
0	0	7.5b	3.349b	14.83d	167.3e	141.6e	40.01d	100c	42b
	30	5.25c	0.767c	33.16c	321c	202.2c	43.17c	75.43d	75a
1.5	0	12.5a	5.02a	11.02f	135.8f	137.5f	18.14f	149.3a	19e
	30	13a	4.77a	14.61e	201.3d	150d	22.01e	121.4b	38c
3	0	6c	0.725c	39.92a	562a	240.1a	125.3a	62.26f	23.5d
	30	5.5c	0.942c	39.15b	451b	212.7b	111.1b	72.34c	24d

Lipid per Oxidation, MDA Content and Chlorophyll Content: Under the effect of 3mM SA treatment MDA content increased significantly compared to control. Results showed that adding SA was positively correlated with Lipid per oxidation (Table 2). Per oxidation of membrane lipids is an indication of membrane damage and leakage under senescence conditions. Table 1 showed a decrease in content of MDA as well, suggesting that oxidative damage was alleviated by the addition of SA. A lower lipid per oxidation resulting from elevated activities of antioxidants was also reported on Cut Gerbera Jamesonii Flower [17-18]. The results of the present experiment were similar with the findings of Mei-hua [8] in Gerbera which showed that added SA decreased the permeability of the plasma membrane of petal cells and decreased MDA level. The evidence suggests that SA decreases the permeability of plasma membranes and membrane lipid per oxidation and maintains the membrane integrity. In this study, a significant increase in activities of the anti oxidative enzymes was observed in cut lisianthus. Increases in activities of these enzymes in response lipid per oxidation may be decreasing the toxicity of ROS. Result showed that the expression levels of the anti oxidative enzymes increased after producing ROS. Two treatments (3mM and 3mM+SU3%) increased MDA content and decreased chlorophyll content in lisianthus cut flower, but SA at 1.5 mM and SA 1.5 mM +SU 3% decreased MDA content and prevented the decrease of chlorophyll content in lisianthus cut flower (Table 1,3). Chlorophyll contents were lower in both SA at 3mM and SA+SU(SA3mM+SU3%) treatments compared to control values; SA at 1.5mM and Salicylic acid 1.5 mM +sucrose 3% improved chlorophyll content in lisianthus fower. Maximum increase was noticed when SA1.5 mM was supplied. (Table 1). adding SA was found to be negativly correlated with Chlorophyll (a+b)content of the lisianthus cut flower (Table 2). SA treatments with sucrose lead to a considerable delay in degradation of Chlorophyll total(a+b)compared to control (Table 3).

Anthocyanin Leakage and ACC-oxidase Activity: Two treatments (SA 1.5 mM and SA+SU(SA1.5+SU3%) improved membrane permeability by decreasing anthocyanin leakage(Table 1,3). Two treatments (3mM and 3mM+SU3%) impaired membrane permeability by increasing anthocyanin leakage and increasing the production of ethylene (Table 1 and 3). Addition of 1.5mM SA maintained membrane permeability. 1.5 mM SA with SU could alleviate or decrease cell wall damages (Table 3). It is evident from the data presented in Table 1 that the maximum anthocyanin leakage was recorded in 3 mM SA adding SA in vase water can prevent anthocyanin leakage by maintaining the pH of the vase solution. Mei-hua *et al*. [8] reported that SA can extend the vase life of cut flowers with by increasing membrane stability. adding a suitable ethylene inhibitor can prevent accelerated flower senescence, anthocyanin leakage and cell death. These results are in agreement with previous reports that have reported decreased accelerate flower senescence, anthocyanin leakage and cell death of cut flowers when

placed in solutions of a suitable ethylene inhibition [8]. It seems that in high concentrations of SA, pH increased and affected vacuoles pH and which resulted in lower anthocyanin leakage (Table 1). The action of ethylene in enhancing the rate of leakage of this pigment can be interpreted as an effect of the gas on senescence and the membrane permeability of cut flower. Treatment with salicylic acid 1.5 mM + sucrose 3% higher delayed the climacteric ethylene production and extended vase life of the lisianthus (Table 1,3),While Treatment with salicylic acid 3 mM increased ACC-oxidase activity and senescence.

Superoxide Dismutase and Proline Content: Under the effect of SA at 1.5mM treatment increase SOD activity and decreased accumulation proline significantly compared to control. SA at 1.5mM +sucrose 3% treatment improved membrane permeability by increasing SOD activity and decrease accumulation proline compared to control. SA treatments significantly reduced accumulation proline of cut flower in all treatments except 3mM SA and SA+SU(SA3mMol+SU3%) (Table 3). Addition of SA and sucrose maintained SOD activity and decrease accumulation proline.

Microbe Population: The Microbe population vase solution of lisianthus cut flowers was decreased by the concentration of salicylic asid 1.5 and 3 mM (Table 1). The Microbe population was lower in salicylic acid at 1.5 and 3 mM compared to salicylic combined with sucrose treatment and control (Table 3). The higher Microbe population was attained when sucrose was use compared to control (Table 3). Means of Microbe population in various salicylic acid+sucrose containing vase solutions was slightly significantly higher than control. Adding SA was found to be negativly correlated with Microbe population vase solution of the lisianthus cut flower (Table 2). This indicates that with SA concentration increased, the Microbe population vase solution was decreased.

CONCLUSION

From the results of the present study, it can be concluded that SA+sucrose treatments significantly decrease bacteria populations in vase flower preservative solution, produce MDA and ACC-oxidase activity, reduce the membrane permeability and per oxidation of lipids and increase SOD activity compared to the control. SA at 1.5 mM +sucrose also proved more effective in delaying petal senescence and/or flower wilting. However, our results showed that SA treatments maintained the vase life of flowers for a longer period.

REFERENCES

1. Ohkawa, K., A. Kano, K. Kanematsu and M. Korenaga, 1991. Effects of air temperature and time on rosette formation in seedlings of Eustoma grandiflorum (Raf.) Shinn. Sci. Hort., 48: 171-6.
2. Halevy, A.H. and A.M. Kofranek, 1984. Evaluation of lisianthus as a new flower crop. Hort. Sci., 19: 845-47.
3. Sankat, C.K. and S. Mujaffar, 1994. Water balance in cut anthurium flowers in storage and its effect on quality. Acta Hort., 368: 723-32.
4. Ketsa, S., 1989. Vase life characteristics of inflorescences of dendrobium 'Pompadour. Hort. Sci., 64: 611-5.
5. Van Doom, W.G. and Y. Witte, 1991. The mode of action of bacteria in the vascular occlusion of cut rose flowers. Acta Hort., 298: 165-7.
6. Wu, M.J., W.G. Van Doom and M.S. Reid, 1991. Variation in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars. II. Comparison of sensitivity to exogenous ethylene and of ethylene binding. Sci. Hort., 48: 109-16.
7. Tang, X., A. Gome, A. Bhatia and W. William, 1994. Pistil-specific and ethylene-regulated ex-pression of 1-aminocyclopropane-1-carboxylate oxidase genes in petunia flowers. The Plant Cell, 6: 1227-1239.
8. Mei-hua, FAN., Jian-xin, WANG, S.H.I. LI-na, S.H.I. Ge. fan and L.I. Ruo, 2008. Salicylic Acid and 6-BA Effects in Shelf-life Improvement of Gerbera jamesonii Cut Flowers. Anhui Agricultural Science Bulletin.
9. Han, S.S., 2003. Role of sugar in vase solution on postharvest flower and leaf quality of Oriental Lily Stargazer. Hort. Sci., 38: 412-6.
10. Bates, L., L. Waldren and J. Teare, 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.
11. Poovaiah, B.W., 1979. Increased levels of calcium in nutrient solution improves the postharvest life of potted roses. J. Amer. Soc. Hort. Sci., 104: 164-166.
12. Hodges, D.M., J.M. Delong, C.F. Forney and R.K. Prange, 1999. Improving the thiobarbituric acid-reactivesubstances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta, 207: 604-611.

13. Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Ann. Biochem., 44: 276-287.
14. Maye-Lean, M.A. and P. John, 1994. Activity of 1-Aminocyclo propane-1carboxylate (ACC)oxidase (ethylene-forming enzyme) in the pulp of. riepening bananase. J. Horst. Sci., 69: 243-250.
15. Ichimura, K. And T. Hiraya, 1999. Effect of silver thiosulfate complex (STS) in combination with sucrose on the vase life of cut sweet pea flowers. Hort. Sci., 68: 23-7.
16. Yamane, K., T. Kawauchi, Y.T. Yamaki and N. Fujishige, 2005. Effects of treatment with trehalose and sucrose on sugar contents, ion leakage and senescence of florets in cut gladiolus spikes. Acta. Hort., 669: 351-8.
17. Shakirova, F.M., 2007. Role of hormonal system in the manifestation of growth promoting and antistress action of salicylic acid. In: S. Hayat, A. Ahmad, Salicylic Acid-A Plant Hormone, Springer, pp: 69-89.
18. Yuping, Z., 2009. Effects of Salicylic Acid on Fresh Keeping of Cut Gerbera Jamesonii Flower. Anhui Agricultural Science Bulletin.