American Journal of Plant Physiology

ISSN 1557-4539

Academic Journals Inc.

www.academicjournals.com

American Journal of Plant Physiology 6 (2): 120-125, 2011
ISSN 1557-4539 / DOI: 10.3923/ajpp.2011.120.125

Interaction Between Glutamin and Different Chemicals on Extending the Vase Life of Cut Flowers of 'Prato' Lily

[1]M. Kazemi, [1]S. Zamani and [2]M. Aran

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

[2]Department of Landscape, College of Agriculture, University of Zabol, Zabol, Iran

Corresponding Author: M. Kazemi, Department of Horticulture Sciences, Faculty of Agriculture and Natural Resources, Islamic Azad University, Karaj Branch, Rajaee St., Eivan, Ilam, Postal Code 69417-47611, Iran Tel: +98 842 3230192

ABSTRACT

In this study, effects of different concentrations of glutamin, malic acid, Salicylic acid and their interaction on extending the vase life, total chlorophyll content, ACC-Oxidase activity, anthocyanin leakage, membrane stability and malondialdehyde content of Cut Flowers of 'Prato' Lily was investigated. The vase were placed in chambers at 25°C, relative humidity about 70% and 14 h photoperiod that was maintained using fluorescent lamps (light intensity of 15 μ mol m^{-2} sec^{-1}) at the top of the corolla. The results showed that glutamin, malic acid and Salicylic acid treatments increased the vase life and decrease the percentage of wilting compared to the control. The vase solution containing 3 mM glutamin and 4 mM malic acid with 2 mM Salicylic acid significantly increased vase life compared to the control, in addition, the malondialdehyde accumulation and ACC-Oxidase activity reduced in the same solution while membrane stability was improved. Results suggest that glutamin and malic acid along with salicylic acid increases vase life by affecting many of the age-related changes associated with Lily petal senescence.

Key words: Lily, malic acid, salicylic acid, glutamin, vase life

INTRODUCTION

Flowers are extremely perishable; maintaining their physiological functions vary actively even after harvest and the beginning of their senescence very often depends on ethylene. A rise in ethylene production that accelerates senescence has been found in cut flowers (Mayak and Halevy, 1980; Halevy and Mayak, 1981). Ethylene enhanced flower senescence and wilting (Halevy and Mayak, 1979), increased permeability of petal cells and accelerated the decrease in cell membrane fluidity (Mayak *et al*., 1977). The other consequences include increase in cell membrane permeability and solute uptake capacity (Doyle and Kubitschek, 1976), degradation of membrane lipids and MDA production (Wright *et al*., 1981). Ethylene production causes a sharp increase in production of oxygen free radicals which is responsible for stress dependent peroxidation of membrane lipids. 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 (Liu *et al*., 1987). To scavenge ROS, plants posses specific mechanisms, which include activation of antioxidant enzymes (Jaleel *et al*., 2006) and non enzymatic antioxidants such as, carotenoids and ascorbic acid (Mittler, 2002). SA is a well known phenol that can prevent ACC-oxidase activity that is the direct precursor of ethylene and decrease ROS with increase enzyme antioxidant

activity (Ansari *et al*., 2007; Mba *et al*., 2007; Mahdavian *et al*., 2007; Canakci, 2008). Fan *et al*. (2008) showed that SA along with 6-BA could extending the vase life of cut flowers with decrease ROS and ethylene production. presumably, SA acid can with increases the enzyme antioxidant activity cause delay the onset of hydrolysis of structural cell components, decrease ROS production, ACC-oxidase activity and sensitivity. MA is a well known organic acid that can reduced the number of bacteria in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ACC-oxidase activity and sensitivity (Kazemi *et al*., 2010). Other important factor in the deterioration of cut flowers involves the diminishing of respiration substrates, the speed of these changes depend, at least in part, on the amount of reserves that are present in the flower when they are cut (Rogers, 1973). Carbohydrates are important reserve compounds, being sucrose the most abundant soluble carbohydrate, sometimes the only one in the phloem sap (Figueroa *et al*., 2005). The senescence of cut flowers is closely related to a considerable reduction of the energy needed for synthesis reactions. Therefore, an exogenous carbohydrate supplementation would be enough to delay the senescence, considering that the main effect would be to maintain the structure and activity of the mitochondria (Coorts, 1973; Kaltaler and Steponkus, 1976). 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 α-ketoglutarate and glutamate. Glutamine plays an important role in the nitrogen and carbonskeleton exchange among different tissues, where this amino acid fulfils many different physiological functions (Kovacevic and McGivan, 1983). 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 (Sigmaaldrich, 2010). As Glutamine and MA are readily metabolized by plants but not by many microorganisms, so we considered using it as a possible substitute for sucrose (Kazemi *et al*., 2010). 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 (Hojjati *et al*., 2007). Therefore, in this study, the preservative effects of SA and MA with Glutamine on the vase life of cut Lily flowers was compared with emphasis on the possibility of SA and MA with Glutamine effect on antioxidative indicators of cut flower.

MATERIALS AND METHODS

Plant material and storage conditions: The experiment was started on september 10, 2010 and chlorophyll content, Membrane stability, MDA content and ACC Oxidase activity were measured at 14th day of vase life.

Lily were obtained from local commercial greenhouses (Tehran, Iran). Following harvest and transport to the laboratory, the stems were recut to 40 cm length. In this study, three levels of MA (0, 2 and 4 mM), three levels of glutamin (0, 1.5 and 3 mM) and three levels of SA (0, 2 and 4 mM) were applied on 162 Lily cut flowers. 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.

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 (1979).

Determination of acc-oxidase activity: For the measurement of ACC oxidase activity use the method described by Maye-Lean and John (1994).

Assays of mda content (lipid peroxidation): Oxidative damage to lipids was measured based on the method of Heath and Packer (1968).

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 (1971).

Experimental design and statistical analysis: Experiment was arranged in a factorial test with complete randomized design with six 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 Duncan analysis in the same software ($p = 0.05$).

RESULTS

Anthocyanin leakage and ACO activity: The results indicate that 4 mM MA caused significant decrease in anthocyanin leakage and ACO activity compared to control (Table 1). Addition of 3 mM glutamin and 2 mM SA to 4 mM MA significant decrease in anthocyanin leakage and ACO activity compared to control ($p = 0.05$) (Table 1). Highest means of ACO activity was found in cut flowers treated with 4 mM SA (Table 1).

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

Superoxide dismutase activity: Lily flowers treated by MA alone or together with glutamin and SA had more Superoxide dismutase activity. The maximum Superoxide dismutase activity was recorded in 4mM MA +2 mM SA+3 mM glutamin compare other treatments and control (Table 1). Statistically significant differences existed among 4 mM MA +2 mM SA + 3 mM glutamin compared to other treatments and control ($p = 0.05$). The minimum Superoxide dismutase activity was noted in 4 mM SA (Table 1).

MDA and total chlorophyll content: The results indicate that 4 mM MA caused significant decrease MDA content compared to control (Table 1). Addition of 3 mM glutamin and 2 mM SA to 4 mM MA significant decrease MDA content compared to control (Table 1). A significant negative correlation was observed between MA concentration and the MDA content in Lily cut flowers (Table 1). Total chlorophyll content increased along with MA, SA and glutamin

Table 1: Mean comparisons of chlorophyll content, Vase life, MDA, SOD activity, Membrane stability and ACC Oxidase activity in MA, SA and glutamin treatments

MA (mM)	SA (mM)	Glutamin (mM)	Vase life (day)	Chlorophyll total (a+b) content (spad reading)	ACC oxidase activity (nmol/gFW/h)	Membrane stability (Anthocyanin leakage OD 525)	MDA (μmol/mg protein)	SOD (U g^{-1} protein)
0	0	0	6AC	1AC	65.28AC	174.12D	315.74D	50.01AB
		1.5	8AB	2.64AB	40.11AB	154D	176.39AB	70.07C
		3	8AB	2.7AB	38.15AB	150.65D	170AB	84.56C
	2	0	8AB	3AB	20C	55.14C	63B	100B
		1.5	10C	3.54C	18.14B	50.14C	58.74B	125.36B
		3	11C	3.58C	18B	50.5C	50B	130B
	4	0	4D	0.41D	90AD	200AD	400AD	20.08AC
		1.5	5AC	1.6AC	78.54D	168.25D	180AB	30.17AB
		3	6AC	1.09AC	78.48D	170.14D	200.71AC	33.65AB
2	2	0	10C	4.87C	23.01C	55.8C	70.56C	75.8C
		1.5	11C	4.6C	21C	55C	70.25C	75.14C
		3	11C	5.03B	22C	55C	68.6C	73.56C
	4	0	6AC	2AB	60.3D	70.68AC	100.1C	80.3C
		1.5	8AB	1.94AB	54.12D	69.78AC	87.64C	81.15C
		3	9AB	2.89AB	55D	70AC	80C	87.6C
4	0	0	12B	6.14B	17.41B	40.31C	45.12B	100B
		1.5	12B	5.94B	17.12B	41.56C	45B	106.04B
		3	12B	6.08B	16.89B	40C	43.14B	110B
	2	0	12B	6.06B	14B	20.14B	35.1B	164.45B
		1.5	12B	6.72B	14.5B	22.3B	30.17B	170.36B
		3	14A	7.86A	7.11A	10.08A	21.14A	200.19A
	4	0	8AB	4C	48.7AB	81.5AC	90.15C	70.12C
		1.5	9AB	3.67C	45.6AB	75.6AC	86C	75.6C
		3	8AB	3.45C	45AB	76.84AC	84.15C	70.68C
F-test probabilities								
	MA	0.03	0.02	0.001	0.04	0.03	0.02	0.001
	SA	0.2	0.001	0.008	0.04	0.005	0.04	0.01
	Glutamin	0.01	0.04	0.02	0.04	0.03	0.001	0.02

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

concentrations in Lily cut flower (Table 1). Result showed that 4mM MA with 3 mM glutamin and 2 mM SA leaded to a considerable delay in degradation of chlorophyll compared to other concentrations (SA 4 mM and 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. Production of MDA as a known biomarker for oxidative stress is another consequence (Yildirim *et al*., 2008). In present study use of MA, SA and glutamin as a preservative mixture ingredient increased vase life of cut flowers significantly. 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. The protective function of SA includes the regulation of ROS and antioxidant enzymes (Khan *et al*., 2003; El-Tayeb *et al*.,

2006; Shi and Zhu, 2008; Joseph *et al*., 2010). Fan *et al*. (2008) and Yuping (2009) reported that treatment with salicylic acid 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. Kazemi *et al*. (2010) reported that treatment with MA acid significantly extends the vase life. Previous work had revealed that MA sprays during growth period increased chlorophyll content of cut flowers while citric acid spray caused extended post harvest vase life. Darandeh *et al*. (2010). The observed reduction in MDA content and anthocyanin leakages by MA application supports our conclusion of considering it as a practical agent for retarding of Lily cut flower senescence. Presumably, the relatively low level of MDA in MA 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 MA application could be at least one mechanism through which MA has affected on the senescence process. The effect of MA 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 MA as a new potent agent to be applied in preservative mixtures used for cut flowers.

CONCLUSION

From the results of the present study, it can be concluded that SA, glutamin and MA treatments significantly increase vase life in vase flower preservative solution, reduced MDA content, ACC-oxidase activity and the membrane permeability and per oxidation of lipids. However, present results showed that SA, glutamin, MA treatments maintained the vase life of flowers for a longer period.

REFERENCES

Ansari, M.S. and N. Misra, 2007. Miraculous role of salicylic acid in plant and animal system. Am. J. Plant Physiol., 2: 51-58.

Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-286.

Canakci, S., 2008. Effects of salicylic acid on fresh weight change, chlorophyll and protein amounts of radish (*Raphanus sativus* L.) seedlings. J. Biol. Sci., 8: 431-435.

Coorts, G.D., 1973. Internal metabolic changes in cut flowers. Hort. Sci., 8: 195-198.

Da Silva, J.A.T., 2003. The cut flower: Postharvest considerations. J. Biol. Sci., 3: 406-442.

Doyle, R.J. and H.E. Kubitschek, 1976. Near ultraviolet light inactivation of an energy-independent membrane transport system in *S. cerevisiae*. Photochem. Photobiol., 24: 291-293.

El-Tayeb, M.A., A.E. El-Enany and N.I. Ahmed, 2006. Salicylic acid-induced adaptive response to copper stress in sunflower (*Helianthus annuus* L.) Plant Growth Regul., 2: 372-379.

Fan, M.H., J.X. Wang, G. Shi, L.N. Shi and R.F. Li, 2008. Salicylic acid and 6-BA effects in shelf-life improvement of *Gerbera jamesonii* cut flowers. Anhui Agricultural Science Bulletin. http://en.cnki.com.cn/Article_en/CJFDTOTAL-BFYY200808060.htm.

Figueroa, I., M.T. Colinas, J. Mejia and F. Ramirez, 2005. Postharvest physiological changes in roses of different vase life. Cien. Inv. Agric., 32: 167-176.

Halevy, A.H. and S. Mayak, 1981. Senescence and postharvest physiology of cut flowers, Part 2. Hortic. Rev., 3: 59-143.

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125: 189-198.

Hojjati, Y., A. Khalighi and A.R. Farokhzad, 2007. Chemical treatments of *Eustoma* cut flower cultivars for enhanced vase life. J. Agric. Soc. Sci., 3: 75-78.

Jaleel, C.A., R. Gopi, G.M.A. Lakshmanan and R. Panneerselvam, 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Sci., 171: 271-276.

Joseph, B., D. Jini and S. Sujatha, 2010. Insight into the role of exogenous salicylic acid on plants grown under salt environment. Asian J. Crop Sci., 2: 226-235.

Kaltaler, R.E.L. and P.L. Steponkus, 1976. Factors affecting respiration in cut roses. J. Am. Soc. Hortic. Sci., 101: 352-354.

Kazemi, M., E. Hadavi and J. Hekmati, 2010. The effect of malic acid on the bacteria populations of cut flowers of carnations vase solution. World Applied Sci. J., 10: 737-740.

Khan, W., B. Prithiviraj and D.L. Smith, 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. J. Plant Physiol., 160: 485-492.

Kovacevic, Z. and J.D. McGivan, 1983. Mitochondrial metabolism of glutamine and glutamate and its physiological significance. Physiol. Rev., 63: 547-605.

Liu, Y.L., C.L. Mao and L.J. Wang, 1987. Advances in salt tolerance in plants. Commun. Plant Physiol., 23: 1-7.

Mahdavian, K., K.M. Kalantari and M. Ghorbanli, 2007. The effect of different concentrations of salicylic acid on protective enzyme activities of pepper (*Capsicum annuum* L.) plants. Pak. J. Biol. Sci., 10: 3162-3165.

Mayak, S., Y. Vaadia and D.R. Dilley, 1977. Regulation of senescence in carnation (*Dianthus caryophyllus*) by ethylene: Mode of action. Plant Physiol., 59: 591-593.

Mayak, S. and H. Halevy, 1980. Flower Senescence. In: Senescence in Plants, Thiman, K.V. (Ed.). CRC Press, Boca Raton, Florida, USA., pp: 131-156.

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.

Mba, F.O., Z.T. Xiong and H.J. Qiu, 2007. Salicylic acid alleviates the cadmium toxicity in Chinese cabbages (*Brassica chinensis*). Pak. J. Biol. Sci., 10: 3065-3071.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.

Poovaiah, B.W., 1979. Increased levels of calcium in the nutrient solution improves the postharvest life of potted roses. J. Am. Soc. Hort. Sci., 104: 164-166.

Rogers, M.N., 1973. An historical and critical review of postharvest physiology research on cut flowers. Hort. Sci., 8: 189-194.

Shi, Q. and Z. Zhu, 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. Environ. Exp. Bot., 63: 317-326.

Wright, L.A. Jr., T.M. Murphy and R.L. Travis, 1981. The effect of ultraviolet radiation on wheat root vesicles enriched in plasma membrane. Photochem. Photobiol., 33: 343-348.

Yildirim, B., F. Yasar, T. Ozpay, D. TurkOzu, O. Terziolu and A. Tamkoc, 2008. Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. *Arvense* L.). J. Anim. Vet. Adv., 7: 907-910.

Yuping, Z., 2009. Effects of salicylic acid on fresh keeping of cut gerbera jamesonii flower. Anhui Agricultural Science Bulletin. http://en.cnki.com.cn/Article_en/CJFDTOTAL-AHNB200913063.htm.