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Effect of Some Treatment Chemicals on Keeping Quality and Vase-life of Gebrera Cut Flowers

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ABSTRACT

The experiment was carried out to investigate the effect of different concentrations of Salicylic acid, Malic acid, Citric acid and Sucrose on keeping quality and vase life of gebrera cut flowers. In this study three levels of malic acid (0, 100 and 150 mg L^{-1}), two levels of sucrose (0 and 3% w/v), three levels of salicylic acid (0 , 1.5 and 3 mM) and two levels of citric acid (0 and 150 mg L^{-1}) were applied in a factorial arrangement, carried out in a complete randomized design on 144 gebrera cut flowers. The vase were placed in chambers at 19°C, relative humidity about 70% and 14 h photoperiod that was maintained using fluorescent lamps (light intensity of 15 μmol m^{-2} sec^{-1}) at the top of the corolla. The recorded traits included Vase life, total chlorophyll content (SPAD reading), anthocyanin leakage, MDA content, ACC-Oxidase activity and water absorption. The results showed that malic acid, salicylic acid and sucrose treatments increased cut-flower water absorption, fresh weight and vase life, while decreasing MDA content, ACC-oxidase activity and membrane permeability together with total delaye of senescence and peroxidation of lipids. Maximum flower vase life was recorded in treatment with 150 mg L^{-1} malic acid+1.5 mM salicylic acid+sucrose 3%. A direct relationship between vase life and increasing of fresh weight and water uptake was observed as well.

Key words: Vase life, gebrera, chemical treatments, ACC-oxidase activity, anthocyanin leakage

INTRODUCTION

Gerbera, also known as Transvaal daisy or Barberton daisy Gerbera jamesonii (Bol. ex Adlam) is a member of the composite family. 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 (Sankat and Mujaffar, 1994), carbohydrate depletion, microorganisms (Witte and Van Doom, 1991) and ethylene effects (Wu *et al*., 1991; Da Silva, 2003). 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 (Tang *et al*., 1994; Da Silva, 2003). Ethylene production causes a sharp increase in production of oxygen free radicals which is responsible for stress dependent peroxidation of membrane lipids. 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). The effects of Senescence can be reduced by inhibitors of ethylene biosynthesis and increase enzyme antioxidant activity (Khan *et al.*, 2003; El-Tayeb *et al.*, 2006; Shi and Zhu, 2008; Joseph *et al.*, 2010). 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 and Misra, 2007; Mba *et al.*, 2007; Mahdavian *et al.*, 2007; Canakci, 2008). Ethylene production of cut Gerbera flowers increased with flower senescence and treatment with Salicylic Acid (SA), an ethylene produce inhibitor, extended flower longevity (Fan *et al.*, 2008). 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). 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 postharvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amount of metabolic sugars is factors that affect the rate of senescence. Keeping the flower in vase solutions containing sucrose has been shown to extend their vase-life. Han (2003) found that addition of sugar to vase solution improved the intensity of the petal color but did not improve bud opening, longevity, or size of non-cold-stored Oriental Lily cv. Stargazer cut flower harvested at 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 (Han, 2003). The purpose of this study was to find responses of the cut gebrera flowers to salicylic acid application and its effect on vase life, ACC-oxidase activity, MDA accumulation and enzyme antioxidant activity.

MATERIALS AND METHODS

The experiment was started on september 10, 2010 and chlorophyll content, Membrane stability, MDA content and ACC Oxidase activity were measured at 13th day of vase life. Gerbera (Gerbera, jamesonii) were obtained from local commercial greenhouses (Pakdasht, Tehran, Iran). Following harvest and transport to the laboratory, the stems were recut to 40 cm length. In this study three levels of malic acid (0,100 and 150 mg L^{-1}), two levels of sucrose (0 and 3% w/v), three levels of Salicylic acid (0, 1.5 and 3 mM) and two levels citric acid (0 and 150 mg L^{-1}) were applied on 144 gebrera cut flowers. After recording the fresh weight, each flower was placed in a 250 mL bottle containing preservative solutions.

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 leaves was considered.

Determination of anthocyanin leakage: Anthocyanin leakage was measured based on the method of Poovaiah (1979).

Determination of Acc-oxidase activity: ACC oxidase activity was assayed by measuring to the method described by Maya-Lean and John (1994).

Assays of MDA content (Lipid peroxidation): Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according to Heath and Packer (1978).

Microbe population: Test Microbe population were isolated from vase solutions of gebrera by measuring to the method described by Zagory and Reid (1986).

Water uptake and weight fresh: The volume of water uptake was calculated by subtracting the volume of water evaporated from a control bottle without cut flowers from the amount of water decreased in bottles containing flowers. The fresh weight of the cut flowers also measured in initial day and terminal day of experiment.

Superoxide dismutase: The activity of superoxide dismutase was measured based on the method described by Beauchamp and Fridovich (1971).

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 ($p = 0.05$).

RESULTS AND DISCUSSION

Anthocyanin leakage and ACO activity: The results indicate that Anthocyanin leakage and ACO activity was not affected by citric acid treatment. Treatment with 150 mg^{-1} MA+0 mM SA+3% sucrose solution slightly reduced the Anthocyanin leakage and ACO activity and extended vase life of the gebrera (Table 1). While the improvement in membrane stability with increased concentrations of SA and MA solution, flowers treated with 150 mg^{-1} MA+1.5 mM SA and 3% sucrose solution significantly decreased Anthocyanin leakage and ACO activity ($p = 0.05$). Highest means of ACO activity was found in cut flowers treated with 3 mM SA (Table 1). 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 the same treatment with salicylic acid significantly extends the vase life with reduced the Anthocyanin leakage and ACO activity. Also, Kazemi *et al*. (2010) reported that treatment with malic acid significantly extends the vase life with decrease Acc-oxidase activity. In our study use of MA, SA and sucrose as a preservative mixture ingredient increased vase life of cut flowers significantly with reduced the Anthocyanin leakage and ACO activity.

Water uptake and weight fresh and Microbe population: Results regarding the water uptake by the gebrera flowers show that maximum Water uptake and weight fresh was in gebrera flowers that treated by 150 mg^{-1} MA+1.5 mM SA+Suc 3% and 150 mg^{-1} MA+1.5 mM SA and these treatments differed non-significantly with each other (Table 1). The minimum Water uptake and weight fresh was noted in 3 mM SA compared to control. Adding MA was found to be positively correlated with water uptake of the gebrera cut flower (Table 1). MA and SA affected on the Microbial population in vase solution of gebrera cut flowers significantly, the Microbial population decreased with the increase in concentrations of MA and SA and the lowest microbial concentration

Table 1: Mean comparisons of chlorophyll content, Vase life, MDA content, SOD activity, Microbe population, Membrane stability and ACC Oxidase activity in MA, SA, Citric acid, Sucrose and their Interaction

Treatment					ACC oxidase					
MA (mg^{-1})	SA (mM)	Suc (w/v)	Vase life (day)	Chlorophyll total (a+b) content (spad reading)	activity (nmol g^{-1} FW h^{-1})	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol mg^{-1} protein)	Microbe population (cfu)	Water uptake (mL)	SOD (U g^{-1} Protein)
0	0	0	6^{AB}	2^{C}	35^{AB}	111^{AB}	104^{AC}	45^{AB}	30^{AB}	50^{AB}
	0	3	8^{C}	3^{B}	33^{AB}	54.32^{C}	62.11^{AB}	60^{AC}	45^{C}	72.14^{C}
	1.5	0	9^{C}	3.14^{C}	18^{B}	33.12^{B}	43.74^{C}	15^{A}	60^{B}	126.36^{B}
	3	0	4^{AC}	1^{AB}	48.35^{AC}	218^{AC}	119.14^{D}	12^{A}	15^{AC}	20.12^{AC}
100	0	0	7^{C}	2.33^{C}	27^{C}	55.12^{C}	51.17^{C}	20^{B}	55^{B}	100^{B}
	1.5	0	8^{C}	3.18^{B}	23.14^{C}	30.14^{B}	40.12^{C}	20^{B}	65^{B}	120.14^{B}
		3%	9^{C}	3.9^{B}	22.78^{C}	30^{B}	41.25^{C}	30^{C}	65^{B}	121^{B}
	3	0	6^{AB}	2^{C}	30.14^{AB}	110^{AB}	81^{AB}	21^{B}	20^{AB}	60.12^{C}
		3%	6^{AB}	2^{C}	$31A^{B}$	80.08^{C}	70.39^{AB}	33^{C}	30^{AB}	65^{C}
150	0	0	10^{B}	4^{B}	16.14^{B}	25.14^{B}	44.18^{C}	20^{B}	70^{B}	124^{B}
	1.5	0	11^{B}	4.5^{B}	10^{A}	22.11^{B}	30.12^{A}	10^{A}	70^{B}	130^{B}
		3%	13^{A}	5^{A}	15^{B}	14.35^{A}	29^{B}	28^{B}	90^{A}	179.61^{A}
	3	0	6^{AB}	1.8^{C}	29.17^{C}	50.12^{B}	49.12^{C}	11^{A}	40^{C}	54.17^{AB}
		3%	7^{C}	2^{C}	25.14^{C}	40.39^{B}	50^{C}	24^{B}	45^{C}	69.78^{C}
F-test probabilities										
	MA		0.02	0.0001	0.03	0.04	0.03	0.02	0.02	0.01
	SA		0.001	0.03	0.002	0.004	0.001	0.002	0.03	0.01
	sucrose		0.01	0.02	0.04	0.03	0.04	0.6	0.04	0.04

Means in each column followed by similar letters are not significantly different at 5% level. Data recorded on day 11 of experiment

was evident when cut flowers were treated with 150 mg^{-1} MA+1.5 mM SA (Table 1). Anjum *et al.* (2001) reported Adding a suitable germicide in vase water can prevent the growth of microbes and increased water uptake. Kazemi *et al.* (2010) showed that the treatment of MA reduced Microbial population in vase solution of carnation cut flowers and increased Water uptake in carnation cut flowers. Also salicylic acid seems to act by germicide the decrease of Microbial population. In contrast, the sucrose in vase solution rapidly increased microbial population in vase solution of gebrera cut flowers.

MDA content and Superoxide dismutase activity: MDA accumulation was inhibited in the flowers of gebrera by MA, SA and sucrose to a larger extent when compared to control plant (Table 1). The results indicate that MDA accumulation was not affected by citric acid treatment. A significant negative correlation was observed among MA and SA concentrations and the MDA content in gebrera cut flowers (Table 1). Highest means of MDA accumulation was found in cut flowers treated with 3 mM SA (Table 1). The results indicate that gebrera flowers treated by SA alone or together with sucrose and MA had more Superoxide dismutase activity. The maximum Superoxide dismutase activity was recorded in flowers treated by 150 mg^{-1} MA+1.5 mM SA+3% compared other treatments and control ($p = 0.05$) (Table 1). Statistically significant differences existed among 150 mg^{-1} MA+0 mM SA+3% compared to other treatments and control. The minimum Superoxide dismutase activity was noted in 3 mM SA (Table 1). The protective function of SA includes the regulation of ROS and antioxidant enzymes (Khan *et al.*, 2003); Shi and Zhu, 2008). SA acid with increases the enzyme antioxidant activity cause delay the onset of hydrolysis of structural cell components, decrease ROS production and sensitivity (Mba *et al.*, 2007; Mahdavian *et al.*, 2007; Canakci, 2008). Other studies have shown that exogenous SA can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress (He *et al.*, 2002).

Yuping (2009) reported that treatment with salicylic acid significantly extends the vase life with increases the enzyme antioxidant activity and decreased ROS production. Also, present result showed that adding MA in vase water significantly increases the enzyme antioxidant activity, this result indicates MA inhibits the conversion of ACC to ethylene in gebrera petals. Present results showed adding SA, MA and sucrose was found to be positively correlated with increases the enzyme antioxidant activity of the gebrera cut flower (Table 1).

Total chlorophyll content: MA, SA and SUC treatments significantly increased the total chlorophyll content to a larger extent when compared to control ($p = 0.05$). The maximum total chlorophyll content was noted in 150 mg^{-1} MA+1.5 mM SA+Suc 3% and 150 mg^{-1} MA+1.5 mM SA treatments compared to control. The minimum total chlorophyll content was noted in 3 mM SA compared to control. Canakci (2008) reported that treatment with salicylic acid significantly extends the vase life with increases total chlorophyll content. Kazemi *et al.* (2010) reported that treatment with salicylic acid significantly extends the vase life with increases total chlorophyll content in carnation cut flowers. Previous study 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). Present results showed adding SA, MA and sucrose was found to be positively correlated with total chlorophyll content of the gebrera cut flower (Table 1).

Vase life: Flower stems kept in water containing MA and SA had significantly increased vase life relative to the water control for all concentration of MA and SA except than 3 mM Salicylic acid ($p = 0.05$) (Table 1). The use of 150 mg^{-1} MA+1.5 mM SA+Suc 3% resulted in a greater extension in vase life than other treatments. The results indicate that Vase life was not affected by citric acid alone treatment. Fan *et al.* (2008) 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 (1999) reported that treatment with sucrose extends the vase life of florets harvested at a bud stage. Keeping the flowers in vase solutions containing sucrose has been shown to extend their vase-life (Han, 2003; Yamane *et al.*, 2005). Kazemi *et al.* (2010) showed that the treatment of MA increased vase life in carnation cut flowers. Our results showed adding SA, MA and sucrose was found to be positively correlated with vase life of the gebrera cut flower (Table 1).

CONCLUSION

From the results of the present study, it can be concluded that salicylic acid and malic acid with sucrose treatments significantly decrease produce MDA and ACC-oxidase activity, bacteria populations in vase flower preservative solution, reduce the membrane permeability and peroxidation of lipids compared to the control. Salicylic acid, malic acid and sucrose also proved more effective in delaying petal senescence and/or flower wilting. However, result our showed that salicylic acid, malic acid and sucrose treatments maintained the vase life of flowers for a longer period.

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