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Delaying of Postharvest Senescence of Lisianthus Cut Flowers by Salicylic Acid Treatment

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Abstract

Salicylic acid (SA) is considered to be plant signal molecule that plays a key role in plant growth, development, and defense responses. The physiological mechanism of exogenous SA to affect the senescence of cut lisianthus flowers during vase life was investigated. Fresh cut lisianthus flowers were treated with distilled water (control), 0.5, 1 and 2 mM SA and then held at 25 ˚C up to 12 days. Exogenous SA supply at 1 mM extended vase life, which was associated with reduced electrolyte leakage and MDA content. SA treatment also reduced activity of lipoxygenase (LOX), which is responsible for membrane lipid peroxidation. SA treatment also enhanced activities of catalase (CAT) and ascorbate peroxidase (APX) and decreased H_2O_2 accumulation during vase life. Thus, exogenous SA supply could maintain membrane integrity by increasing antioxidant system activity, thereby retarding the senescence of cut lisianthus flower during vase life.

Keywords: Antioxidant enzyme, Lipoxygenase, Lisianthus, Salicylic acid, Vase life.

INTRODUCTION

Lisianthus (*Eustoma grandiflorum*) is becoming one of the most highly ranked cut flowers in international markets, due to its rose-like flower shapes and beautiful colors (Bahrami *et al.*, 2013). Vase life as a commercial attribute determines the flexibility of the market at any one time, particularly for cut flowers. The short vase life of cut flowers is related to physiochemical processes which affect senescence. These attributes are highly influenced by water loss and wilting during transportation. Water deficit and consequent precocious senescence result in poor quality of cut flowers and loss of markets, and there are many reports on these effects (Ezhilmathi *et al.*, 2007). Maintaining the quality of cut flowers is one of the main challenges of florists in the flower trade worldwide. In floriculture, delaying the onset of senescence in order to prolong the vase life of cut flowers is the focus of many researchers (Hassan and Ali, 2014).

Membrane deterioration is an early and characteristic feature of petal irreversible senescence of cut flowers. Increased lipid peroxidation, mediated and sustained by phospholipid-degrading enzymes, such as phospholipase D (PLD) and lipoxygenase (LOX), results in a loss of membrane integrity, which has been noted in the senescing petal tissues (Brown *et al*., 1990). It has been observed that flower senescence is accompanied with increased permeability of petal cells and increased ROS production (Reezi *et al*., 2009). Accumulation of harmful ROS such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) lead to the oxidation of the cells vital molecules such as loss of membrane integration via lipid peroxidation, protein oxidation, enzymatic activity inhibition, and, finally, damage to DNA and RNA and dictates, ultimately, oxidative stress. In plant cells, ROS accumulation may be due to the inescapable leakage of electrons onto O_2 from the electron transport chain in chloroplasts and mitochondria and/or by the activation of LOX or NADPH oxidases located in cell membranes (Aghdam and Bodbodak, 2014). Cellular antioxidants are an important buffer against free radical-induced oxidations (Smith *et al*., 1989). For the survival of plants, appropriate functioning of the antioxidant system is important to maintain a balance between ROS production and scavenging. Several enzymes such as catalase (CAT) and ascorbate peroxidase (APX) are involved in the scavenging of ROS in plant systems (Scebba *et al*., 1999).

Postharvest treatments have been used to increase cut flowers vase life by regulating water balance, distribution of assimilates, delaying senescence and blocking microbial agents. However, use of nontoxic, easy to use and inexpensive molecules is always crucial in this respect for large-scale applications. Salicylic acid (SA) has been considered a new potential alternative for this purpose and has been found to affect physiological and biochemical functions in plants (Asghari and Aghdam, 2010). In addition, a potential role of SA in response to stresses and gene expression during senescence has been demonstrated (Morris *et al*., 2000). Recently, it has been found that SA delayed gladiolus and rose cut flower senescence (Ezhilmathi *et al*., 2007; Alaey *et al*., 2011). Alaey *et al*. (2011) suggested that the SA is able to increase the vase life of cut rose flowers and delay senescence by regulating the plant water and increasing the scavenging capacity of cells. In the present study, we investigated the effects of pulse treatment with SA on the vase life of cut lisianthus flowers, as well as physiological and biochemical changes during its petal senescence.

MATERIALS AND METHODS

Flowers and treatments

Cut flowers of lisianthus (*Eustoma grandiflorum*) 'Miarichi Grand White' were obtained from a commercial greenhouse and were re-cut under tap water to have uniform length of 30 cm. Flowers were then placed in a preservative solution containing distilled water (control), 0.5, 1 and 2 mM SA. All treatments were kept at 25 ± 1°C under a 16:8 h light/dark cycle and 60 ± 5% RH for 24 hours. Subsequently, flowers were transferred to flasks containing only 200 ml^{-1} distilled water. The end of vase life was evaluated as the time which 50% of the open flowers had wilted (Cho *et al*., 2001).

Membrane integrity evaluation

Membrane permeability, expressed by relative electrolyte leakage rate, was measured by the method of Jiang and Chen (1995). Thirty petal discs were immersed in 20 mL of 0.3 M mannitol solution at 25 °C, followed by shaking for 30 min. Electrolyte leakage was determined with a conductivity meter. Total electrolyte leakage was determined after boiling the samples for 10 min. and cooling to 25 °C. Relative electrolyte leakage rate was expressed as a percentage of total electrolyte leakage. MDA content was measured according to the method of Heath and Parker (1968). Frozen petal tissues (1 g) from 10 flowers were ground finely in liquid nitrogen, then homogenized in 15 mL of 10% trichloroacetic acid (TCA) and finally centrifuged at 5000 × g for 10 min. The supernatant phase was then collected. MDA content was determined by adding 5 mL of 0.5% thiobarbituric acid (dissolved in 10% TCA) to 0.5 mL supernatant. The solution was heated at 95 ◦C for 20 min, quickly cooled, and centrifuged at 10,000 × g for 10 min to clarify precipitation. Absorbance at 532 nm was measured and subtracted from the non-specific absorbance at 600 nm. The concentration of MDA was calculated with an extinction coefficient of 1.55 n mol $L^{-1}m^{-1}$. MDA content was expressed as n mol g^{-1} fresh weight (FW).

According to method of Doderer *et al*. (1992), for analysis of LOX activity, frozen petal tissues (1 g) from 10 flowers were ground finely in liquid nitrogen and then homogenized in 15 mL of 50 mM phosphate buffer (pH 7.0). After centrifugation at 10,000 × g and 4 ◦C for 20 min, the supernatant was collected and then used as the crude enzyme extract. LOX activity was assayed at 25 ◦C by monitoring the formation of conjugated dienes from linoleic acid at 234 nm according to the method of Axelrod *et al*. (1981). The reaction mixture (3 mL) contained 2.8 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.1 mL of 10 mM sodium linoleic acid solution and 0.1 mL of the crude enzyme solution. One unit of LOX activity was defined as a change of 0.01 in absorbance per minute at 25 ◦C. The specific LOX activity was expressed as U mg^{-1} protein.

Antioxidant system activity evaluation

Frozen petal tissues (2 g) from 10 flowers were ground finely in liquid nitrogen and then homogenized in 15 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) PVP. The homogenate was centrifuged at 10,000 × g for 15 min at 4 ◦C and then the supernatant was used to determine activities of CAT and APX. CAT activity was assayed by measuring the disappearance of hydrogen peroxide (H_2O_2) according to the method of Oracz *et al*. (2009). The assay mixture (3 mL) contained 2.95 mL of 44.25 M H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 0.05 mL of enzyme extract. CAT activity was calculated by a decrease in absorbance at 240 nm for 3 min at 25 ◦C. One unit of CAT activity was defined as the amount of the enzyme that caused a change of 0.001 in absorbance per minute and the specific activity was expressed as U mg^{-1} protein. APX activity was determined by the method of Nakano and Asada (1981). The reaction mixture (3 mL) consisted of 1.5 M ascorbic acid, 0.3 M EDTA and 0.3 M H_2O_2 solution in 50 mM phosphate buffer (pH 7.0) and 0.1 mL of enzyme extract. Ascorbate concentration was followed by the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM cm^{-1}). One unit of APX activity was defined as 1 M ascorbate oxidized per minute at 290 nm and the specific activity was expressed as U mg^{-1} protein. The protein concentration of petal extracts was estimated using the method of Bradford (1976) by BSA as standard. The H_2O_2 content measured according to Patterson *et al*. (1984). Frozen petal tissues (1 g) from 10 flowers were homogenized with 10 ml of acetone at 0 °C. After centrifugation for 15 min at 6000 × g at 4 °C, the supernatant was collected. The supernatant (1 ml) was mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml ammonia, and then centrifuged for 10 min at 6000 × g and 4 °C. The pellets were dissolved in 3 ml of 10% (v/v) H_2SO_4 and centrifuged for 10 min at 5000 × g. Absorbance of the supernatant phase was measured at 410 nm. H_2O_2 content was calculated using H_2O_2 as a standard and then expressed as µmol g^{-1} fresh weight (FW).

For physiological parameters, results were expressed as mean ± SE from 3 replications.

Statistical significance between mean values was assessed using one way analysis of variance with SAS (Version 9.1) statistical software. Means were compared using the LSD test.

RESULTS AND DISCUSSION

Vase life

As shown in Fig. 1, treatment with postharvest SA at 1 mM resulted in a higher lisianthus cut flowers vase life ($P<0.01$). Based on these results, 1 mM SA for postharvest treatment was chosen for further analyses.

Salicylic acid treatment and membrane integrity

Electrolyte leakage of the lisianthus cut flowers increased during vase life (Table 1). The electrolyte leakage of lisianthus cut flowers treated with 1 mM SA at postharvest stage remained lower than that in untreated control flowers ($P<0.01$; Table 1). As well, during vase life, the MDA content in the lisianthus cut flowers increased (Table 1). Compared to the controls, a lower content of MDA was found in the lisianthus cut flowers treated with postharvest 1 mM SA ($P<0.01$; Table 1). There was a significant increase in the activity of LOX in lisianthus cut flowers during vase life (Table 1). The treatment with SA caused reduction in LOX activity in comparison to the control for the whole 12 days of vase life ($P<0.01$; Table 1).

Electrolyte leakage is an effective parameter to assess membrane permeability and therefore is used as an indicator of membrane integrity. In addition, lipid peroxidation, responsible for loss

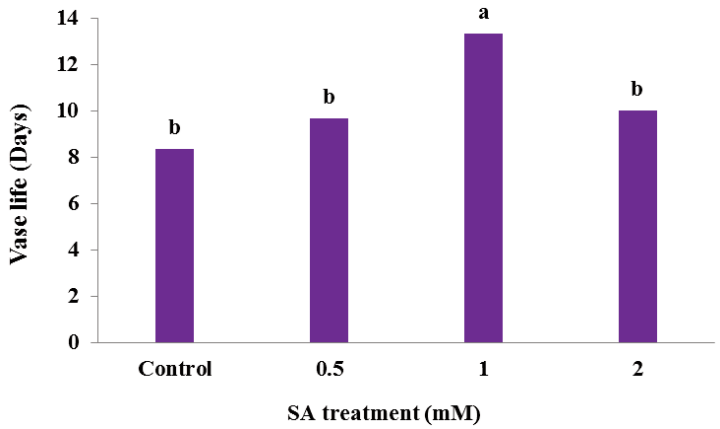

Fig.1. Effects of salicylic acid treatment at 0.05, 1 and 2 mM on vase life of lisianthus cut flowers.

Table 1. Effect of postharvest salicylic acid treatment at 1 mM on electrolyte leakage, MDA content and LOX enzyme activity of lisianthus cut flowers for 12 days.

Time (day)	Treatmenta	Membrane integrity		
	SA (mM)	EL (%)	MDA (n mol g^{-1} FW)	LOX (U mg^{-1} protein)
3	0	18.55 ± 1.231 ^{c}	2.125 ± 0.751 ^{d}	0.908 ± 1.531 ^{d}
	1	19.62 ± 1.186 ^{c}	1.432 ± 0.477 ^{e}	0.686 ± 1.486 ^{e}
6	0	22.15 ± 0.342 ^{b}	3.172 ± 0.282 ^{c}	1.876 ± 0.382 ^{c}
	1	19.94 ± 0.935 ^{c}	2.052 ± 0.154 ^{e}	0.758 ± 0.935 ^{e}
9	0	26.48 ± 0.472 ^{ab}	5.353 ± 0.354 ^{b}	2.650 ± 0.452 ^{b}
	1	20.37 ± 0.337 ^{c}	3.752 ± 0.753 ^{c}	1.131 ± 0.317 ^{c}
12	0	37.43 ± 1.652 ^{a}	7.785 ± 0.425 ^{a}	3.589 ± 1.128 ^{a}
	1	22.44 ± 1.286 ^{b}	5.423 ± 0.236 ^{b}	2.149 ± 1.682 ^{b}
Significant	df			
Treatment	1	**	**	**
Time	3	**	**	*
T × T	3	*	*	*

Mean separation by Duncan's Multiple Range Test at P = 0.05. The same letters within a column are not significantly different. *P < 0.05; **P < 0.01; ns: not significant

of cell membrane integrity, could be evaluated by the content of malonyldialdehyde (MDA; Aghdam and Bodbodak (2014). Lipid peroxidation could be carried out by enzymatic oxidation of unSFA by LOX or by non-enzymatic oxidation by ROS. MDA is the end product of the peroxidation of membrane fatty acids. The quantity of MDA is used as a marker of oxidative stress and a rise of MDA indicates damage of cell membrane integrity. The main result of both events is the loss of the biomembrane functionality (Sevillano *et al*., 2009). Hassan and Ali (2014) reported that the 1-MCP or SA treatments significantly prolonged the vase life and minimized the weight loss of gladiolus spikes compared with the control. Both treatments enhanced the relative water content (RWC) of leaves and maintained chlorophyll content compared with the control values, which were decreased. Ethylene production, proline accumulation and MDA content were increased in florets of untreated spikes. 1-MCP or SA reduced ethylene production, decreased both proline content and MDA level and hence maintained membrane stability. The increment in MDA has been described as a biomarker of lipid peroxidation (Bailly *et al*., 1996) and thus decreased its level in lisianthus cut flowers treated with SA indicates reduced lipid peroxidation. Reduced lipid peroxidation participates to decreased electrolyte leakage in response to SA treatment. Such effect of SA as lipid peroxidation reduction and maintained cell stability was previously reported by Ezhilmathi *et al*. (2007) and Hatamzadeh *et al*. (2012). Reduced lipid peroxidation and retained membrane stability have been demonstrated to be inversely proportional with flower senescence in gladiolus (Hatamzadeh *et al*., 2012).

Mansouri (2012) reported that the SA at 0.1 and 1.0 mM and nitric oxide at 0.1 mM increased vase life and decreased fresh weight loss of chrysanthemum flowers. Anthocyanin content increased in chrysanthemum flowers treated with 1 mM SA and nitric oxide. The electrolyte leakage associated with MDA accumulation reduced in chrysanthemum flowers treated with SA and nitric oxide treatments. Reducing sugar contents increased with SA treatment. Postharvest SA and nitric oxide application at low concentration prolonged vase life of cut chrysanthemums by improving the membrane stability and decreasing the lipid peroxidation. Mansouri (2012) suggested that the extended vase life in SA treated chrysanthemums is associated with decreased fresh weight loss, improved membrane permeability and decreased lipid peroxidation. According to our results, SA might extend vase life through improving membrane permeability and decreasing of lipid peroxidation. Since lipid peroxidation is mediated by ROS (Kellogg, 1975), therefore SA may either be directly scavenging ROS and thus decreasing lipid peroxidation, or it may be modulating the activity of antioxidant enzymes. Senescing plant tissue also experiences an increase in LOX activity, which also promotes the process of membrane polyunsaturated fatty acid peroxidation (Lynch and Thompson, 1984). Similar to lipid peroxidation (MDA content), SA caused a decrease in LOX activity during vase life (Table 1). An increase in LOX activity has been correlated with an increase in cell membrane permeability and senescence in daylily and rose (Panavas and Rubinstein, 1998; Fukuchi-Mizutani *et al*., 2000).

Salicylic acid treatment and antioxidant system activity

As shown in Table 2, lisianthus cut flowers treated with SA showed higher activities of CAT and APX associated with lower H_2O_2 accumulation during vase life ($P<0.01$; Table 1). Hassan and Ali (2014) reported that the 1-MCP or SA treatments significantly prolonged the vase life and minimized the weight loss of gladiolus spikes compared with the control. An increase in floret antioxidant enzyme activities (CAT, SOD and POX) was observed in 1-MCP or SA treated spikes compared with the control. The effects of 1-MCP or SA on floret senescence seemed not entirely limited due to their effects on ethylene, but they most likely had a sustainable impact on the membrane integrity. Hassan and Ali (2014) reported that the 1-MCP or SA treatments alleviated the oxidative stress in cut flowers during postharvest senescence. The role of SA treatment in scavenging the ROS and preventing flower senescence is previously indicated (Ezhilmathi *et al*., 2007;

Table 2. Effect of postharvest salicylic acid treatment at 1 mM on antioxidant enzymes CAT and APX activity and H_2O_2 accumulation of lisianthus cut flowers for 12 days.

Time (day)	Treatmenta	Antioxidant system activity		
	SA (mM)	CAT (U mg^{-1} protein)	APX (U mg^{-1} protein)	H_2O_2 (µ mol g^{-1} FW)
3	0	0.921 ± 0.151 ^{a}	2.43 ± 0.234 ^{a}	59.44 ± 7.235 ^{a}
	1	1.242 ± 0.180 ^{a}	2.82 ± 0.193 ^{a}	49.26 ± 2.460 ^{a}
6	0	0.865 ± 0.381 ^{b}	1.36 ± 0.564 ^{b}	65.46 ± 3.875 ^{b}
	1	0.882 ± 0.482 ^{c}	2.21 ± 0.342 ^{ab}	51.87 ± 5.539 ^{a}
9	0	0.785 ± 0.175 ^{c}	1.22 ± 0.457 ^{c}	70.63 ± 7.789 ^{c}
	1	0.821 ± 0.645 ^{cd}	1.88 ± 0.367 ^{b}	56.79 ± 4.124 ^{a}
12	0	0.687 ± 0.156 ^{d}	0.88 ± 0.456 ^{d}	92.66 ± 5.724 ^{d}
	1	0.985 ± 0.613 ^{c}	1.34 ± 0.269 ^{c}	78.76 ± 6.533 ^{c}
Significant	df			
Time	3	**	**	*
Treatment	1	**	**	**
T × T	3	*	**	*

Mean separation by Duncan's Multiple Range Test at P = 0.05. The same letters within a column are not significantly different. *P < 0.05; **P < 0.01; ns: not significant

Hatamzadeh *et al*., 2012). The activities of antioxidant enzymes are considered as a response against oxidative stress (Zhou *et al*., 2014). SA treatments enhanced the production of antioxidant enzymes which scavenge the ROS, as indicated by the decreased level of MDA (Table 1 and 2).

Cellular membranes are highly prone to ROS such as H_2O_2 attack, and it is reasonable to propose that progressive decline in membrane stability assayed by MDA content is probably the consequence of enhanced ROS attack under decreasing antioxidant activity such as CAT and APX enzymes activity during vase life (Table 2). Senescence of flowers has been delayed by the use of commercial ROS scavengers, such as SA (Alaey *et al*., 2011). In the present study, the decline in membrane integrity of lisianthus cut flowers was alleviated by treatment with SA, which was associated with an increase in CAT and APX activity in treated flower. It is therefore reasonable to propose that SA has a role in the induction of antioxidant enzymes and/or might also be acting as a scavenger of ROS, thus maintaining membrane integrity for extended period. Ezhilmathi *et al*. (2007) reported that petal wilting in *Gladiolus* is associated with ROS induced lipid peroxidation, enhanced LOX activity, and decrease in ROS scavenging system in the form of SOD and CAT. Also, Ezhilmathi *et al*. (2007) reported that the *Gladiolus* cut flowers treated with 5-sulfosalicylic acid (5-SSA) exhibited significantly higher water uptake, vase life, number of opened florets and lower number of unopened florets. *Gladiolus* cut flowers treated with 5-SSA also exhibited lower respiration rates, lipid peroxidation and LOX activity, and higher membrane stability, soluble protein concentration, and activity of SOD and CAT. Results suggested that 5-SSA increased vase life by increasing the ROS scavenging activity of the gladiolus cut flowers. Promyou *et al*. (2012) reported that postharvest treatment with salicylic acid (2 mM for 15 min) alleviated CI in anthurium cut flower, an effect associated with decreasing electrolyte leakage, MDA content and lipoxygenase (LOX) activity, and increasing catalase (CAT) and superoxide dismutase (SOD) activities, which led to a decreasing of spathe browning and fresh weight loss, two detrimental effects of CI on this ornamental. Alaey *et al*. (2011) reported that the SA treated cut rose flower showed higher water uptake, relative fresh weight, and CAT activity. SA retarded the decrease of CAT activity during flowers senescence. Alaey *et al*. (2011) suggested that the postharvest SA application prolonged vase life in cut rose flowers by improving the ROS scavenging capacity related to CAT activity and by better regulation of the water balance.

CONCLUSION

In conclusion, the study was an attempt to investigate the potential roles of SA in delaying the senescence of cut lisianthus flowers. SA was able to prolong the vase life and delay flower

senescence by maintaining membrane integrity, which was result from decreasing LOX enzyme activity as responsible for membrane lipid peroxidation and increasing the antioxidant enzymes CAT and APX activities, which was led to diminishing H_2O_2 accumulation. The effects of SA treatment on retarding flower senescence was due to increased antioxidant enzyme activities and thus reduced lipid peroxidation and maintained membrane stability, assayed by electrolyte leakage and MDA content.

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