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Full Length Research Paper

The Effectiveness of Pre-Harvest Salicylic Acid Application on Physiological Traits in Lilium (*Lilium longiflorum* L.) Cut Flower

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Abstract. The aim of the study was to determine the effect of foliar application of salicylic acid (SA) on Lilium cut flower quality. At the stage of bud initiation, four concentrations (0, 50, 100 and 200 ppm) of SA were sprayed on shoot. After treatment, morphological and physiological characteristics were measured within 7 days. It is resulted that SA and Time have significant effect on flowering stem length (FSL), flowering stem diameter (FSD) and bud volume (BV) ($P<0.01$) and also the interaction between SA*Time has a significant effect on bud volume ($P<0.05$). According to the results, flowering stem length, flowering stem diameter and bud volume were significantly influenced by SA pretreatment and time. SA pretreatment at 50 ppm concentration had a positive effect on mentioned traits however at high concentration, especially at 200 ppm concentration, inhibitory effects were observed. There was an increasing trend in values for these traits over time and the highest values were obtained in the last day. Leaf chlorophyll and malondialdehyde content were not significantly affected by exogenous SA treatment hence promotion effects of SA on oxidative stress was not clear. Membrane stability of the younger leaves were greater than the older leaves furthermore the response of these leaves, against SA treatment, was more tolerable. Overall, the damaging effect of high dosages of SA is more on middle leaves and also this leaves showed more response to SA spray. In this case probably SA pre-harvest spray can done on the stem middle leaves and upper leaves and avoid of damaging effects of SA on other parts of plants specially flower bud induction or initiation. It is concluded that SA at 50ppm concentration improves morphological characteristics of Lilium cut flower so the flower quality increases in response to these modifications.

Key words: *Lilium longiflorum*, membrane stability, morphological characteristics, salicylic acid, bud size

1. INTRODUCTION

Short postharvest vase life of *Lilium longiflorum* is one of the most important problems in production of this valuable cut flower (Da Silva, 2003; Kader, 2003). To control this natural phenomenon, many studies are performed and some findings are improved vase life. Within those investigations the use of plant growth regulators such as GA_{4+7} plus benzyladenine (Catherine et al., 2001) and GA with 75 ppm concentration (Emami et al., 2011) has a remarkable position in reducing foliar chlorosis and increasing vase life of *Lilium longiflorum*, respectively. The contribution of cytokinin in flower longevity has been proved for dicotyledonous flowers (Mayak and Halevy, 1970) but the evidence on monocotyledonous flower is not clear.

Petals senescence commonly is accompanied by morphological, biochemical and biophysical deterioration which consists of declining protein content, increase in protease activity and decline in lipid fluidity in membranes (Arora et al., 2007). Furthermore, initiation of senescence in plant tissues is involved with reactive oxygen species (ROS) (Dhindsa et al., 1981). Activated oxygen species such as O_2^- and H_2O_2 and their interaction product, hydroxyl radical (OH^-), react with DNA, degrade proteins, lipids and nucleic acids leading to senescence (Arora et al., 2002). According to Mayak et al. (1983) superoxide anions (O_2^-) that are producing during senescence of carnation petal induce the degradation of phospholipids and the fatty acids released by this breakdown are then peroxidase, which in turn affects membrane permeability (Simon, 1974). The senescence of cut flowers is closely related to many factors that some factors such as variety, pre-harvest factors, food Supply, water supply (Sankat and Mujaffar, 1994) and mechanical damage are critical in life span of cut flowers. There are several documents based on using different treatments in postharvest for delaying senescence and enhancing cut flower vase life such as *Rosa hybrida* (Hajizadeh et al., 2012), *Eustoma grandiflorum* (kazemi et al., 2012) specially SA (kazemi et al., 2011; Gerailoo and Ghasemnezhad, 2011). However plant growth situation during pre-harvest can effect on the quality of cut flowers about

%30-%70 (Halevy and Mayak, 1981) since, using of some treatments during pre-harvest is reasonable.

It has been found that (salicylic acid) SA plays a role during the plant response to abiotic stresses and regulates physiological and biochemical processes during the entire lifespan of the plant (Rivas-San Vicente and Plasencia, 2011). It has also improved in some vase life of cut flowers. For instance, Hatamzadeh et al. (2012) reported that Post-harvest treatment of SA with 150 mg/L concentration on cut gladiolus flowers caused an effective increasing on vase life, maintains higher spike fresh weight, antioxidant enzyme, stability of membrane and leading to delay in petal senescence.

Salicylic acid (SA) is a phenolic compound and natural constituent of plant (Raskin, 1992) which is recognized as an endogenous regulator in plants after the finding that it is involved in many plant physiological processes (Pancheva et al., 1996). Plants possess a well-defined enzymatic antioxidant defense system to protect themselves against these deleterious effects by scavenging ROS (Hatamzadeh et al., 2012). SA can neutralize oxidative stress by increasing in the activity of anti-oxidant enzymes (Tayeb et al., 2006). In recent years, several reports have identified the beneficial effects of salicylic acid in maintaining the quality of several species such as *Rosa hybrida* (Yongping et al., 2000) and *Gerbera jamesonii* (Yuping, 2009) cut flowers. For this reason, the present study aimed to investigate the effect of pre-harvest foliar application of salicylic acid (SA) on the growth, flowering and cut flower quality of *Lilium longiflorum* L. cv. Tressor. Furthermore, the effect of SA on oxidative stress in different parts of flower stem was studied.

2. MATERIALS AND METHODS

2.1. Sample preparation

A split plot experiment with two factors was performed in randomized complete block design with three replications to evaluate acid salicylic effects on growth and flowering performance of *Lilium longiflorum* L. The experiment was carried out in green house. The temperature of the greenhouse was kept at 24±4 ºC with 60-70 percent humidity. *Lilium longiflorum* L. cv. Tressor, flower F1 bulbs were planted to the experiment plots in a 8:8:1 (v/v) mixture of pit, perlite and sand. They were then irrigated with 1/2 Hoagland solution every 3 days. At the stage of bud initiation, four concentrations (0, 50, 100 and 200 ppm) of salicylic acid were sprayed on stem and sampling was done from three different parts of stem (lower, middle and upper). Distilled water was considered as control.

2.2. Analytical method

The following morphological and physiological characteristics were measured during 7 days after treatment;

Flowering characteristics:

Flowering Stems Length (FSL): Flowering stem length were measured by ruler from the basal point of stem until the beginning of inflorescence.

Flowering Stems Diameter (FSD): Diameter of flowering stem was evaluated by a coulis in the middle of stem during 1 week as daily recordings.

Number of Buds per inflorescence (NB): For this character the number of buds per each stem was counted.

Bud Volume (BV): For the measurement of bud volume the diameter and length of first bud on the inflorescence was measured in mm from the middle of a bud and through the bud from tip to end of bud, respectively on the first day before treatment until 6 day after treatment. Then the volume of bud calculated as an equation for volume of cylinder, approximately.

Chlorophyll Index: Chlorophyll index was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan); this is presented by SPAD value. Average of 3 measurements from different spots of a single leave was considered.

Membrane stability index (MSI): For determination of membrane stability index (MSI), fresh leaf samples were cut into small discs of uniform size. Then samples were weighed and taken in test tubes containing10 ml of double distilled water. These tubes were incubated at 40°C in a water bath for 30 minutes and electrical conductivity of the samples (C) was measured using conductivity bridge. The samples were transferred to the other test tubes and incubated at 100°C in the boiling water bath for 15 minutes and their electrical conductivity (EC) was measured as above. Membrane stability index was calculated and expressed in percentage using the formula (Premachandra et al., 1989), MSI= [1-(C1/ C2)] x100.

Lipid peroxidation (MDA): Lipid peroxidation analysis was performed according to the method of Heath and Packer (1968) using 0.2 g of fresh leaf tissue from each treatment. 1 ml MDA extract was added to 4 ml trichloroacetic acid containing 0.5% thiobarbituric acid. The solution was heated at 95 ˚C for 30 min and then quickly cooled in running water. The solution was centrifuged at 10000 g for 10 min. The absorbance of the supernatant was measured at 532 and 600 nm. The concentration of MDA was calculated by subtracting absorbance at 600 nm from absorbance at 532 nm, and expressed as mg MDA g fresh weight (= 155 mM^{-1} cm^{-1}).

2.3. Statistical analysis

The data were analyzed for variance using ANOVA procedure in SAS software version 9.1 (SAS Institute, Cary, North Carolina, USA). The differences between means were compared by Duncan's multiple range tests. Statistical significance was considered at $p<0.05$. Note: in this study effect of main plots was not significant hence the analysis of data were reported in factorial.

3. RESULTS AND DISCUSSIONS

3.1. Effect of SA on flowering characteristics

According to table 1 it is resulted that SA and Time have significant effect on flowering stem length (FSL), flowering stem diameter (FSD) and bud volume (BV) and also the interaction between SA*Time has a significant effect on bud volume.

3.2. SA and Bud Volume (BV)

Salicylic acid treatment at 50 ppm concentration did not increase bud volume significantly in comparison to the control. While with increasing SA to higher concentrations BV was decreased significantly (Table 2) and the highest inhibition effect obtained by 200 ppm concentration (Tab 2). Our findings are agree with Sabzi et al. (2012) which suggested 1mM SA similar to control had the most effect on rose flower diameter compare to 2 mM SA. According to Table 1 the interaction between SA and Time on Bud Volume (BV) was significantly different. As illustrated in figure 1 the volume of Lilium buds increased along with time as bud volume at before treatment until 6th day increased from 20.973 mm to 53.799 mm.

Table 1: Analysis of variance for flowering characteristics

Source of variation	Means of Squares				
	df	**FSL**	**FSD**	**NB**	**BV**
SA	3	93.24**	1.23**	1.04	201.79**
Time	6	67.69**	0.87**	1.09	1791.64**
SA*Time	18	4.85	0.04	0.84	56.25*
E	84	13.5045	0.1027	1.4521	12.9144

** significant at p<0.01, * significant at p<0.05, SA: Salicylic acid, BV: Bud Volume, FSD: Flowering stem diameter, FSL: Flowering stem length, NB: Number of buds

Table 2: Means comparison of salicylic acid treatments on flowering characteristics

Treatment Salicylic acid (ppm)	Flowering characteristics Bud Volume (mm)	Flowering Stem Length(cm)	Flowering stem Diameter (mm)
Control	38.785^{a}	57.530^{bc}	9.503 ^{b}
50	38.945^{a}	60.529^{a}	10.006^{a}
100	34.219^{b}	59.291^{ab}	9.623 ^{b}
200	32.931^{b}	55.696^{c}	9.485 ^{b}

3.3. SA and flowering stem diameter (FSD)

Salicylic acid at 50 ppm concentration affected flowering stem diameter positively but other concentrations of SA had no significant differences with control (Table 2). Results obtained from figure 1 showed that there is an increasing trend in flowering stem diameter from start till 4th day but there was no change in stem diameter after that. It is suggested that foliar SA applications on *Saintpaulia* significantly improved some plant characteristics such as the number of leaves, rosette diameter and the number of days from potting to anthesis (Jabbarzadeh et al., 2009). It seems that positive effect of SA on growth parameters are attributed to enhanced CO_2 assimilation, chlorophyll concentration and photosynthetic rate (Karlidag, 2009).

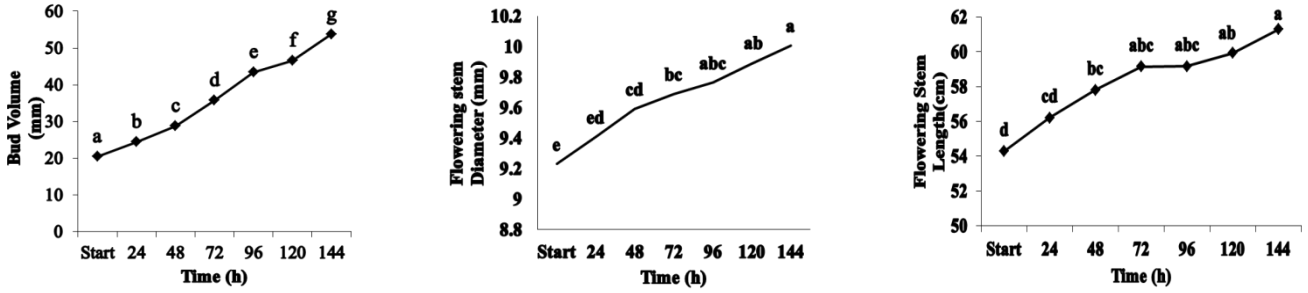

Fig. 1: Change in inflorescence traits during SA treatments; Bud volume (Left), flowering stem diameter (Center) and flowing stem length (Right).

3.4. SA and flowering stem length (FSL)

According to Table 2 it is resulting that there is a significant difference between SA at level of 50 ppm and 200 ppm as well as control in flowering stem length, thus Liliums which are treated with 50 ppm SA have the most stem length in comparison with others. The same results obtained in Gladiolus which sprayed with 50 ppm SA by Ram et al. (2012). On the other hand, the inhibitory effect of SA on stem length is clearer at level of 200 ppm. The stimulatory effect of SA has been demonstrated before by Handro et al. (1997) who reported that using of SA at (0.5-1µM) in medium culture of *Ullucus tuberosus* caused to more elongated axillary shoots than controls. As shown in figure 1 flowering stem length had the increasing trend until 3th day bud no significant difference in flowering stem length was observed after that. As Salicylic acid is a growth promoting chemical (Ram et al., 2012) has a favorable effect on growth parameters and it seems that accelerates the cell divisions and cell elongation in the apical portion of stem.

3.5. Effect of SA on physiological parameters

Besides the effect SA on flowering parameters other observations was carried out about the role of SA in preserving quality of leaves and preventing of lipid peroxidation among tree different parts of the stem leaves (lower, middle and upper). According to table 3 the effect of leaf position (LP) on Chlorophyll ($p<0.01$) and the interaction between SA×LP on membrane stability Index (MSI) ($p<0.05$) were significant. Means comparison for chlorophyll content showed that the amount of chlorophyll in leaves harvested from different part of stem is significantly different. The lower, middle and upper leaves chlorophyll content were 43.93, 59.60 and 62.91 (µg cm^{-2}), respectively. Also there was no significant difference in Malondialdehyde (MDA) between 3 levels of leaf position and also different concentrations of SA treatments (Table 3).

Table 3: Analysis of variance for physiological parameters

Source of variation	df	Means of Squares		
		Chl	MDA	MSI
SA	3	14.89	0.132	123.46
LP	2	1121.42**	0.060	178.52
SA×LP	6	24.20	0.205	162.46*
E	24	444.86	0.0849	59.6

** significant at $p<0.01$, * significant at $p<0.05$SA: salicylic acid, LP: leaf position, Chl: Chlorophyll content, MDA: Malondialdehyde, Membrane stability index: MSI

Results from table 3 showed that the interaction between SA×LP was significantly different ($p<0.05$) on membrane stability index. According to figure 2 the least and highest membrane stability index were related to lower stem leaves and stem upper leaves, respectively. It appears that deterioration effects of SA on older leaves are greater that the younger leaves and with increasing SA concentration those effects are severe (Fig 2).

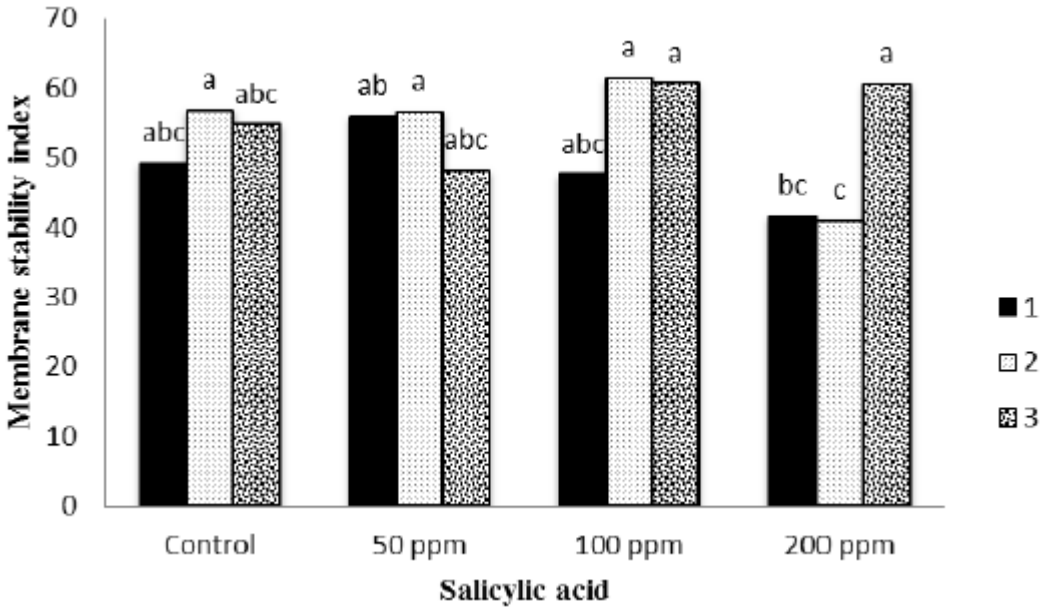

Fig. 2: Means comparison of SA×LP on membrane stability index; lower leaves (1), middle leaves (2) and upper leaves (3)

It seems that the effect of SA at high dosages not only is advantage but also is very injurious. However upper stem leaves seems to have constant membrane stability for different applications of SA concentrations. It is suggested that juvenile leaves have less susceptibility to different levels of SA treatment. At middle leaves which seems to be more active in photo assimilation there was no significant difference between control and SA 50 ppm and 100 ppm treatments in MSI but an increasing trend in membrane stability index was observed with increasing level of SA to 50 and 100 ppm but not for 200 ppm. Overall, the damaging effect of high dosages of SA is more on middle leaves and also this leaves showed more response to SA spray. In this case probably SA pre-harvest spray can done on the stem meddle leaves and upper leaves and avoid of damaging effects of SA on other parts of plants specially flower bud induction or initiation.

4. CONCLUSIONS

It is clear that SA has the important role in the control of several physiological and biochemical processes in plants especially flower-inducing but this phenomenon can occur in combination with other plants growth regulators (e.g. gibberellins) and also it is related to day length strongly. So, having no effect on bud number in our findings is acceptable and needs to be studied along with other factors. On the other hand it seems that the inhibitory effect of SA on ethylene production resulting in stimulation of growth and regenerative capacity. Salicylic acid applied to the foliage of intact plants induced positive effects on the bio-productivity of horticultural and ornamental plants. Moreover, in order to get a desired effect it was observed that lower concentrations of salicylic acid are needed.

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