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*Full Length Research Paper*

# Efficiency of salicylic acid delay petal senescence and extended quality of cut spikes of *Gladiolus grandiflora* cv 'wing's sensation'

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**Gladiolus (Wing's sensation) is one of the popular cut flowers that demonstrates postharvest problems which cause shorter vase life and loss of quality. In this research, effect of salicylic acid (SA) continuous treatment on the quality and vase life of cut *Gladiolus* cv 'wing's sensation' flowers over four developmental stages (bud stage; half bloom; full bloom; senescence) were investigated. This research was conducted in a split plot in time experiment based on completely randomized design with 3 replications. The flowers were treated in different concentrations of SA (50, 100, 150 and 200 mg/L). Results showed that the SA delayed flower senescence and leakage of ion in petals, as well as decreased fresh weight loss and lipid peroxidation. In addition, these treatments also increased antioxidant enzyme activities of peroxidase (POD) and maintain protein content. The (150 mg/L) SA treatment was the most effective on vase life of cut gladiolus flowers. Moreover, the results showed that the postharvest application of SA (150 mg/L) maintain higher spike fresh weight, antioxidant enzyme, stability of membrane and leading to delay in petal senescence.**

**Key words:** Antioxidant enzymes, cut flower, lipid peroxidation, salicylic acid.

## INTRODUCTION

Gladiolus is a popular cut flower in the world, but flowers longevity is very short. The vase life of individual florets is 4 to 6 days (Mayak et al., 1973). The life of the flower is a function both of the life of individual florets, the postharvest expansion and opening of the buds remaining on the spike (Serek et al., 1994). Short postharvest vase life is one of the most important problems of the cut flowers. However, longevity of vase life is an important factor in consumer preference (Da Silva, 2003; Kader, 2003). The previous report showed that exogenous ethylene and also ethylene inhibitors have no effect on petal senescence of gladiolus (Ezhilmathi et al., 2007). So, an alternative system must exist to regulate senescence in gladiolus. For example, the ion leakage from gladiolus tepal starts to increase prior to the onset of wilting (Yamane et al., 1993). But the increase in membrane permeability and subsequent wilting are delayed by treatments with cycloheximide (CHI) as protease inhibitors (Yamane et al., 1999).

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 $O_2^-$ or $H_2O_2$ and their interaction product, hydroxyl radical (OH), react with and degrade proteins, lipids and nucleic acids leading to senescence (Arora et al., 2002; Thompson et al., 1987). Oxidative stress arises from an imbalance in generation and metabolism of (ROS) that is, more ROS being produced than are metabolized (Neill et al., 2002). Plants possess a well-defined enzymatic antioxidant defence system to protect themselves against these deleterious effects by scavenging ROS. Membrane breaks down and ethylene biosynthesis, which appears to be closely linked, seems to involve free radicals (Paulin et al., 1986). According to Mayak et al. (1983) superoxide anions ($O_2^-$) that are producing during senescence of carnation petal induce

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**Figure 1.** Different developmental stages of senescence; Stage 1: bud stage, Stage 2: half bloom, Stage 3: full bloom, and Stage 4: senescence.

the degradation of phospholipids and the fatty acids released by this breakdown are then peroxidase, which in turn affects membrane permeability (Simon, 1974). This membrane deterioration is a prerequisite for ethylene synthesis (Doorn and Stead, 1994). There are different compounds that are used in postharvest for suppressing senescence.

SA is a phenolic compound that inhibits ethylene production, the inhibitory actions of SA most closely resembled with that of dinitro phenol, a known inhibitor of ethylene forming enzyme (Leslie and Romani, 1988). Ezhilmathi et al. (2007) reported that 5-sulfosalicylic acid as a salicylate derivative was effective in extending vase life of cut gladiolus. Treatment with sodium benzoate and n-propyl gallate delayed slightly the onset of wilting in gladiolus florets (Yamane et al., 1999). Singh et al. (2008) showed that vase solution containing $GA_3$ (50 mg/L), followed by BA (50 mg/L) with sucrose (50 g/L) significantly enhanced the vase life of gladiolus flowers. The objectives of this study was to investigative the optimum concentration of SA application for improving postharvest of cut flowers, and delaying senescence of gladiolus florets.

## MATERIALS AND METHODS

Fresh cut spikes of gladiolus cv. 'Wing's sensation' were harvested in the morning from a commercial field in Karaj and transferred immediately to the laboratory, University of Guilan, Iran. Cut spikes at tight bud stage, having 16 to 18 buds each and the basal two to three buds showing colour were sorted and selected for uniform size (30 ± 5) cm and placed in holding solutions containing SA (50, 100, 150, 200 mg/L). Distilled water was considered as control. Vase solutions were changed after every 24 h and the volume of the remaining solution was recorded. All solutions were prepared with distilled water. The flowers were held under fluorescent lighting (12 h) at 20°C and 60±5% relative humidity. Observations were recorded on vase life, changes in fresh weight, and membrane stability index (MSI), protein content, peroxidase activity (POD), and lipid peroxidation in terms of malondialdehyde substance (MDA) content. All physiological characteristics were measured at four developmental stages with three replications. Eighteen spikes from each treatment were used for biochemical analysis (POD activity, lipid peroxidation and membrane stability index and protein amount). All the parameters were studied using the tepals of the lower most flower of the spike at four developmental stages, bud stage; half bloom; full bloom; senescence (Figure 1).

### Vase life

Vase life was determined as the time period for which a spike retained fresh weight similar to that at harvest; this coincided with the stage where spikes showed wilting in 3 to 4 lower florets. Forty five spikes (9 in each replication) were exposed to different treatments.

### Changes in fresh weight

15 selected spikes were weighted individually daily and the changes in fresh weight were calculated as a percentage, on the basis of initial fresh weight.

### Lipid peroxidation

Lipid peroxidation was carried out by the method of Heath and Packer (1968). Petal sample (0.5 g) was homogenized in 5 ml 0.1% trichloracetic acid (TCA). The homogenate was centrifuged at 14000 g for 15 min, and then 1 ml of supernatant was added, 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 10000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The MDA concentration was calculated according to its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

### Protein content

The protein concentration of the supernatant was estimated using the method of Bradford (1976). The absorbance of blue colour was read at 595 nm using uv-visible spectrophotometer. The amount of protein was quantified by using a standard curve and result were expressed as mg protein per g fresh weight of petals.

### Peroxidase (POD) activity

POD activity was measured according to the method of Yamane et al. (1999) with a little modification. Petals (0.5 g) were homogenized in ice cold 50 mM potassium phosphate buffer (pH 7) containing 0.5 mM EDTA with pre-chilled pestle and mortar. The homogenate was centrifuged at 4°C for 15 min at 14000 g. The supernatant was used as enzyme extract and protein. POD activity was assayed by measuring spectrophotometer the formation of guaiacol ($\Delta A$ 470 = 12.26 $mM^{-1}.cm^{-1}$) in a 1 ml reaction mixture of (100 µl of 0.5 M K-

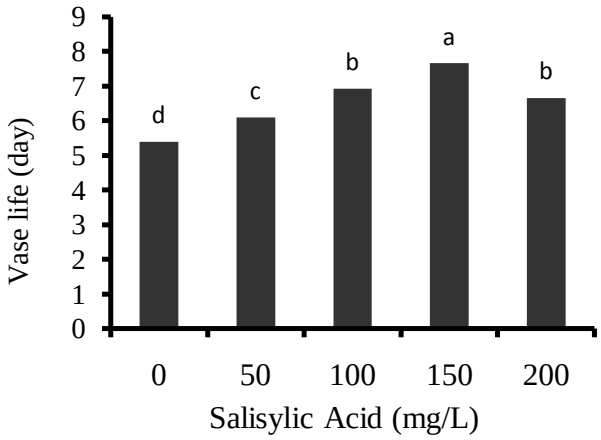


**Figure 2.** Effect of solutions containing salicylic acid on vase life of cut gladiolus 'Wing's sensation'. Similar letters indicate treatments that are not significantly different from one another (P≤0.05).

phosphate buffer pH 7), 450 µl of 25 mM guaiacol, 450 µl of 45 mM $H_2O_2$ and 100 µl crude enzyme according to the method of Yamane et al. (1999) with a little modification. The activity is expressed as mg of protein.

#### Statistical analysis

Treatments were arranged in a split plot in time experiment, based on a completely randomized design with three replications for each treatment. Treatment includes SA in five levels (0, 50, 100, 150 and 200 mg/L) All studied traits were subjected to analysis of variance. Significant effects of treatments were identified by analyzing data using SAS software version 9.1 (SAS Institute, Cary, North Carolina, USA). Mean comparisons to identify significant differences among treatment were performed using least significant difference (LSD method and graphs were plotted using Microsoft Excel.

## RESULTS AND DISCUSSION

It is desirable to improve the longevity of cut flowers for commercial purpose. However, practical control of vase life is still difficult in ethylene-insensitive flowers because the process of cell death leading to petal senescence is incompletely understood (Yamada et al., 2003). Our results showed that cut spikes that were continuously treated with SA delayed petal senescence as compared to control. Among all vase solution treatments, 150 mg/L (SA) was found to be the most effective, followed by 100 and 200 mg/L for improving vase life of cut spikes of gladiolus (Figure 2). SA has been tested for control of post harvest diseases. Most of the research using SA is directed to induction of systemic acquired resistance (SAR) in hosts against the attack of pathogens. Our results show that with increase of SA concentrations, vase life of cut spikes decreased. Probably high concentrations of SA may have injured xylem vessels and collapsed the water flux up to the petals. This finding is in harmony with the results of Guy de capdevilie (2003) that long pulsing time with SA at 7.2 mM caused flowering, while dehydration reduced the vase life of the flowers. The present studies have reconfirmed the role of ROS in senescence of cut spike of gladiolus. Therefore, antioxidant activity of SA may help to enrich antioxidant system activity like POD and improved longevity of cut flowers. A gradual decrease in fresh weight was observed over the senescence period in control and treated flower, but cut spikes that were treated with 150 mg/L (SA) suppressed declining of fresh weight in early days of vase life (Figure 3).

Similar results have also been reported in gladiolus (Ezhilmathi et al., 2007) and in petunia (Serek et al., 1995). SA have important role in decreasing transpiration and evaporation of tissues, as well as decreasing respiration, so caused preventing from loss of fresh weight in cut flowers. Our results coincide with Hossain et al. (2006) in gladiolus flowers. Electrolyte leakage is often used as a parameter for determining tissue damage as the loss of membrane's selective permeability (Bartoli et al., 1995). Loss of membrane integrity is the final and irreversible phase of senescence associated with membrane lipid peroxidation (Paulin et al., 1986). The lipid peroxidation (MDA concentration) increased after full bloom (stage 3) and the maximum MDA was recorded in senescence stage. Lipid peroxidation and membrane stability were inversely proportional, and closely associated with flower senescence. Similar profiles had been observed in chrysanthemum petal senescence by Bartoli et al. (1995). Treated flowers with SA maintained a significantly lower level of lipid peroxidation at over vase life stages (Figure 4). Lipid peroxidation is mediated by ROS (Kellog, 1975).

So, SA may be scavenging ROS and lead to decreasing lipid peroxidation. Analysis of variance for cut spike of gladiolus to evaluate effects of SA treatments on

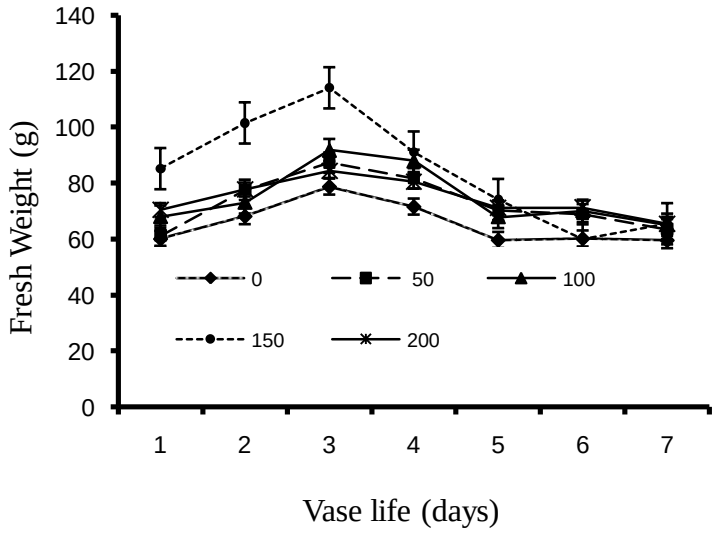


**Figure 3.** Effect of salicylic acid on fresh weight of cut gladiolus 'Wing's sensation'. Similar letters indicate treatments that are not significantly different from one another (P ≤0.05).

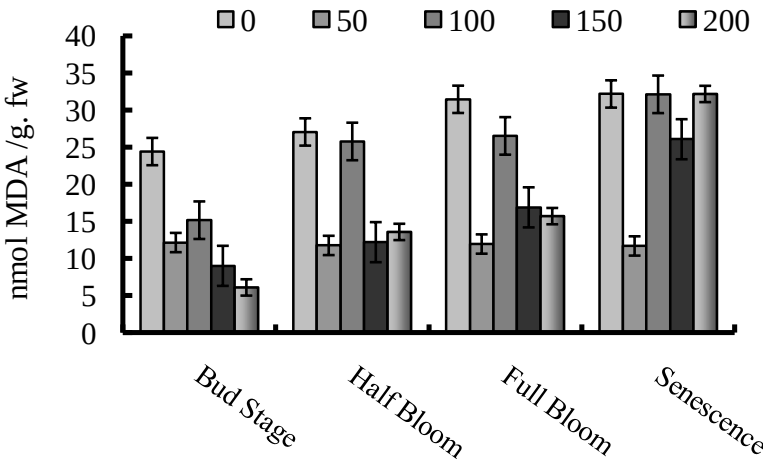


**Figure 4.** Effect of salicylic acid on lipid peroxidation (MDA) of cut gladiolus 'Wing's sensation' during different developmental stages. Bar indicates standard error of mean.

physiological traits was shown in (Table 1). The effect of SA on total soluble protein concentration of petal at four developmental stages is shown in Figure 5. As the results indicated, protein concentration decreased after flower bud stage in treated and untreated flowers. But protein content of treated flowers with SA was higher than control at 1, 2, 3 stages of flower development. Our finding coincides with Van-Doorn and Stead (1997), which indicated that during petal senescence, protein content declined and membrane permeability increased. The results showed that peroxidase (POD) activity in gladiolus petal was very low during early developmental stages, but with beginning of senescence, its activity increased significantly (Figure 6). Treatment with SA as free radical scavengers might delay slightly the onset of wilting by maintaining membrane integrity, in gladiolus florets. An increase in POD activity in petals may strengthen vascular cells, which remain functional during the later stage of senescence (Panavas and Rubinstein, 1998). Previous results showed that POD is involved in the senescence of gladiolus, because it catalyzes the decomposition of $H_2O_2$ (Yamane et al., 1999).

Different studies obtained the idea that vase life of flower is modulated by antioxidants (Baker et al., 1977; Baker et al., 1978) due to involvement of ROS in wilting. POD enzyme uses $H_2O_2$ as a substrate for several reactions and its specific activity increases during senescence. The actual function of POD in senescence

**Table 1.** Analysis of variance for cut spike of gladiolus to evaluate effects of SA treatments on studied traits.

| Source of variation | df | Mean squares | | |
|---|---|---|---|---|
| | | Lipid peroxidation (nm MDA/g fresh weight) | Protein (mg/fresh weight) | POD (Unit mg.protein$^{-1}$) |
| Salicylic acid (A) | 4 | 0.001** | 325.48** | 0.224ns |
| Error (a) | 8 | 0.10 | 72.51 | 0.051 |
| Time of sampling(B) | 6 | 0.001** | 7114.29** | 34.46** |
| A×B | 24 | 0.001** | 60.01* | 0.32* |
| Error (b) | 12 | 0.38 | 22.87 | 0.39 |

*: Significant at P < 0.05. **: significant at P < 0.01. Ns: not significant.

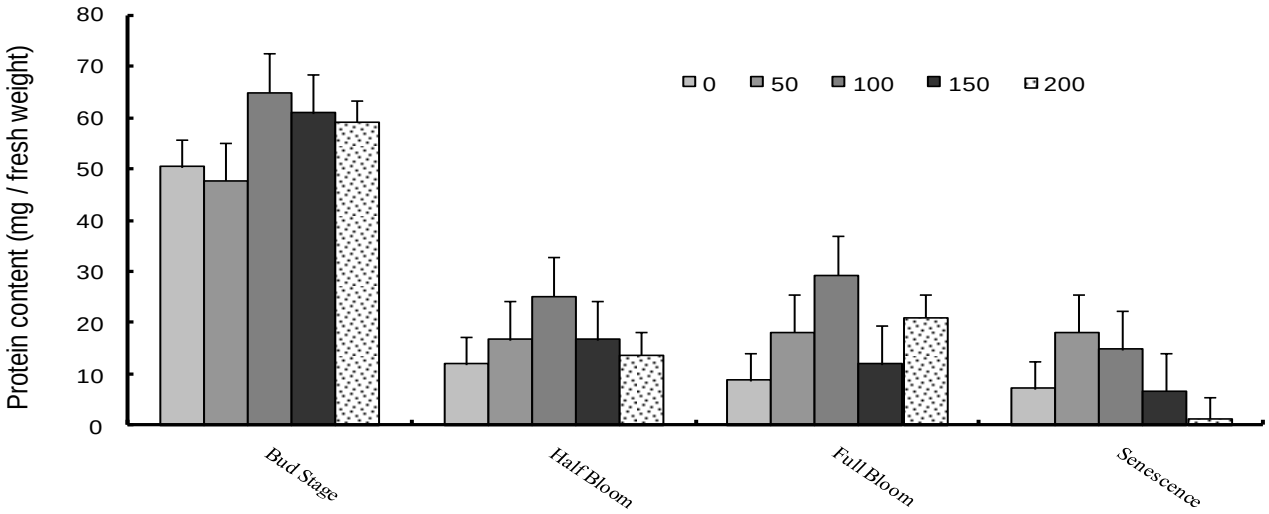


**Figure 5.** Effect of salicylic acid on protein content of cut gladiolus cv Wing's sensation during different developmental stages. Bar indicates standard error of mean.

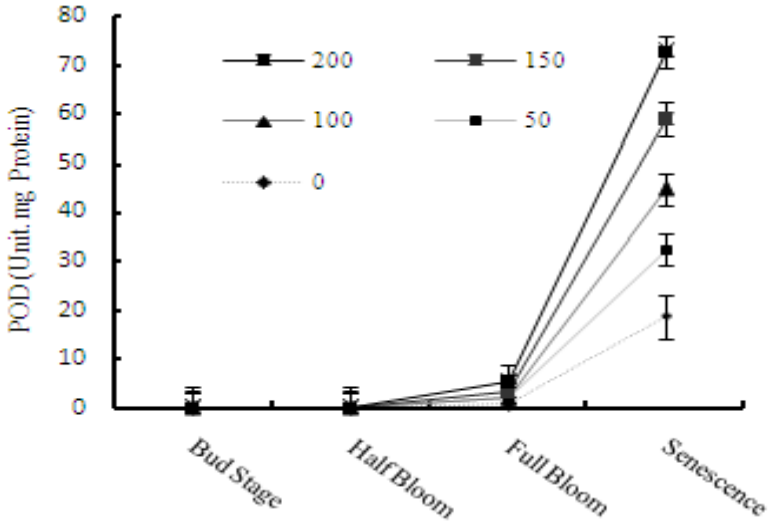


**Figure 6.** Effect of salicylic acid on peroxidase activity (POD) of cut gladiolus 'Wing's sensation' during different developmental stages. Bar indicates standard error of mean.

is very ambiguous because of their ability to produce lignin and to reduce growth by cross-linking wall materials (Lee and Lin, 1995). Increase in specific activity of peroxidase in gladiolus (Yamane et al., 1999) and daylily (Panavas and Rubinstein, 1998) petals may be due to the decrease in total protein, after flower opening.

## Conclusion

Vase solution of 150 mg/L SA has positive influence in vase life of gladiolus cut spikes by retarding fresh weight loss and lipid peroxidation and enrich the antioxidant activity of POD enzyme.

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