EFFECT OF SALICYLIC ACID ON ANTIOXIDANT ENZYME ACTIVITY AND PETAL SENESCENCE IN 'YELLOW ISLAND' CUT ROSE FLOWERS

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A B S T R A C T

The effect of exogenous salicylic acid (SA) on the vase life of cut rose flowers was investigated. 'Yellow Island' roses were obtained from a commercial grower. The roses were pulse treated with SA (0, 50, 100, 150, 200 mg l^{-1}) for 18 hours. Compared to the control treatment (DW), the greatest delay in senescence was obtained in cut roses treated with 150 mg l^{-1} SA. With this treatment, flower vase life was doubled. The physiological characteristics such as protein concentration, lipid peroxidation (MDA) level and enzyme activity such as lipoxygenase (LOX), superoxide dismutase (SOD) and peroxidase (POD) were measured only in the control (DW) and those kept in a solution of 150 mg l^{-1} salicylic acid (SA) for over 8 days of vase life. Degradation of protein and accumulation of MDA during vase life was shown to be suppressed by 150 mg l^{-1} SA. The activity of LOX and POD gradually increased with progressing flower senescence, while SOD activity declined. The flowers which had been pulsed with SA exhibited lower LOX and POD activity, and a higher activity of SOD. Overall, the results suggest that SA increases vase life by improving the antioxidant system and reducing oxidative stress damages during rose flower senescence.

Key words: oxidative stress, salicylic acid, flowers senescence, lipid peroxidation, lipoxygenase

INTRODUCTION

The rose undoubtedly remains the queen of the cut flowers. Most of the commercial cut roses will easily last in a vase for 10 days. Unfortunately, many consumers consider roses to have a very short vase life. This is partly because of the poor water uptake by certain cultivars that often results in the 'bent neck' style (Reid, 2002). Furthermore, many commercial cultivars are quite sensitive to ethylene gas, therefore, appropriate pre-treatment of those sensitive cultivars with ethylene inhibitors, especially if they are to be sold in supermarkets or other ethylene polluted areas, could be helpful (Reid, 2002).

Petals senescence commonly is accompanied by morphological, biochemical and biophysical deterioration. Declining protein content, lipid fluidity in membranes, and an increase in protease activity are what make up the deterioration (Arora et al., 2007). Furthermore, it was also confirmed that reactive oxygen species (ROS) is involved in plant tissues (Dhindsa et al., 1981). Activated oxygen species (AOS) such as O_2^- or H_2O_2 and their interaction product, hydroxyl radical (OH) degrade proteins, lipids and nucleic acids lead to senescence (Thompson et al., 1987; Arora et al., 2007). Oxidative stress arises from an imbalance in generation and metabolism of ROS, i.e. more ROS are being produced than 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 and Adam, (1984) superoxide anions ($0_2^{\circ-}$) that are produced during the senescence of carnation petals, induce the degradation of phospholipids. The fatty acids released by this breakdown are then peroxidized, which in turn affects membrane permeability. This membrane deterioration is a prerequisite for ethylene synthesis (Van Doorn and Stead, 1994). The role of oxidative stress during petal senescence in rose was also investigated by Kumar et al. (2008). They suggested that an increase in endogenous H_2O_2 levels and a decrease in anti-oxidant enzyme activities, may be partly responsible for initiating senescence in rose petals.

The use of a preservation solution is considered a common practice for delaying senescence and extending flower vase life. These treatments control ethylene synthesis, pathogen development, maintenance of hydric and respiration balance, and contribute to colour conservation (Halevy and Mayak, 1981). For these reasons, many floral preservation solutions contain germicides, ethylene synthesis inhibitors, growth regulators, some mineral compounds, and carbohydrates that are essential for extending the vase life of cut flowers (Halevy and Mayak, 1981).

Earlier experiments carried out by Ezhilmathi (2001), showed that 5-

sulfosalicylic acid as salicylate derivatives in vase solution was most effective in extending flower vase life of cut gladiolus. Salicylates increased vase life by increasing ROS scavenging activity of the gladiolus cut flowers (Ezhilmathi et al., 2007).

Senescence of rose flower buds is still not completely understood. In order to understand petal senescence, it is important to explore the mechanisms of oxidative stress management. In this study, the levels of endogenous antioxidant enzymes and other physiological characteristics during senescence of rose flowers pulsed with salicylic acid, was investigated.

MATERIAL AND METHODS

Plant material and treatments

Flower stems of the yellow rose (*Rosa hybrida*), 'Yellow Island' were used. Flower were obtained from a commercial market and transferred immediately to the laboratory. The experiments were carried out the same day. The flower stems were re-cut under water to a uniform length of 30 cm. They were placed in holding solutions containing salicylic acid (SA) (0, 50, 100, 150, 200 mg l^{-1}) plus 200 mg l^{-1} 8- hydroxyquinoline sulphate (8-HQS) and 30 g l^{-1} sucrose at a temperature of 20 °C (±1 °C), under a 16:8 h light/dark cycle (irradiance 25 W m^2) and 60±5% RH for 18 hours. The control flowers were kept in distilled water (DW).

Evaluation

The flowers were considered senescent when showing at least one of the following symptoms of senescence: wilting of leaves or flowers, neck bending and incomplete bud opening (Ketsa and Sribunma, 1985). Physiological characteristics such as protein concentration and lipid peroxidation (MDA) levels and enzyme activity such as lipoxygenase (LOX), superoxide dismutase (SOD) and peroxidase (POD) were determined after 2, 4, 6 and 8 days of treatment. 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 model PG Instrument +80, England. The amount of protein was quantified by using a standard curve and result were expressed as mg protein per g fresh weight of petals.

Lipid peroxidation was carried out by the method of Bates et al. (1973). Petal samples (0.25 g) were homogenized in 1 ml 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 14000 g for 15 min, and then 500 µl of supernatant was added 500 µl 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 content was calculated according to its extinction coefficient of 155 Mm cm^{-1}.

Enzyme extract for LOX, SOD and POD activity was prepared by first freezing a weighed amount of petal tissue (0.5 g) in liquid nitrogen

followed by grinding with 10 ml extraction buffer (50 mM phosphate buffer, pH 7 containing 0.5 mM EDTA and 2% PVPP [w/v]). Homogenate was centrifuged for 20 min at 15,000 g, and the supernatant was used to determine enzyme activity.

LOX activity was estimated according to the method of Bonnet and Crouzet (1977) with some modification. The substrate solution was prepared by adding 10 µl linoleic acid to 4 ml distilled water containing 5 µl Tween-20. The solution was kept at pH 9.0 by adding 1 ml NaOH 0.2 M until all the linoleic acid was dissolved and the pH remained stable, and the total volume was adjusted to 25 ml. LOX activity was determined spectrophotometrically by adding 20 µl of enzyme extract to 80 µl linoleic acid substrate and 900 µl phosphate buffer solution (50 Mm, pH = 7). Absorbance was read at 234 nm, and the activity was expressed as unit per mg fresh weight.

SOD activity was estimated by recording the decrease in absorbance of nitro-blue tetrazolium dye (Dhindsa et al., 1981). Three ml of the reaction mixture contained 13 mM methionine, 25 mM nitro-blue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.1 ml enzyme. The reaction was started by adding 2l M riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. The complete reaction mixture without enzyme, which yielded maximal colour, acted as the control. The reaction was stopped by switching off the light and putting the tubes in the dark. A non-irradiated reaction mixture served as a blank. The activity is expressed as units per mg fresh weight.

POD activity was assayed by measuring spectrophotometrically the formation of guaiacol in l ml reaction mixture of 450 µl 25 mM guaiacol, 450 µl 225 mM H_2O_2 and 100 µl crude enzyme. The activity is expressed as units per mg fresh weight.

Statistical analysis

The recorded data were statistically analyzed (ANOVA analysis) using the software of SAS. Sources of variation were different concentrations of salicylic acid and developmental stages. Means were compared with the LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Based on senescence symptoms, the solutions containing salicylic acid gave a longer vase life for cut roses than the control treatment (Fig. 1). Cut roses that were pulsed for 18 hours in the solution containing 30 g l^{-1} sucrose, 200 mg l^{-1} 8- hydroxyquinoline sulphate (8-HQS) and 150 mg l^{-1} salicylic acid had a maximum vase life of 11 days. Cut roses in the control treatment had a vase life of only 5 days (Fig. 1 and 2). However, cut roses pulsed with solutions containing 100 and 200 mg l^{-1} (together with 30 g l^{-1} sucrose together and 200 mg l^{-1}) 8-HQS were not significantly different from those treated with 150 mg l^{-1} (Fig. 1).

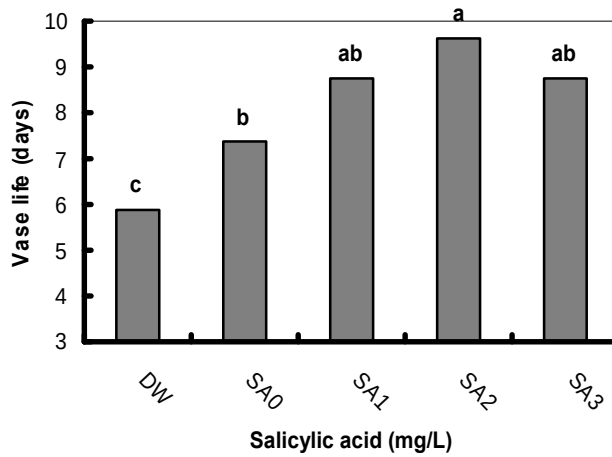

Figure 1. Effect of different salicylic acid (SA) concentrations on the vase life of 'Yellow Island' cut rose flowers
Values labelled with the same letter are not different at the 5% significance level

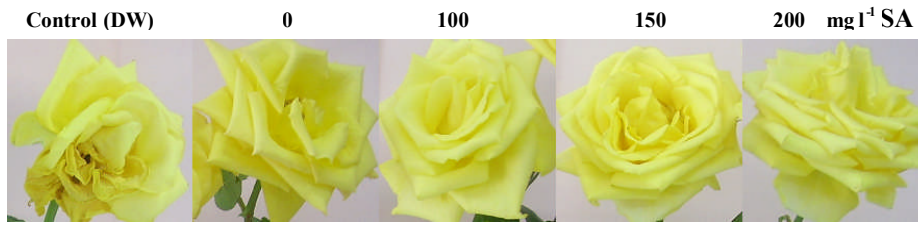

Figure 2. Effect of exogenous salicylic acid (SA) on vase life of 'Yellow Island' cut rose flowers after 9 days. The number at the top of the picture indicates the concentrations of SA

Therefore, for the maximum vase life, 'Yellow Island' cut roses should be pulsed in the solution of 150 mg l^{-1} SA for 18 hours.

Previous reports showed that most flowers can produce ethylene and ethylene shortens vase life (Ketsa and Sribunma, 1985). Salicylic acid might delay senescence by inhibiting ethylene synthesis with ACC oxidase activity (Bueno and Del Rio, 1992). Furthermore, earlier experiments carried out by Ezhilmathi (2001) showed that 5-sulfosalicylic acid as a salicylate derivatives in a vase solution was most effective in extending flower vase life of cut gladiolus. Salicylates increased vase life of gladiolus cut flowers by increasing ROS scavenging activity (Ezhilmathi et al., 2007).

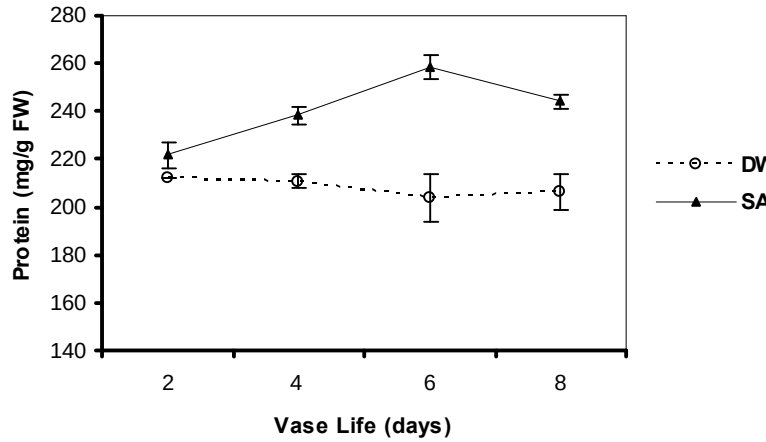

Figure 3. Effect of salicylic acid (SA) on protein content in 'Yellow Island' cut rose flowers

The data on the effect of SA on protein concentration of petals is shown in Figure 3. The protein concentration decreased significantly with the onset of senescence in flowers kept either in distilled water (DW) or in a SA solution. The protein concentration of flowers held in SA was over 8 days of vase life higher than the control. Petal senescence has invariably been associated with the loss of protein (Kenis et al., 1985; Van Doorn and Stead, 1994). The protein concentration of petals in cut daylily flowers rapidly decreased due to little de novo synthesis and considerable protein degradation (Lay-Yee et al., 1992). Protein degradation is an important feature of the dismantling of membranes (Woolhouse, 1984). It is likely that the progressive destabilization of membrane bilayer accompanying senescence may be due to loss of membrane protein function (Thompson, 1974).

Lipid peroxidation (MDA) concentration rapidly increased for up to 4 days in distilled water (DW) and salicylic acid (SA) treatments, but declined thereafter (Fig. 4). The SA treated flowers maintained a significantly lower level of MDA for up to 4 days, and thereafter there was no significant difference between the treated and untreated flowers. Since lipid peroxidation is mediated by ROS (Arora et al., 2007), SA may either be directly scavenging ROS and thus decreasing lipid peroxidation, or it may be modulating the activity of antioxidant enzymes (Ezhilmathi et al., 2007).

Lipoxygenase activity (LOX) activity slightly increased in the control and SA treated flowers. The treatment with SA caused reduction in LOX activity in comparison to the control for up to 8 days of vase life (Fig. 5). Senescing plant tissue experiences an increase in LOX activity,

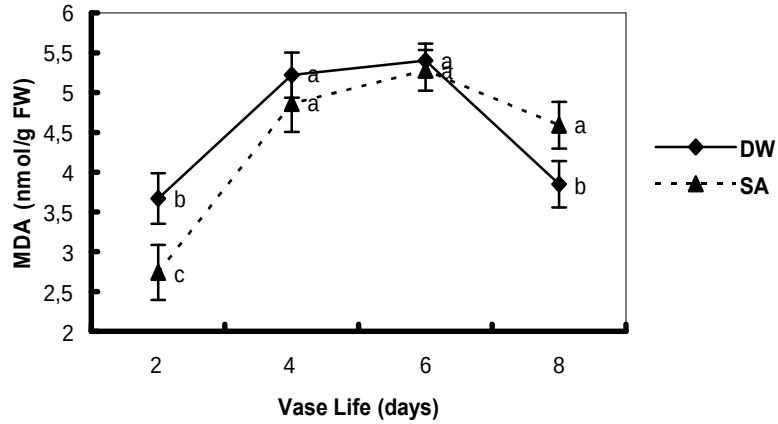

Figure 4. Effect of salicylic acid (SA) on MDA content in 'Yellow Island' cut rose flowers

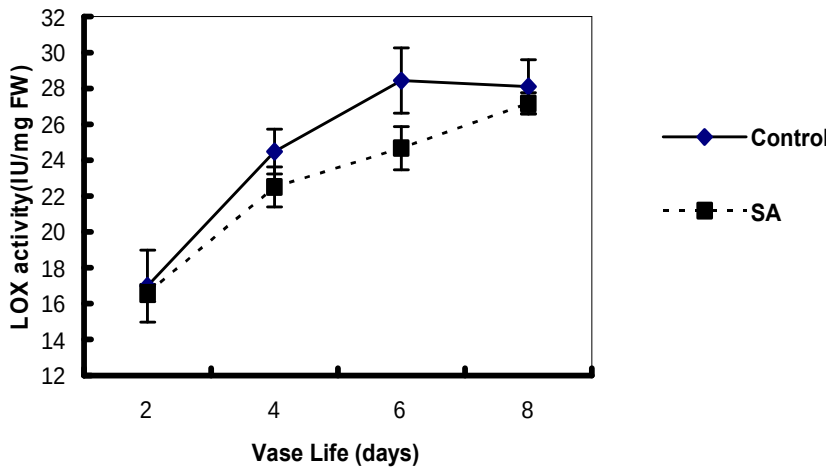

Figure 5. Effect of salicylic acid (SA) on lipoxygenase (LOX) activity in 'Yellow Island' cut rose flowers

which promotes the process of membrane polyunsaturated fatty acid peroxidation (Lynch and Thompson, 1984). In the present study, the LOX activity was found to increase gradually after harvest. Similar results were observed in tulips (Jones and McConchie, 1995) and gladiolus (Ezhilmathi et al., 2007). An increase in LOX activity has been correlated with an increase in cell membrane permeability and senescence in daylily (Panavas and Rubinstein, 1998) and rose (Fukuchi-Mizutani et al., 2000).

Superoxide dismutase (SOD) activity increased initially up to 4 days

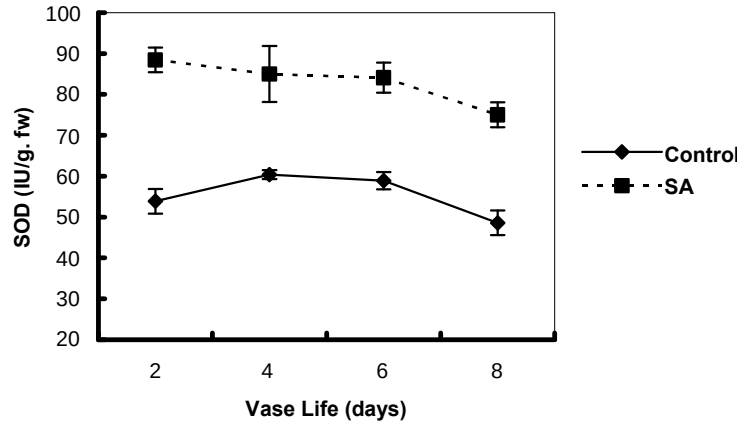

Figure 6. Effect of salicylic acid (SA) on superoxide dismutase (SOD) activity in 'Yellow Island' cut rose flowers

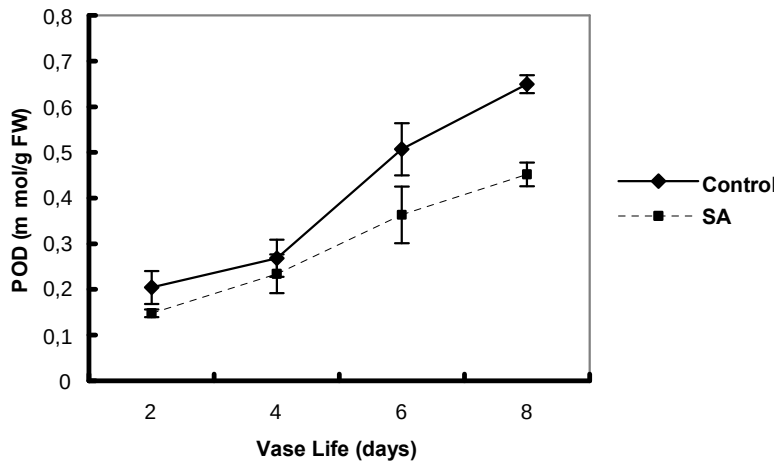

Figure 7. Effect of salicylic acid (SA) on peroxidase (POD) activity in 'Yellow Island' cut rose flowers

and thereafter declined in distilled water (DW), but in SA pulsed flowers SOD activity significantly decreased after 8 days of vase life. The results also showed that SA treated flowers showed significantly higher SOD activity compared to the control for 8 days of vase life (Fig. 6). Various studies have demonstrated that vase life of flowers is modulated by antioxidants (Baker et al., 1978), suggesting the involvement of ROS in senescence. Prochazkova et al. (2001) have reported the participation of ROS in plant senescence. In the present study SOD activity de-

clined during the vase life of the flowers. These results are consistent with the pattern of SOD activity during senescence in carnation petals (Ezhilmathi et al., 2007). Similar results have been reported in carnation petals (Sylvestre et al., 1989) and daylily (Panavas and Rubinstein, 1998), although in both cases the changes occur rather later in the progression of senescence.

Peroxidase (POD) activity increased significantly both in distilled water (DW) and SA pulse flowers, for 8 days. The results also showed that SA pulsed flowers showed significantly lower POD activity compared to the control (Fig. 7). An increase in POD activity in petals may strengthen vascular cells, which remain functional during the later stage of senescence (Panavas and Rubinstein, 1998). These results and ours indicated that POD is involved in the senescence of gladiolus because it catalyzes the decomposition H_2O_2. POD enzyme uses H_2O_2 as a substrate for several reactions and its specific activity increases in both carnation (Bartoli et al., 1995) and daylily (Panavas and Rubinstein, 1998) during senescence. Different studies have shown that flower vase life is modulated by antioxidant (Baker et al., 1978), due to involvement of ROS in wilting.

In conclusion, salicylic acid enhanced the vase life of 'Yellow Island' roses by maintaining a higher activity of SOD enzyme and reducing oxidative stress damages such as lipid peroxidation, LOX activity during flower senescence of cut rose.

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WPŁYW KWASU SALICYLOWEGO NA AKTYWNOŚĆ ENZYMÓW OKSYDACYJNYCH ORAZ STARZENIE SIĘ PŁATKÓW CIĘTEJ RÓŻY ‘YELLOW ISLAND’

Somayeh Gerailoo i Mahmood Ghasemnezhad

S T R E S Z C Z E N I E

Badanie miało na celu określenie wpływu egzogennego kwasu salicylowego (SA) na kwiaty ciętej róży. Róże ‘Yellow Island’ pochodzące od hodowcy komercyjnego traktowane były w sposób pulsacyjny kwasem salicylowym (0, 50, 100, 150, 200 mg l^{-1}) przez 18 godzin. W porównaniu z grupą kontrolną (DW) największe opóźnienie w procesie starzenia, i dwukrotne wydłużenie żywotności kwiatów po ich ścięciu, wykazały róże traktowane 150 mg l^{-1} SA. Fizjologiczne cechy, takie jak stężenie białek, poziom utlenienia lipidów (MDA) i aktywność enzymów – lipoksygenazy (LOX), dysmutazy ponadtlenkowej (SOD) i peroksydazy (POD) były oznaczone jedynie w grupie kontrolnej (DW) oraz u roślin trzymanych w 150 mg l^{-1} roztworze kwasu salicylowego (SA) przez ponad 8 dni po zbiorze. Rozpad białek i gromadzenie się MDA po ścięciu zostały zahamowane po zastosowaniu 150 mg l^{-1} SA. Aktywność LOX i POD stopniowo zwiększyła się wraz z postępującym starzeniem się kwiatów, podczas gdy aktywność SOD się zmniejszyła. Kwiaty traktowane pulsacyjnie SA wykazały niższą aktywność LOX i POD, natomiast wyższą SOD. Wyniki wskazują, że SA przedłuża żywotność kwiatów po ich zbiorze wzmacniając działanie przeciwutleniaczy i redukując stres oksydacyjny w czasie starzenia się kwiatów róży.

Słowa kluczowe: stres oksydacyjny, kwas salicylowy, starzenie się kwiatów, utlenianie lipidów, lipoksygenaza