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Postharvest application of hydrogen peroxide and salicylic acid differently affects the quality and vase life of cut rose (*Rosa hybrida* L.) petals and leaves

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Rose represents an important product in the market of cut flowers. Its quality is related to visual appearance and is affected by oxidative stress and senescence. To maintain the quality and extend the product vase life, innovative technical solutions to be applied in postharvest are needed. In this work the effectiveness of hydrogen peroxide (H_2O_2) and salicylic acid (SA), was assessed on cut rose leaves and petals. Several physiological indicators of quality were studied, including chlorophyll content, chlorophyll *a* fluorescence, color, lipid peroxidation, phenolic compounds and anthocyanins. After 7 days of vase life 73.33% of untreated roses were not marketable anymore, while the percentage was lower in response to treatments (47.06% in SA and 25.53% in H_2O_2). The application of H_2O_2 induced a reduction in leaf chlorophyll and in the performance index which, at the end of the vase life decrease by 76% and 49% respectively. Consistently, the lipid peroxidation in leaves treated with H_2O_2 increased by a 53%. After 4 days of vase life, SA allowed maintaining higher levels of anthocyanins in petals compared to H_2O_2 and to controls. The results obtained allowed individuating different responses, depending on the treatment applied as well as on the plant organ.

1. Introduction

The market of cut flowers is characterized by the high perishability of the commercialized products. The pipeline from growers to the final consumers, is often long and at the same time, the item must have very high-quality standards. Flowers showing discoloration, shriveling, bending or any other visible sign of damage can no longer be commercialized, and product losses represents a crucial problem for this sector.

Rose (*Rosa hybrida* L.) is one of the most important species in the flower market and it is particularly appreciated by the consumers as cut flower. The quality of cut roses is strictly related to the integrity and quality of petals, but also to the color of leaves, which contributes to their visual appearance. Chlorophyll degradation and leaf yellowing reduce the

ornamental value of cut flowers and compromise their commercialization. During transportation and handling, leaves and petals can undergo mechanical damage, which can lead to tissue browning with negative effect consequences on quality.

On the physiological point of view, the loss of quality is a direct consequence of oxidative stress which in turn can lead to senescence (Kumar *et al.*, 2008). These phenomena are characterized by the activation of several physiological responses at cellular level and involve signaling molecules, enzymes, and transcriptional regulators such as genes and transcription factors (Gregersen *et al.*, 2013; Thomas, 2013). In the recent years, several efforts have been made to maintain the quality of cut roses and extend their vase life. These efforts include breeding programs the improvement of growing conditions, and the optimization of transportation. Moreover, the effectiveness of several treatments applied during the vase life has been assessed (Reid and Jiang, 2012).

Salicylic acid (SA) is a plant hormone involved in several plants functions, including stress responses, development, and plant signaling (Hayat *et al*., 2010; An and Mou, 2011; Rivas-San Vicente and Plasencia, 2011). Spray application of SA has been reported to prolong vase-life in cut rose flowers by increasing the activity of the ROS-scavenging enzyme catalase and improving the water balance. Interestingly, the lowest dosage applied (50 µM) was the most effective (Alaey *et al.*, 2007). Also, in other studies, low concentrations of SA (0.01 mM) have been reported as the most effective in inducing plants responses to stress (Horváth *et al*., 2007).

Hydrogen peroxide (H_2O_2) is an important molecule produced and accumulated in plant cells because of the aerobic metabolism. It belongs to the reactive oxygen species (ROS) and can play a key role in stress metabolism and senescence. The increment in ROS levels is, in fact, a typical feature of senescence and can lead to cellular damage and finally to cell death (Panieri *et al*., 2013). However, slight alterations of the ROS balance, including H_2O_2 accumulation, can be considered as important signals in plant cells and can trigger a series of defense responses, which in some case can also involve SA (Kalachova *et al*., 2013; Herrera-Vásquez *et al*., 2015). While high concentrations of H_2O_2, can directly induce oxidative stress in plant tissue, low doses can lead to the activation of positive physiological responses. It has been suggested that H_2O_2 priming can help in modulating ROS-mediated stress tolerance in plants (Hossain *et al*., 2005). Non-toxic levels of this molecule must be maintained to have beneficial effects and to improve the quality and vase life of cut flowers, as reported for lily (Liao *et al*., 2012).

The aim of this work was to evaluate the effect of low concentrations of H_2O_2 and SA when applied to cut roses during the vase life. Attention has been given to the individuation of specific and common responses induced by these two molecules. To achieve this goal, several indices in both petals and leaves were considered, including chlorophyll content, chlorophyll *a* fluorescence, color, lipid peroxidation, phenolic compounds and total anthocyanins.

2. Materials and Methods

Plant material

Cut rose stems (*Rosa hybrida* L. 'Tacazzi') at the commercial developmental stage were purchased on July 20th, 2017, from the Milan flowers market and brought to the laboratory within one hour. Each stem was around 60 cm-long. Once in the laboratory, 20 stems were randomly selected from a pool of 60 and placed in a beaker filled with 2 L of distilled water (control). Similarly, two other groups of 20 stems each, were placed in a SA (0.01 mM) and H_2O_2 (0.1 mM) solution respectively. The concentrations of the treatments were chosen among the lowest effective dosage found in the literature to reduce the risk of toxicity. After 24 hours, all the solutions were substituted with 2 L of fresh distilled water and the volume was maintained until the end of the trial. The vase life conditions were: 20±2°C, 55% relative humidity (RH), 18 µmol m^{-2} s^{-1} light intensity, with a photoperiod of 12 hours. Samples of petals and leaves were collected at the beginning of the trial as well as after 4 and 7 days of vase life. After 7 days the remaining stems were maintained until all of them were considered unmarketable.

Vase life and damaged flowers (%)

At each time point, the number of damaged flowers were estimated based on the visual appearance. Those flowers showing wilting, shriveling or desiccation were considered as not marketable anymore and thus counted as damaged. This information was used to calculate the percentage of damaged flowers and the average vase life (reported as days).

Water content (%)

At harvest and after 7 days three roses for each treatment (the whole stem) were weighed and

immediately placed in an oven at 115°C until the complete drying. The water content was calculated as percentage of water respect to fresh weight.

Chlorophyll and chlorophyll a fluorescence-related parameters

During vase life *in vivo* determination of leaves chlorophyll content was performed using a CL-01 chlorophyll mater (Hansatech, United Kingdom). Similarly, chlorophyll *a* fluorescence was measured on dark adapted leaves using a portable fluorimeter (Handy PEA, Hansatech, United Kingdom). The parameters measured were: the quantum maximum efficiency of PSII (Fv/Fm), the performance index (PI), the number of reactive centers per cross section (RC/CSm), and the amount of energy dissipated per reaction center (DIo/RC).

Color measurement

Changes in petal coloration in response to treatments and to vase life were evaluated at each time point by using a Minolta CR-400 colorimeter (Konica Minolta, Inc., Japan). The color parameters were described using the Lab* and the Munsell (L*C*h) color spaces. Briefly, in the Lab*color space the lightness is represented by L* (with 0 indicating black and 100 white). The colors in the red green region are represented by a* (red with positive values and green with negative values) while b* describes the interval from yellow (positive values) to blue (negative values). In the Munsell color space h represents the hue while C* is the chroma (or color purity).

Phenolic index and total anthocyanins

For the extraction of phenolic compounds and anthocyanins, around 1 g of petals and leaves, collected at each time point, were homogenized in 8 mL of acidified methanol (1% HCl V/V) and extracted overnight in the dark. The phenolic index was calculated as the absorbance at 320 nm of the diluted extracts, normalized to fresh weight (Ke and Saltveit, 1989). Total anthocyanins were determined spectrophotometrically at 535 nm using an extinction coefficient (ε) of 29,600 mM^{-1} cm^{-1} and expressed as cyanidin-3-glucoside equivalents and (Klein and Hagen, 1961).

Lipid peroxidation

The level of lipid peroxidation was measured with the thiobarbituric acid reactive substances (TBARS) assay (Heath and Packer, 1968). Briefly, around 1 g of petals and leaves was homogenized in 5 mL 0.1% trichloroacetic acid (TCA) solution. The extract was mixed with 4 mL of 20% (W/V) TCA, 25 µL of 0.5% thiobarbituric acid (TBA) and distilled water. After vortexing, the mixture was heated at 95°C for 30 minutes in a water bath and then cooled on ice. Absorbance at 600 nm was subtracted from the absorbance at 532 nm (as an index of non-specific turbidity) and the concentration of TBARS was expressed as malondialdehyde (MDA) equivalents (nmol g^{-1} FW), calculated using an extinction coefficient (ε) of 155 mM^{-1} cm^{-1}.

Statistical analysis

All data were subjected to two-way ANOVA followed by Bonferroni's multiple comparisons test, except for vase life data which were subjected to a one-way ANOVA. Statistics were performed using GraphPad Prism version 6 for Windows, GraphPad Software, La Jolla, California, USA (www.graphpad.com).

3. Results

Vase life and water content

After 4 days of vase life all the flowers treated with H_2O_2 and SA were considered marketable, while 10% of untreated flowers showed a marked loss of quality. After 7 days, the higher rate of damage was recorded in the controls (73.33%), followed by SA (47.06%) and H_2O_2 (25.53%) (Fig. 1 A). After 8 days all

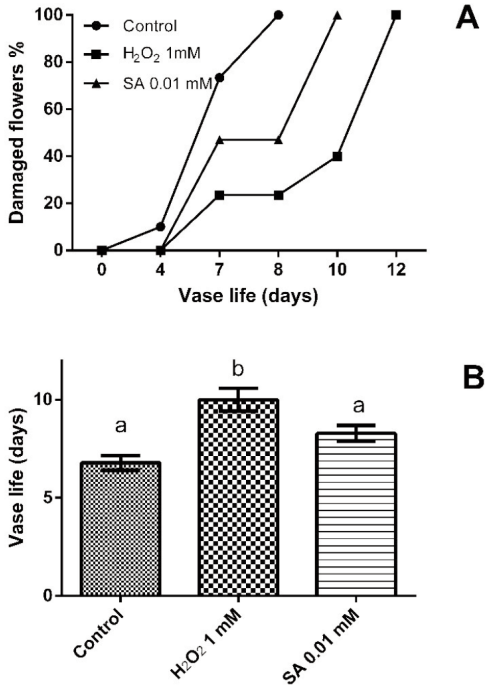

Fig. 1 - Percentage of damaged flowers (A) and average vase life (B) measured in cut 'Tacazzi' rose. Data are means ± SE (B: n=14; C: n=3). Different letters indicate significant differences ($p<0.05$).

the control flowers were already damaged, while 6 flowers treated with SA were still marketable until 10 days, and 6 among those treated with H_2O_2 lasted until 12 days.

The treatment with H_2O_2 determined a significant increment in the average vase life compared to SA and control (Fig. 1 B).

The initial water content was 77.86% and showed a slight progressive decline without any significant difference among the treatments. After 7 days the values were: 70.01%, 70.61%, 70.64%, in control, SA and H_2O_2 respectively. The flowers treated with H_2O_2 showed a reduced opening compared to those treated with SA or to controls (Fig. 2 A, C).

Color

After 7 days of vase life, all the petal samples looked paler compared with to the beginning of the trial and an increment in the L* value was recorded despite the treatments applied (Table 1). H_2O_2-treated flowers did not show any change in the color-related parameters compared to controls. On the other hand, after 7 days, SA induced a decrement in all the color attributes compared to the beginning of the trial, including a*, b*, hue (h) and chroma (C*). This variation was consistent with the visual observation of the petals which appeared darker and turned from red to a more violet coloration (Fig. 2 C).

Chlorophyll content and chlorophyll a fluorescence-related indexes

Treatment and vase life duration significantly affected the chlorophyll content of cut rose leaves. After 4 days of vase life, the chlorophyll content was

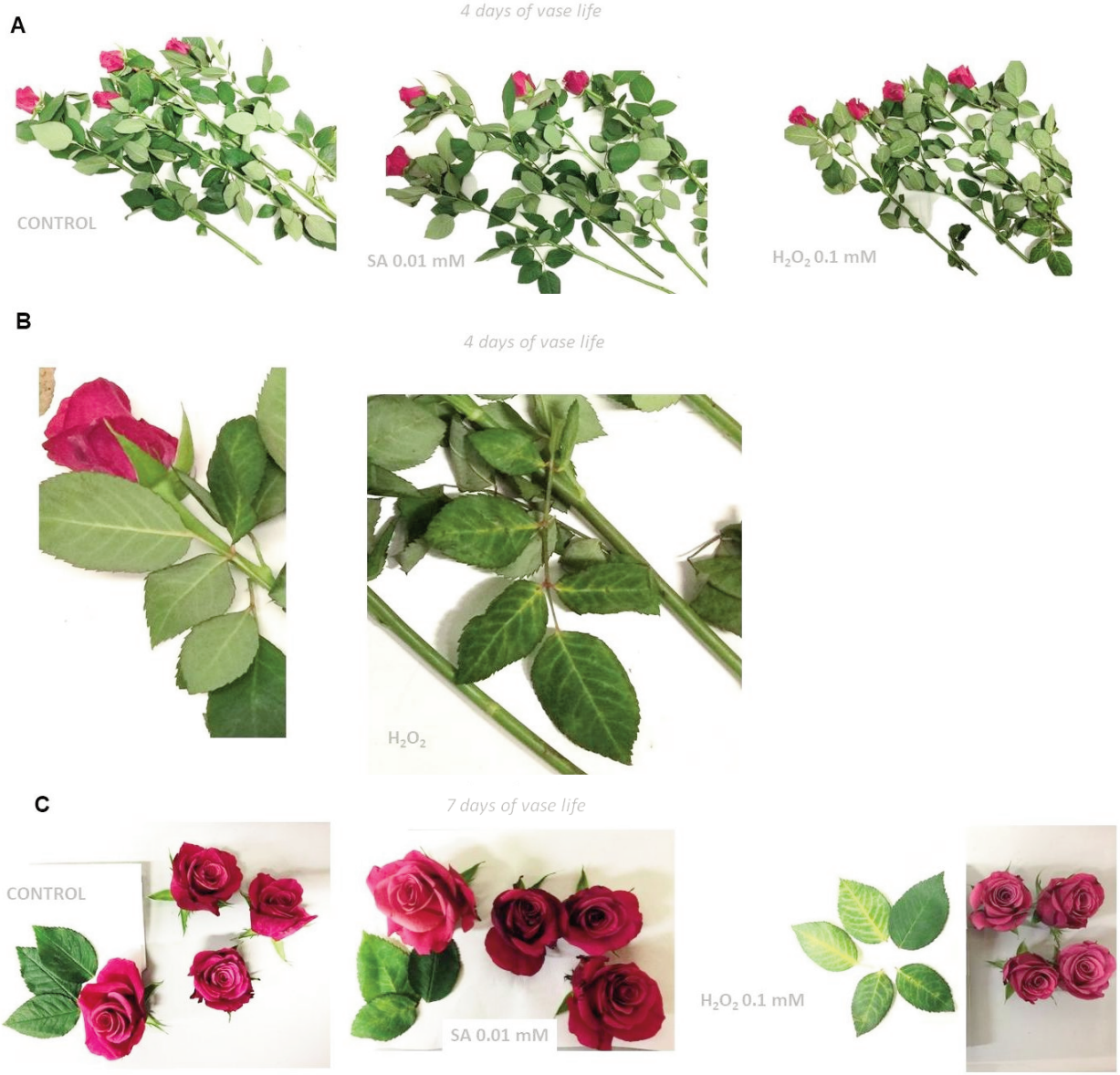

Fig. 2 - Visual appearance of cut roses (cv. Tacazzi) as affected by H_2O_2 or SA application after 4 (A, B) and 7 (C) days of vase life.

Table 1 - Changes in petal color parameters of cut 'Tacazzi' rose during vase life and in response to treatments

Treatment	Days of vase life	L*	a*	b*	h	C*
-	0	41.17±0.64 a	55.93±0.31 a	14.10±.01 a	14.14±0.98 a	57.70±0.37 a
Control	4	34.13±1.93 a	48.57±1.89 ab	9.75±1.12 ab	11.29±1.05 ab	49.56±2.00 ab
H_2O_2 0.1 mM	4	39.00±2.16 a	49.08±1.69 ab	10.46±2.13 ab	11.81±2.09 ab	50.77±2.30 ab
SA 0.01 mM	4	36.09±1.18 a	50.09±1.78 ab	10.12±1.94 ab	11.21±1.77 ab	51.17±2.11 ab
Control	7	47.73±3.02 b	48.12±2.83 ab	9.45±0.56 ab	11.16±0.63 ab	49.05±2.84 ab
H_2O_2 0.1 mM	7	51.12±1.22 b	50.13±1.67 ab	9.80±0.99 ab	11.00±0.81 ab	50.97±1.78 ab
SA 0.01 mM	7	50.19±2.67 b	44.57±3.14 b	6.49±1.02 b	8.52±1.64 b	45.10±3.04 b

Data are means ± SE (n=6). Different letters indicate significant differences ($p<0.05$).

significantly lower in the controls and in SA-treated leaves compared to H_2O_2-treated ones. However, H_2O_2 induced a pronounced discoloration of leaves, particularly at the end of the trial (Fig. 2 B, C). This observation is confirmed by the significant reduction in chlorophyll content which has been recorded after 7 days of vase life (Fig. 3 A).

All the parameters related to chlorophyll *a* fluorescence showed a trend consistent with the one observed in chlorophyll content. In particular, stems treated with H_2O_2 showed a loss in the leaf PSII functionality after 7 days of vase life, evidenced by a significant decrement in the PI value (Fig. 3 C). No significant changes were observed in Fv/Fm, RC/CSm and in DIo/RC (Fig. 3 B, D, E), even though these indexes showed a trend similar to the one observed in the PI.

Lipid peroxidation

The level of lipid peroxidation in leaves was significantly affected by treatment and by vase life duration. In leaves treatments induced higher oxidative damage compared to control, already after 4 days

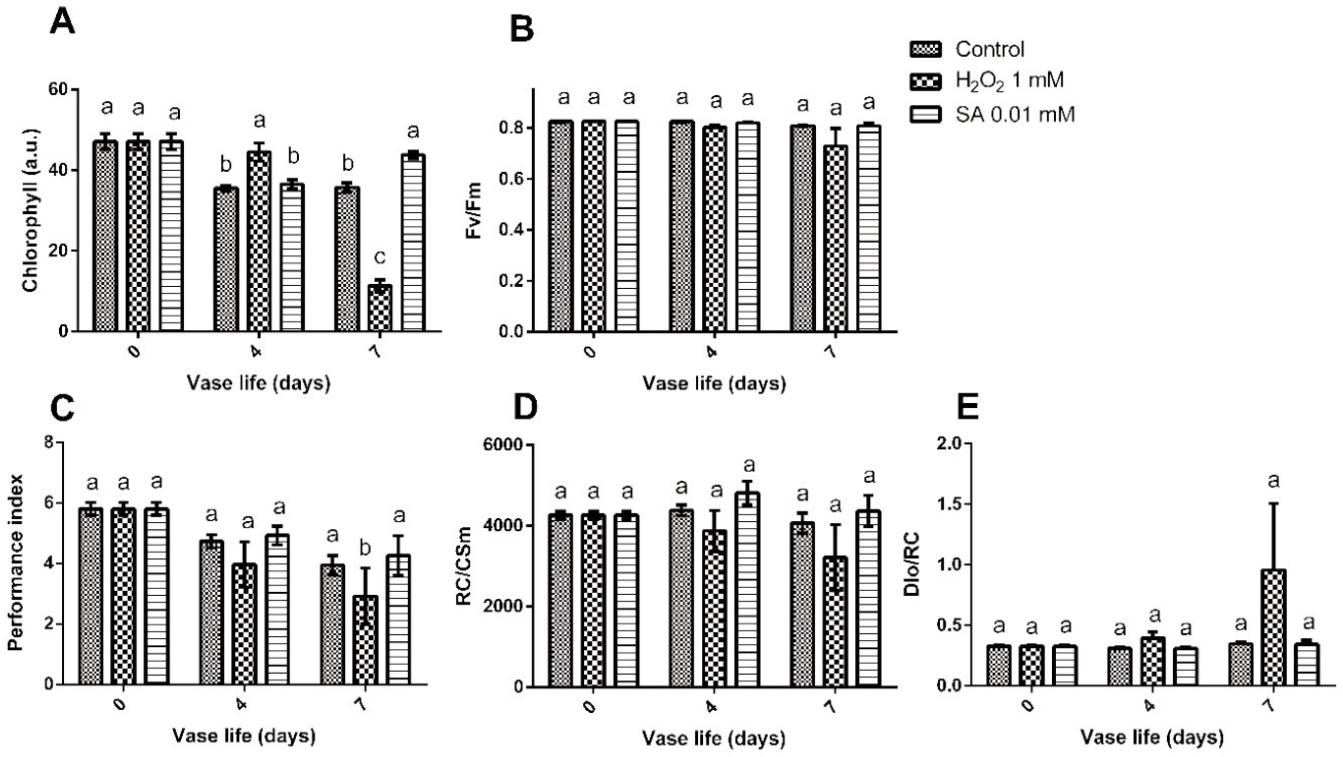

Fig. 3 - Chlorophyll content (A) and chlorophyll a fluorescence-related indexes: maximum quantum efficiency of PSII (Fv/Fm) (B), performance index (PI) (C), number of reaction centers per cross section (RC/CSm) (D), energy dissipated per reaction center (DIo/RC) (E). Measurements were conducted on leaves of cut 'Tacazzi' rose. Data are means ± SE (n=6). Different letters indicate significant differences ($p<0.05$).

and lipid peroxidation reached the maximum level in H_2O_2-treated leaves after 7 days of vase life (Fig. 4 A). The amount of MDA equivalents was markedly higher in flowers (Fig. 4 B) compared to leaves, but no significant changes were observed in petals in response to vase life or to treatments.

Phenolic index and total anthocyanins

The levels of total phenolic compounds, estimated with the phenolic index, were stable, with similar values between leaves and flowers and without showing any significant change in response to treatment or to vase life (Fig. 5 A and B).

On the other hand, total anthocyanins, which were highly accumulated in petals, progressively declined during the vase life (Fig. 5 C and D), with a significant effect of vase life time in both leaves and petals. This trend was more pronounced in petals, which showed a significant decrement already after 4 days in controls and in response to H_2O_2 treatment.

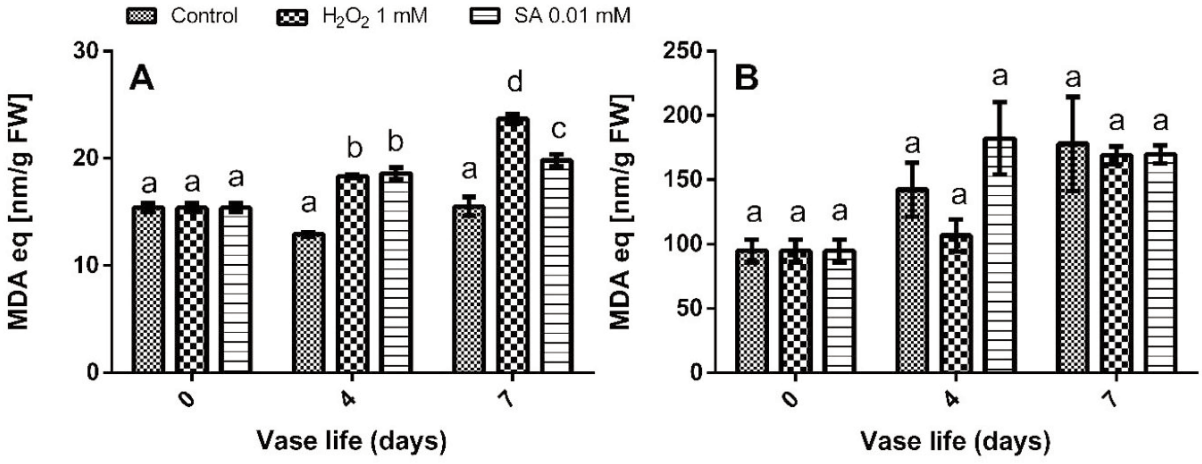

Fig. 4 - Changes in lipid peroxidation in leaves (A) or petals (B) of cut 'Tacazzi' rose during vase life and in response to treatments. Data are means ± SE (n=3). Different letters indicate significant differences ($p<0.05$).

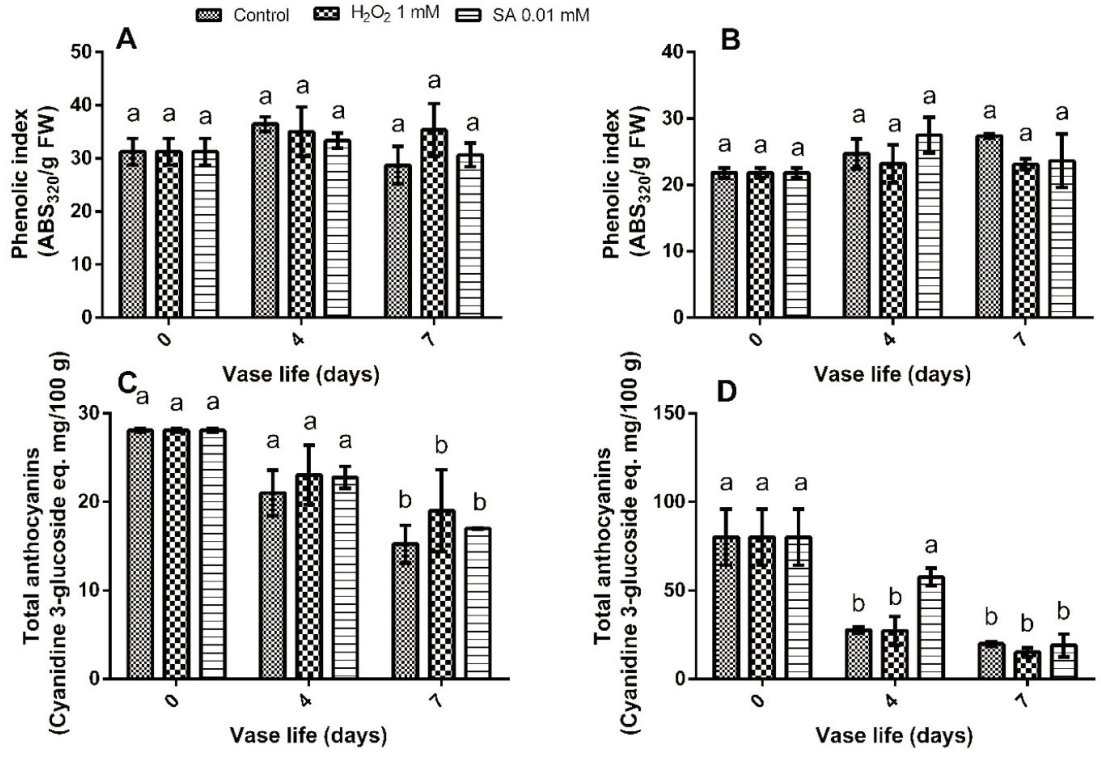

Fig. 5 - Changes in the phenolic index (A: leaves; B: petals) and in total anthocyanins levels (C: leaves; D: petals) of cut 'Tacazzi' rose during vase life and in response to treatments. Data are means ± SE (n=3). Different letters indicate significant differences ($p<0.05$).

Then again, after 4 days SA-treated petals showed significantly higher levels of anthocyanins compared to H_2O_2 and to control.

4. Discussion and Conclusions

The results obtained in this work allowed individuating independent ways of action for the two treatments and evidenced positive and negative aspects of both molecules.

As a first consideration is important to observe that both treatments determined a reduction in the product losses compared to control and in case of H_2O_2, a significant extension of the vase life. This positive result is consistent with other previously published works (Gerailoo and Ghasemnezhad, 2011; Liao *et al.*, 2012). It is also interesting to point out that the two treatments applied, induced different responses in rose leaves and petals.

The application of H_2O_2 determined a delay in the flower opening compared to control and to SA, this is consistent with what already observed in cut lily (Liao *et al.*, 2012) and in tree peony (Shi *et al*., 2015), and can have positive implications in the postharvest management of cut roses. In fact, the possibility of modulating or delaying the flower opening could be successfully be used to extent the commercial life of cut flowers and to better manage the storage and transportation. Moreover, H_2O_2 helped in maintaining low levels of MDA in petals during the first days of vase life. As a drawback, H_2O_2 induced leaves yellowing which in turn can affect the visual appearance of the stem. The detrimental effect of H_2O_2 on leaves has been confirmed by the marked loss of chlorophyll and by the higher level of peroxidation recorded at the end of the vase life. The parallel decrement in the PI, suggests a possible positive association between chlorophyll *a* fluorescence and plant stress, as previously reported (Kalaji *et al.*, 2006). Considering the results obtained in this trial, it appears that H_2O_2 is not affecting the accumulation of phenolic compounds and pigments in cut 'Tacazzi' rose.

It is known that the effect of H_2O_2 depends on the dosage (Liao *et al*., 2012), thus it is possible that the concentration of H_2O_2 applied in this trial was enough to induce oxidative stress and damage in the leaves while having a positive effect on flowers.

There are not many reports about the effect of SA in ornamental leaves, however recently, a role for SA in extending the vase life of *Acacia holosericea* foliage has been suggested (Chen and Joyce, 2017). The positive effect of SA on the leaves of cut roses has been confirmed in the present work, since at the end of the trial, SA-treated leaves showed a better maintenance of chlorophyll and leaf functionality indexes compared to controls and to H_2O_2-treated stems. The positive effect of SA could be due to the activation of plants defenses against ROS, including the increment in antioxidant compounds and the activation of antioxidant enzymes. Treatments with SA have been reported to reduce lipid peroxidation in gladiolus cut flower spikes (Rahmani *et al.*, 2015). Also, SA alone or in combination with sucrose, was shown to affect the rate of lipid peroxidation (MDA) and chlorophyll content in carnation petals (Kazemi *et al.*, 2011), but different results were observed depending on the dosage applied. Unexpectedly, in the present work, the application of 0.01 mM of SA was not effective in preventing lipid peroxidation of cut roses, perhaps because of the too low concentration applied.

The degradation of anthocyanins is a typical phenomenon which negatively affects the quality of ornamental flowers, including rose (Luo *et al.*, 2017). The role of exogenous SA in stimulating the biosynthesis of anthocyanin pigments has been documented (Ram *et al*., 2013), thus it can be hypothesized that SA treatment could delay the degradation of pigments in petals. The increase of phenolic compounds as well as the anthocyanins content could help in counteracting the senescence and extend the flower life as observed in model plants (McNish *et al.*, 2010). Despite the low dose applied, SA helped in maintaining higher levels of anthocyanins in cut rose petals after 4 days of vase life. Also, the petal coloration was affected by SA after 7 days.

In conclusion, the negative effect of H_2O_2 in leaves compared to the beneficial effect observed in flowers is controversial and it would surely worth to be investigated further. Perhaps the negative effects could be circumvented by testing other forms of application, such as direct spraying on the petals avoiding contact with leaves. The application of SA did not induce any damage, but at the same time, the positive effects were not as evident as expected compared to untreated control. This suggests that the conditions of treatment with SA can be further perfectioned. In future experiments the application of higher doses of SA could intensify the positive effects observed in this trial. Given the results obtained, the assessment of the PI, based on the chlorophyll fluorescence mea-

surement, could represent an innovative approach in the evaluation of the vase life of cut flowers, as it followed a trend consistent with the decline observed in chlorophyll content and with the increment in MDA. In fact, the non-destructive estimation of leaf functionality through this index could be further studied, implemented and successfully used for the rapid quality control of cut flowers and ornamentals.

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