



## Impact of GA<sub>3</sub> and spermine on postharvest quality of anthurium cut flowers (*Anthurium andraeanum*) cv. Arizona



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### ABSTRACT

Anthurium cut flowers exposed to low temperatures may be subjected to chilling injury, whereas higher temperatures may accelerate their metabolism and induce premature senescence. Plant growth regulators, as gibberellic acid (GA<sub>3</sub>) and spermine (SPM), have been described to extend the postharvest life of flowers. In this study, both compounds were applied by spraying or pulsing in anthurium cv. Arizona before storage at 20 °C. The solutions were constituted of 144 μM GA<sub>3</sub> and 2 μM SPM, which were used separately or in combination, and analyzed for 12 d. Spraying with GA<sub>3</sub> + SPM extended the vase life and kept the commercial quality. These treatments increase the phenols content, as well as, the activity of polyphenol oxidase (PPO), peroxidase (POD), and superoxide dismutase (SOD). Spadix sprayed with GA<sub>3</sub> or SPM retained high amounts of spermidine (SPD), and in the combination of GA<sub>3</sub> + SPM, there were higher contents of spermidine. These results suggest that the application of GA<sub>3</sub> + SPM by spraying can be used to reduce the senescence in anthurium cut flowers stored at 20 °C, and improve the commercial quality of the inflorescences.

### 1. Introduction

Anthurium (*Anthurium andraeanum* L.) (Araceae) is widely used in floriculture and landscaping and its form, color, size, and spadix and spathe, determine the commercial value and are also indicators of the inflorescences quality. The cut-flowers quickly lose their commercialization characteristics, mainly due to respiratory metabolism which results from the consumption of the energetic reserves (Promyou et al., 2012).

During the senescence of flowers occurs the increase of reactive oxygen species (ROS), such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical (HO·). In plants, the over-production of ROS is regulated by the enzymatic (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)) and/or non-enzymatic systems (Choudhury et al., 2013). In addition, may occur an increase in the phenol levels, due to the loss of the membrane integrity, which together with the increase of the polyphenol oxidase (PPO), contribute to the spathe browning in anthurium cut flowers (Soleimani Aghdam et al., 2016a).

Anthurium cut flowers presents the vase life around 8–68 d after the

harvest, according to the increase of the respiration, even with the low production of ethylene during the postharvest (Paull et al., 1992). In order to decrease the metabolic activity, one alternative is the use of low temperatures. However, these inflorescences may present chilling injury (CI) at temperatures below 12 °C (Soleimani Aghdam et al., 2016a, b), and recommended storage temperature is 12.5–20 °C (Promyou et al., 2012).

In order to increase the vase life, it is necessary to use other techniques to delay the senescence, in detrimental of low temperatures. Preservative solutions have been employed in the preservation of the cut-flowers quality. These solutions can be constituted by carbohydrates, ethylene inhibitors, growth regulators, and germicides used individually or in combination.

Plant growth regulators such as GA<sub>3</sub> and polyamines (PAs) have been described to extend the postharvest life of flowers (Nisar et al., 2015; Saeed et al., 2014). GA<sub>3</sub> is a growth regulator that has been used to extend the vase life of cut flowers. According to Emongor (2004), GA<sub>3</sub> is suggested to enhance the membrane stability and delays senescence in cut-flowers. Studies show that GA<sub>3</sub>, at lower concentrations can be used to prolong the postharvest life of several flowers (Imsabai

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and van Doorn, 2013). GA<sub>3</sub> exogenous induced an increase in the SOD and POD activities in gladiolus (Saeed et al., 2014), and improve the defense systems against ROS generated during postharvest. The spraying of anthurium with 200 mg L<sup>-1</sup> GA<sub>3</sub> extended the vase life for twenty two days (Marsala et al., 2014).

PAs such as putrescine (PUT), spermidine (SPD), and spermine (SPM), take part in many biochemical and physiological processes, including plant growth and development, mediates responses to stresses, cell division, rooting embryogenesis and development of fruit and flowers (Moschou et al., 2012; Nisar et al., 2015). Exogenous application of PAs in flowers has been verified to increase postharvest life. The vase life of *Rosa hybrida* cv. Dolce Vita increased after the application of 0.5 and 1.5 mM SPD and this increase was attributed to the highest content of proteins (Farahi et al., 2013). In *Nicotiana plumbaginifolia*, exogenous PAs (PUT, SPD and SPM) were effective antisenescence agents, retarding protein degradation and retaining the sugar contents (Nisar et al., 2015). Between the most common PAs contents, SPM is a more effective scavenger than other PUT (diamine) and SPD (triamine) (Besford et al., 1993), suggesting that there is a positive relation of the amine group in the elimination of reactive oxygen species (Kubiś, 2008). In this way, the application of SPM in anthurium cut flowers can be a form of avoiding the premature senescence by reducing the oxidative stress effect. However, application of PAs to prolong the post-harvest life of tropical flowers generates an extra cost of production and the use of low concentrations could be ideal to increase the commercial value. Due to the GA<sub>3</sub> action, as well as some PAs, in extending the vase life of cut flowers, our aim was to verify whether the separated or combined application of SPM and GA<sub>3</sub> at low concentrations can retard the senescence of anthurium cv. Arizona inflorescences. In addition, the endogenous content of PUT, SPD, and SPM were analyzed in order to establish a possible relation with the anthurium vase life.

## 2. Material and methods

### 2.1. Plant material and treatments

Anthurium cv. Arizona was acquired from local farmers in Recife city, Pernambuco state (8°03'14" S latitude and 34°52'52" W longitude) when 50% of the true flowers on the spadix had fully opened and were transported at 13 ± 2 °C in individual containers with water. After the selection, the flowers were recut in the base to maintain the pattern of 45 cm in length and placed individually in vases.

For the treatments, 240 anthuriums flowers (30 flowers per treatment) were spraying (150 mL) or submitted to pulsing (1 L) treatment with distilled water (control), 144 μM gibberellic acid (GA<sub>3</sub>), 2 μM spermine (SPM), and 144 μM GA<sub>3</sub> + 2 μM SPM, for 24 h at 20 ± 2 °C and 70% room humidity. After the treatment, the stems were transferred individually to containers with 1 L of distilled water. The stems were stored at 23 ± 2 °C and 70% room humidity, for 12 d. The visual analysis (using a subjective scale of grades) was performed each 3 d.

Analysis of total soluble proteins, total soluble carbohydrates, SOD activity and PAs contents were performed in the spathe. In the stem base, the total phenol content and the PPO and POD activities were analyzed. For the analysis, all tissues were powered by an analytical mill (IKA, A11) with liquid nitrogen, stored at -80 °C, and expressed as fresh weight.

### 2.2. Visual analysis

The commercial quality was evaluated each 3 d using a scale from 1 to 3, according to Table 1.

### 2.3. Electrolyte leakage

Samples were washed 4 times with deionized water, placed in closed vials and incubated for 6 h at 25 ± 2 °C on a rotatory shaker.

After the measurement of electrical conductivity of the solution (C1) with a conductivitymeter (DDS-12DW), the vials were incubated for 1 h at 100 °C and the electrical conductivity of the solution was measured again (C2) after the solution reached the equilibrium. The electrolyte leakage was calculated as follows: electrolyte leakage (%) = (C1 / C2) × 100.

### 2.4. Weight loss

Samples were weighed at 0, 3, 6, 9 and 12 days. The difference between of the initial fresh weight and the fresh weight of the day of analysis were utilized to calculate the percentages of fresh-weight loss (AOAC, 2005).

### 2.5. Soluble proteins

Soluble proteins extraction was carried out in 100 mg of spathe, homogenized in 0.1 M sodium phosphate buffer pH 7.0, and centrifugated at 13,000 g (Hettich Zentrifugen, Mikro220R), for 20 min at 4 °C. The soluble proteins determination was performed (Bradford, 1976), using bovine serum albumin (Sigma-Aldrich, Brazil). The results were expressed in mg g<sup>-1</sup> FW.

### 2.6. Soluble carbohydrates

Soluble carbohydrates extraction and determination were performed according DuBois et al. (1956). Spathe was homogenized in distilled water and centrifugated at 13,000 g (Hettich Zentrifugen, Mikro220R) for 20 min at 4 °C. The supernatant was collected, mixed with phenol (Sigma, Brazil) and sulfuric acid (Merck, Brazil), and incubated for 10 min. The results were expressed in mg g<sup>-1</sup> FW.




### 2.7. Polyamines

The PAs were extracted according to Lima et al. (2008) and determined by high-performance liquid chromatography (HPLC) with diode array detector in column C18 according to Ferreira et al. (2016). The extraction was carried out using 20 mg of spathe and 2 mL 5% perchloric acid (Merck, Brazil). The suspension was mixed, held in ice bath for 30 min, and centrifuged for 10 min at 6,000 g (4°). 4.5 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> and 18.5 mmol L<sup>-1</sup> dansyl-chloride in acetone was added to the supernatant (200 μl). After 120 min incubation in the dark at room temperature, it was added 100 μl of 0.87 mol L<sup>-1</sup> of proline (99%). 1000 μl toluene was used to extract the dansylated PAs and the sample aliquots were subjected to drying with N<sub>2</sub> and resuspended in 3 mL of HPLC grade acetonitrile. Twenty microliters were injected into a UHPLC system (Ultimate 3000 BioRS, Dionex-Thermo Fisher Scientific Inc., USA) equipped with a diode array detector set at 225–300 nm, run at a flow rate of 0.7 mL min<sup>-1</sup> using an Ace 5 C18 (Advanced Chromatography Technologies, UK) column (5 μm, 25 cm × 4.6 mm). The chromatographic run gradient scheme performed was established with different proportions of (A) acetonitrile 100% and (B) acetonitrile 50% as follows: 0–4 min, 40% A + 60% B; 4–8 min, 60% A + 40% B; 8–12 min, 65% A + 35% B; 12–15 min, 85% A + 15% B; 15–21 min, 95% A + 5% B; 21–22 min, 85% A + 15% B; 22 min, 75% A + 25% B. The results were expressed in mg 100 g<sup>-1</sup> FW of spermine, spermidine, putrescine, cadaverine and serotonin.

### 2.8. Total phenols (TP)

The TP was determined using Folin-Ciocalteu reagents, following the method described by (Singleton and Rossi, 1965). Samples of stem base were extracted in methanol, read at 725 nm in spectrophotometer UV/VIS (Amersham-Pharmacia-Biotech), using gallic acid as standard, and the results were expressed as mg 100 g<sup>-1</sup> FW.

**Table 1**  
Rating for visual analysis of Anthurium cv. Arizona.

Rate 1	Rate 2	Rate 3
 <p>Excellent aspect. Turgid. Inflorescence brightness. Without spots. Excellent for commercialization.</p>	 <p>Good aspect. Slightly altered spathe coloring. Absence of brightness in the spathe. Commercial quality.</p>	 <p>Bad aspect. Withered inflorescence. Color change (purplish). Darkening in the top of the spadix. Not suitable for commercialization.</p>

### 2.9. Polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7) activities

PPO and POD activities were carried out according to Simões et al. (2015). Briefly, 100 mg of stem base and 1.3 mL of 0.2 M sodium phosphate buffer pH 6.0, were vortexed and centrifugated at 13,000 g for 20 min (4 °C). The PPO activity was determined using 0.2 M of catechol. The reading was realized in a spectrophotometer (Amersham-Pharmacia Biotech Ultrospec-2000) at 425 nm. The PPO activity was calculated based on the molar extinction coefficient ( $3.4 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for catechol and expressed in  $\mu\text{mol min}^{-1} \text{ g}^{-1} \text{ FW}$ . The POD activity was assessed using 0.2 M phosphate buffer pH 6.0, 100  $\mu\text{L}$  0.5% guaiacol and 100  $\mu\text{L}$  0.08% hydrogen peroxide (0.08%). The readings were performed in a spectrophotometer (Amersham-Pharmacia Biotech Ultrospec-2000) at 270 nm. The POD activity was calculated based on the molar extinction coefficient ( $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for guaiacol and expressed in  $\mu\text{mol min}^{-1} \text{ g}^{-1} \text{ FW}$ .

### 2.10. Superoxide dismutase (SOD, EC 1.15.1.1) activity

The extraction was performed according to Cavalcanti et al. (2004). The homogenization of 100 mg tissue in 1.3 mL sodium phosphate buffer 0.1 M (pH 7.0) was realized with liquid nitrogen. The extract was centrifugated at 13,000 g for 21 min at 4 °C. The SOD activity was determined as described (Giannopolitis and Ries, 1977) and the readings were performed at 560 nm. The activity was determined based on the inhibition of the nitro blue tetrazolium chloride (NBT) reduction and the quantity of enzyme needed to inhibit 50% of the photoreduction was defined as the activity unit (Beauchamp and Fridovich, 1971). The activity was expressed in A.U.  $\text{min}^{-1} \text{ g}^{-1} \text{ FW}$ .

### 2.11. Experimental design and statistical analysis

The experimental design was entirely randomized in factorial scheme, with two application methods (spraying and pulsing) and 4 treatments (144  $\mu\text{M}$  GA<sub>3</sub>, 2  $\mu\text{M}$  SPM, and 144  $\mu\text{M}$  GA<sub>3</sub> + 2  $\mu\text{M}$  SPM, and control). Each repetition was constituted of six stems, in a total of five repetitions. The graphics were generated using the software Sigma Plot version 12.

## 3. Results and discussion

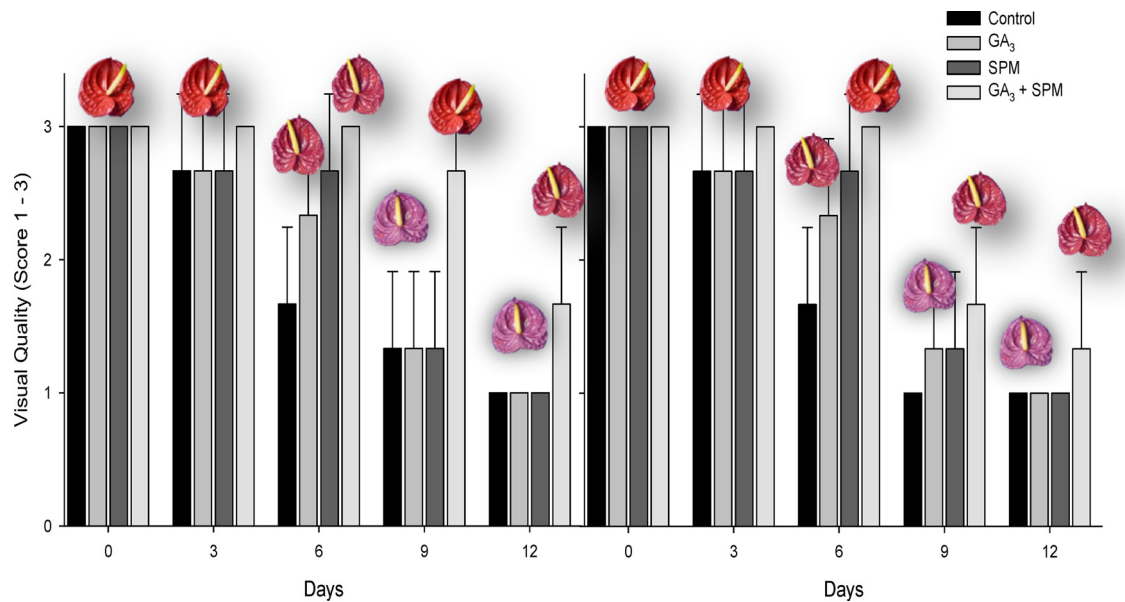
In the harvest day and 3 d after, the flowers stems were turgid, without spots, and with a bright red color, which reflects the good quality for commercialization. No visual differences were observed between control and treatments for these periods (Fig. 1A and B). The visual quality of flowers stems decreased after spraying and pulsing treatments with GA<sub>3</sub> or SPM, similarly to the control, from day 6 of postharvest up to day 12 (Fig. 1A and B). These results showed that GA<sub>3</sub> or SPM, independent of the application method used (spray or pulsing),

were not able to retain the commercial quality of anthurium cut flowers, for a long time.

Anthurium cut flowers sprayed with GA<sub>3</sub> + SPM solution kept better commercial characteristics in comparison to the other treatments, until the end of the experiment (12 d). The same solution applied under pulsing was efficient to retain the commercial quality up to day 9 (Fig. 1B). In the two application methods studied (spray and pulsing), GA<sub>3</sub> + SPM increased the postharvest quality of anthurium cv. Arizona. However, applications made by spray maintained the commercial quality for a longer time (Fig. 1A). The vase life of anthurium cut flowers is generally determined by the color change. These changes occur in the spathe or spadix and there are reports that the inflorescences may be lost from a few days to weeks, depending on the cultivar (Elibox and Umaharan, 2010). In the present work, the changes in pigment content were not measured, but the visual changes were visualized in subjective notes (Fig. 1A and B). In heliconia cv Golden Torch, the pulverization with GA<sub>3</sub> (100 mg L<sup>-1</sup>) extended the vase life for 15.4 d, in comparison to the control (8.5 d) (Mangave et al., 2013). Spermine at 1 mmol L<sup>-1</sup> extended the vase life and decreased the production of ethylene in carnation flowers (Lee et al., 1997). Exogenous SPM application modified the senescence and death of the cells in *Nicotiana tabacum* flowers by executing a pro-survival role, delaying the weight loss, in function of the inhibition and degradation of some pigments and by avoiding de DNA degeneration (Cai et al., 2015). In contrast, the use of SPM in rosa cv. Dolcivita did not promote any vase life extension (Farahi et al., 2013), showing a variable response of flowers to this polyamine application.

Protein levels in the flowers treated with GA<sub>3</sub> and SPM, combined or not, remained more stable than the control when applications were made by spraying (Fig. 2A). For anthurium cut flowers treated by pulsing, protein content decreased in all samples (including control) after day 9 (Fig. 2B). These results suggest that treatments applied had a slightly effect on the protein content.

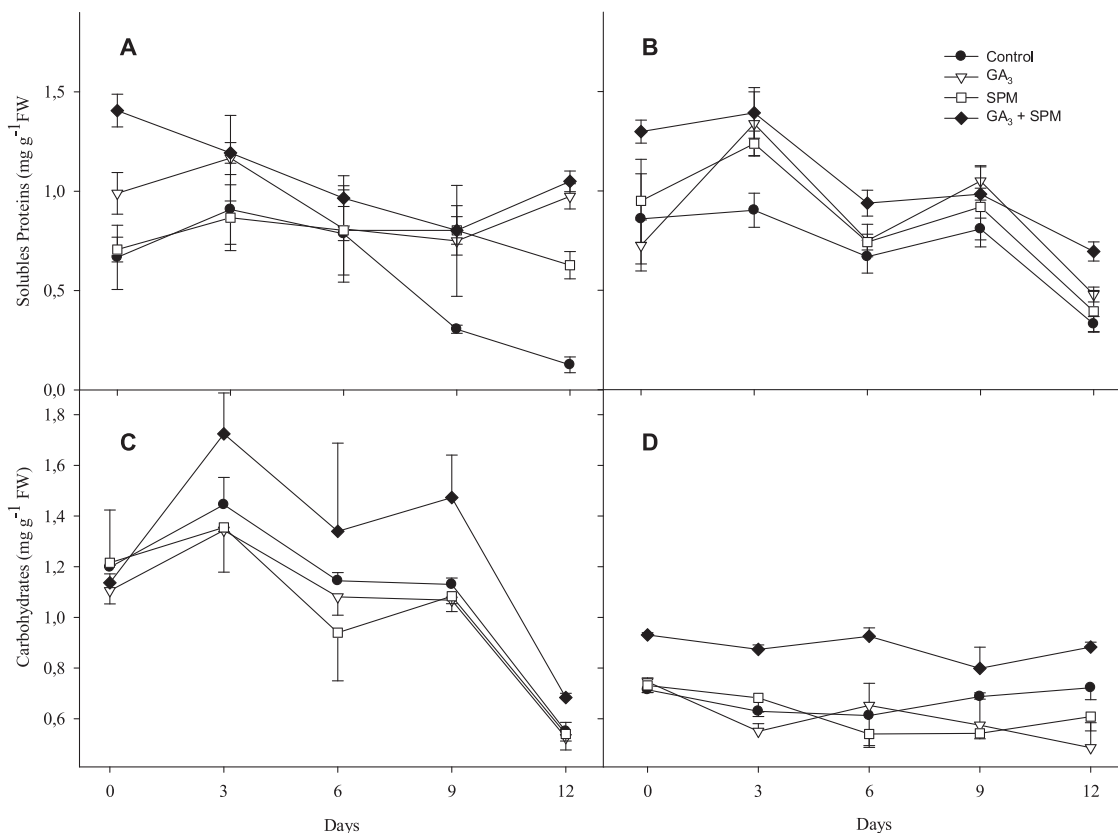
Higher total soluble carbohydrates levels occurred in inflorescences treated with GA<sub>3</sub> + SPM, however, the total carbohydrates contents were lower when the mixture was applied via pulsing (Fig. 2C and D). Usually, the carbohydrates and proteins levels decrease during the senescence process in plants, due to oxidative processes that occur after the harvest. The maintenance of higher carbohydrates levels in cut flowers is essential to prolong vase life and may be related to ethylene synthesis alteration (Pun and Ichimura, 2003). In the research performed by Emongor (2004), the exogenous utilization of gibberellin in gerbera promoted an increase of reducing sugars due to the reserve carbohydrates hydrolysis and, consequently, raising the osmotic potential of the flower head and stems, improving the turgidity and longevity. Spermine is associated to modify the carbohydrates metabolism enzymes and promote the maintenance of higher sugar contents, such as sucrose, during the storage of some species (Song et al., 2015). The results show that the isolated application of GA<sub>3</sub> or SPM were not efficient in the maintenance of carbohydrates levels.



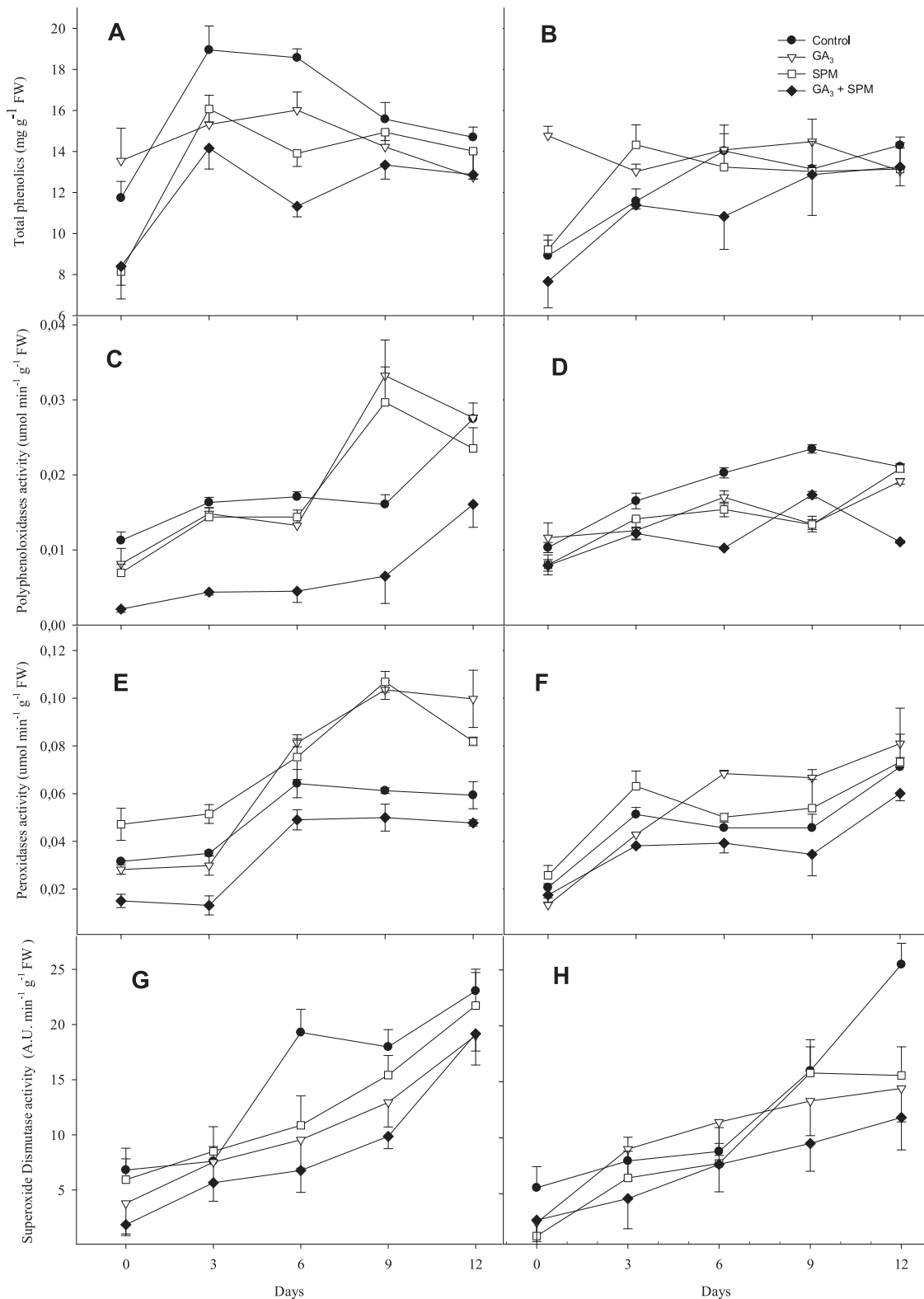
**Fig. 1.** Quality analysis in anthurium cut flowers cv. Arizona after spraying (A) or pulsing (B), with solutions containing gibberellic acid (GA<sub>3</sub>, 144 μM), spermine (SPM, 2 μM), gibberellic acid (GA<sub>3</sub>, 144 μM) + spermine (SPM, 2 μM) and distilled water (control). The flowers were maintained at 20 ± 2 °C, for 12 days.

The spraying or pulsing with GA<sub>3</sub> + SPM induced an increase in the total phenols content (Fig. 3A and B). Phenolic compounds may be synthesized during the normal plant growth or when the plant is submitted to any kind of stress, providing a cell level form of protection against damages from the action of reactive oxygen species (ROS) (Torras-Claveria et al., 2012). Our results showed that the GA<sub>3</sub> + SPM application were followed of increased phenolic content, indicating that

a possible mechanism of cell-membrane defense was activated in flowers and resulted in the absence of darkening in these treatments (Fig. 1A and B). The PPO enzyme activity (Fig. 3C and D) also show an increase in anthurium treated with GA<sub>3</sub> + SPM (spraying or pulsing) during the storage. However, as observed for the total phenol content, the PPO activity in anthurium treated with GA<sub>3</sub> + SPM (spraying or pulsing) was lower than the other treatments and showed an increase



**Fig. 2.** Soluble proteins and carbohydrates in anthurium cut flowers cv. Arizona after spraying (A and C) and pulsing (B and D), with solutions containing gibberellic acid (GA<sub>3</sub>, 144 μM), spermine (SPM, 2 μM), gibberellic acid (GA<sub>3</sub>, 144 μM) + spermine (SPM, 2 μM) and distilled water (control).



**Fig. 3.** Total phenolics, polyphenoloxidases (PPO), peroxidases (POD) and superoxide dismutase (SOD) activities in anthurium cut flowers cv. Arizona under spraying (A, C, E and G) and pulsing (B, D, F and H), with solutions containing gibberellic acid (GA<sub>3</sub>, 144 μM), spermine (SPM, 2 μM), gibberellic acid (GA<sub>3</sub>, 144 μM) + spermine (SPM, 2 μM) and distilled water (control).

during the vase life. These results were similar for enzymes that act in the ROS decrease, such as POD and SOD (Fig. 3E–H). According to Aghdam et al. (2016), the increase of reactive species as H<sub>2</sub>O<sub>2</sub>, which promotes the lipidic peroxidation of the membranes, is one of the factors that decreases the anthurium flowers commercial quality.

Generally, after harvest, it is possible to occur the ROS formation, what damage the cell membranes, including lipidic peroxidation and superoxide radicals, hydrogen peroxide and hydroxyl radicals (Rogers, 2012). In the last years, the ROS effects during the senescence have been intensely studied and the obtained data can confirm the protective

role of the gibberellin combined to spermine when used in order to extend the anthurium vase life at room temperature.  $GA_3$  + SPM induced lower electrolyte leakage and weight loss, which may indicate lower membrane damage (Fig. 7, supplementary material).

Spermine, such as other PAs, have been described for acting as antioxidants, including in flowers such as *Nicotiana plumbaginifolia* L. (Nisar et al., 2015). In addition, this tetramine has been described for presenting an important role in the membranes stabilization (Lester, 2000). Some studies show that  $GA_3$  may have a protective role in the membranes stabilization and, in low concentrations (lower than 200 mg/L), decreased the unsaturated fatty acids oxidation (Saeed et al., 2014). Thus, the beneficial effect of the  $GA_3$  + SPM combination in the used concentrations can be attributed to the membranes stabilization, influencing the anthurium cut flowers senescence. In the  $GA_3$  + SPM combination, the inflorescences showed the lowest activities of PPO (Fig. 3C and D), POD (Fig. 3E and F) and SOD (Fig. 3G and H) during the storage, when compared to the other treatments. This may give indications that this enzymatic antioxidant system may not have been the main protection mechanism for the conservation of these flowers. Inflorescences treated only with distilled water or with  $GA_3$  or SPM isolated, showed an inferior commercial aspect compared to the ones treated with  $GA_3$  + SPM, which showed a cell degeneration, characteristic of the senescence (Fig. 1A and B). Spermine, such as some other PAs, has been described as acting as an antioxidant in various species, including in flowers, as *Nicotiana plumbaginifolia* L (Nisar et al., 2015) and the gibberellic acid, depending on the concentration, may induce tolerance to stress, increasing the antioxidant activity, extending the vase-life (Saeed et al., 2014). The  $GA_3$  isolated concentration used was probably not efficient to decrease the stress and, consequently, to modify the activity of the analyzed enzymes. Thus, the combined action of  $GA_3$  + SPM was efficient to keep the lower enzymes levels than in the other treatments and extend the vase-life.

Inflorescences sprayed with  $GA_3$  contained higher levels of

putrescine at third and ninth day of storage (Fig. 4A). In contrast, in the spadix pulverized with  $GA_3$  and/or SPM presented lower putrescine values compared with the control (Fig. 4B). The inflorescences sprayed with SPM or  $GA_3$  + SPM presented the highest levels of cadaverine (Fig. 4D). The same effect was not observed when SPM was applied via pulsing (Fig. 4D). This effect can be attributed to the senescence, as well as observed in the visual analysis.

From the ninth day, all treatments with  $GA_3$  and SPM via spray, isolated or combined, promoted an increase in the SPD content (Fig. 5A). The application of these products via pulsing did not promote significant alterations in the SPD levels (Fig. 5B). The highest SPM content can be noted in the inflorescences pulverized with  $GA_3$  + SPM, except on the sixth day of storage, when the inflorescences received isolated SPM by spray, showed a significant increase in relation to other treatments (Fig. 5C). Similar to what was found for SPD in inflorescences that were submitted to pulsing, the SPM content was not superior to what was found in the inflorescences treated only with water (Fig. 5D). However, exogenous SPM induced raise in the endogenous SPM content when the inflorescences received the spraying treatment.

Spermidine, spermine and ethylene present the S-adenosyl methionine (SAM) as a common precursor. In the spermidine and spermine synthesis from putrescine, can occur the delay the senescence (Kaur-Sawhney et al., 2003) by using SAM, via SAM decarboxylase. However, a putrescine accumulation may occur in the senescence due to the utilization of SAM in the ethylene synthesis and, therefore, decrease the contents of spermidine and spermine. The PAs can be oxidized by polyamines oxidases, which produce peroxides (Kaur-Sawhney et al., 2003), considered ROS. In order to decrease the peroxides content and possible oxidant actions, peroxidases catalyze the degradation of these compounds. In our study, the levels of POD were similar for all samples and increased during the period of storage. Therefore, increased levels of POD could be associated to ROS scavenging defenses that maintain

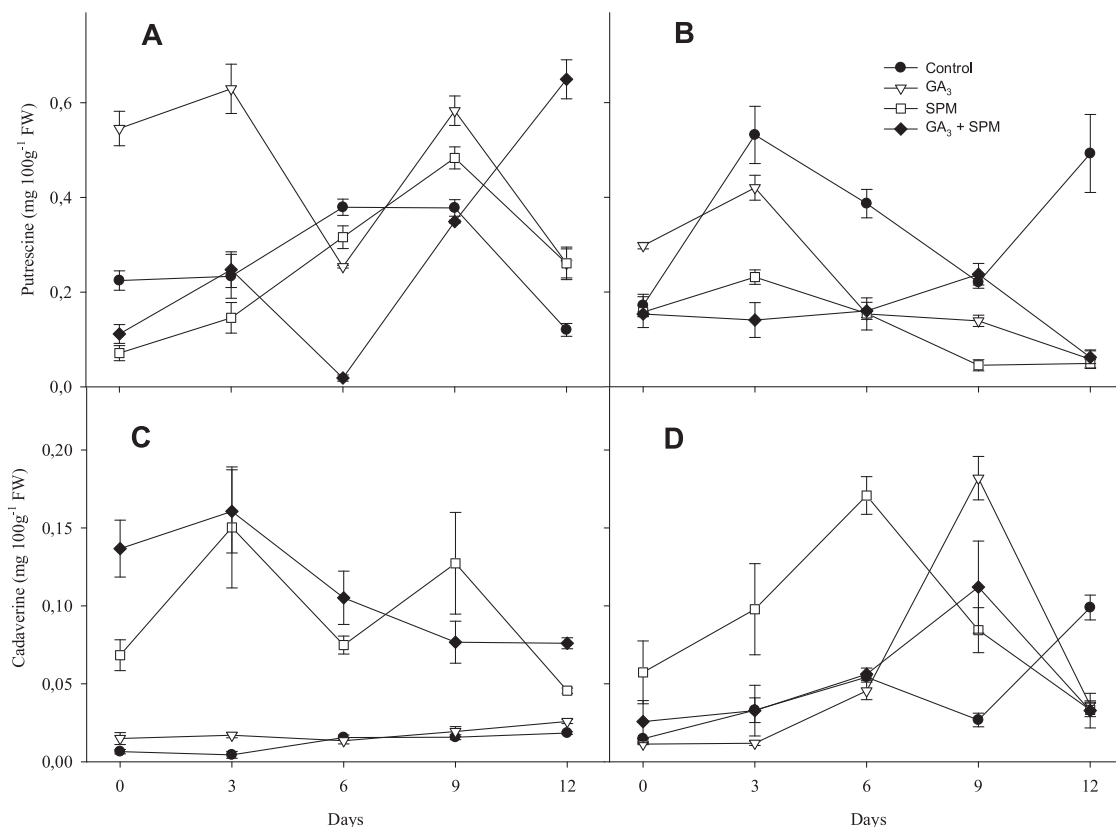


Fig. 4. Putrescine and cadaverine in anthurium cut flowers cv. Arizona subjected to spraying (A and C) and pulsing (B and D), with solutions containing gibberellic acid ( $GA_3$ , 144  $\mu$ M), spermine (SPM, 2  $\mu$ M), gibberellic acid ( $GA_3$ , 144  $\mu$ M) + spermine (SPM, 2  $\mu$ M) and distilled water (control).

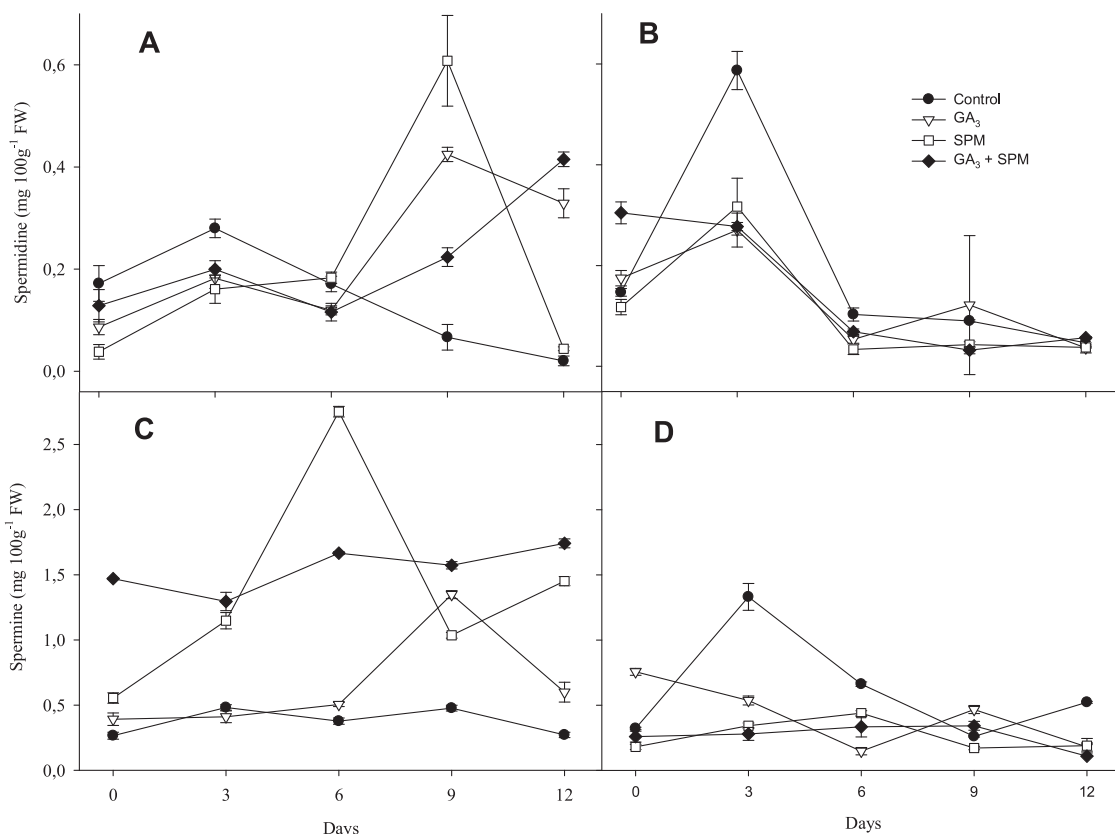


Fig. 5. Spermidine and spermine in anthurium cut flowers cv. Arizona under spraying (A and C) and pulsing (B and D), treated with solutions containing gibberellic acid (GA<sub>3</sub>, 144 μM), spermine (SPM, 2 μM), gibberellic acid (GA<sub>3</sub>, 144 μM) + spermine (SPM, 2 μM) and distilled water (control).

the durability and commercial quality of anthurium (Fig. 1A and 1B). In the PAs content analysis, this treatment via spraying promoted a spermine accumulation in anthurium, as well as the isolated SPM application, which could support the protection against damages promoted by the senescence, including the protection against membrane permeability loss (Lester, 2000).

Few studies describe the serotonin content in flowers or in plants in relation to the postharvest effects. In this study, the serotonin levels were not influenced by the growth regulators (GA<sub>3</sub> and/or SPM) (Fig. 6A and B), except after the GA<sub>3</sub> application at six days in inflorescences (sprayed) and at nine days, in the ones treated by pulsing. Anthurium cv. Arizona treated with GA<sub>3</sub> + SPM presented extended commercial quality and contain lower amounts of serotonin. This

metabolite was found for accumulating in great quantity during senescence of rice leaves, as described by Kang et al. (2009), however, other authors, in spite of noting a correlation between serotonin and the senescence symptoms, do not show clearly how this compound would be involved in the senescence induction or delay. From our results, inflorescences that showed higher serotonin level did not show a good commercial quality at the end of the experimental period, which could be a biochemical indicator for the anthurium cut flowers senescence.

Based on the data obtained, it is believed that in anthurium cut flowers cv. Arizona handling and maintenance at 20 °C stimulates cell damage factors as: dehydration, electrolyte leakage, decrease of protein and sugar levels, and may stimulate oxidative damage by ROS production. However, the handling associated to exogenous application by

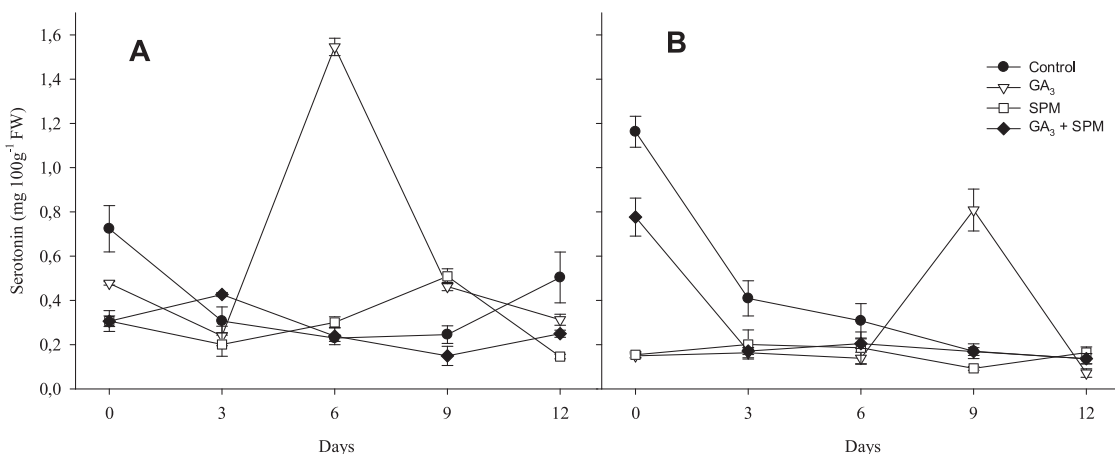


Fig. 6. Serotonin in anthurium cut flowers cv. Arizona under spraying (A) and pulsing (B), treated with solutions containing gibberellic acid (GA<sub>3</sub>, 144 μM), spermine (SPM, 2 μM), gibberellic acid (GA<sub>3</sub>, 144 μM) + spermine (SPM, 2 μM) and distilled water (control).

spraying of GA<sub>3</sub> and SPM in the flower may stimulate synergistically the accumulations of putrescine, cadaverine, spermine, and spermidine more evident and significant than serotonin. The accumulation of these PAs may be related to non-enzymatic antioxidant defense mechanisms than the enzyme mediated by POD and SOD. Furthermore, others mechanism of protection are not completely elucidated, some molecules can be involved in flowers protection, such as nitric oxide (NO<sup>-</sup>), gamma-amino butyric acid (GABA); proline, as well as, products of N<sub>2</sub> metabolism and the tricarboxylic acid citric (Alcázar et al., 2006, 2010). Isolated or together these metabolites and/or metabolic changes should stimulate, even more, the cellular defenses resulting in greater longevity of the anthurium cut flowers cv. Arizona.

#### 4. Conclusion

The treatment with 144 μM GA<sub>3</sub> + 2 μM SPM by spray were efficient to extend the vase life of anthurium cv. Arizona cut flowers stored at 20 °C. In addition, GA<sub>3</sub> and SPM treatment was efficient to maintain the levels of carbohydrates. The flowers treated with the GA<sub>3</sub> and SPM combination promoting increase in the activity of enzymes such as PPO, POD, and SOD; however, with lower activities compared to the other treatments. GA<sub>3</sub> + SPM stimulated endogenous accumulations of spermidine, spermine, putrescine and cadaverine, applied by spraying, which may indicate the participation of these polyamines in the non-enzymatic antioxidant defense mechanism and thus, improving the commercial quality of the inflorescences.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.scienta.2018.06.095>.

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