Scientific Article

Endogenous levels of polyamines during cold storage of bird-of-paradise treated with biocides[1]

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ABSTRACT

This study aimed to analyse the endogenous levels of free polyamines putrescine (Put), spermidine (spd) and spermine (spm) under the effect of 8-HQC (8-hydroxyquinoline citrate) and chlorine during cold storage of bird-of-paradise floral scapes. Flowers were selected, labelled and randomly distributed in recipients for postharvest trials. The base of flower scapes was immersed with pulsing treatments (Control, 100 chlorine, 250 or 500 mg L^{-1} 8-HQC) for 48 h, thereafter, stored at 10.5 °C and 90% relative humidity for a period of 12 days. Flower parts were sampled for polyamines analysis at day 0, 4, 8 and 12 days during storage. All samples had higher Put levels than the control. Treatment with 500 mg L^{-1} 8-HQC showed the highest Spd levels in bracts, while chlorine treatment had the highest Spm levels in stems.

Keywords: *Strelitzia reginae*, inflorescence, ornamental plant, postharvest, senescence.

RESUMO

Teores endógenos de poliaminas durante o armazenamento refrigerado de strelitzia tratados com biocidas

O estudo teve como objetivo analisar os níveis endógenos de poliaminas livres (putrescina, espermidina e espermina) sob o efeito de 8-HQC (citrato de 8-hidroxiquinolina) e cloro durante o armazenamento frio de hastes florais de strelitzia. As flores foram selecionadas, rotuladas e distribuídas aleatoriamente em recepientes com os tratmentos pós-colheita. A base das hastes florais foi imersa em tratamentos de *pulsing* (Controle, 100 cloro, 250 ou 500 mg L^{-1} de 8-HQC) durante 48 h, depois armazenadas a 10,5 °C e 90% de umidade relativa por 12 dias. Análises das partes das flores foram amostradas para análises de poliaminas no dia 0, 4, 8 e 12 durante o armazenamento. Todas as amostras apresentaram maiores níveis de Put que o controle. O tratamento com 500 mg de L^{-1} 8-HQC mostrou os maiores níveis de Spd em brácteas, enquanto o tratamento com cloro apresentou os maiores níveis de Spm em hastes.

Palavras-chave: *Strelitzia reginae*, inflorecência, plantas ornamentais, pós-colheita, senescência.

1. INTRODUCTION

The polyamines (PAs) are low-weight aliphatic molecules present in all organisms. The main PAs in higher plants are free putrescine (Put), spermidine (Spd) and spermine (Spm) or conjugated to phenolic acids and low-weight molecules (BOUCHEREAU et al., 1999; KUZNETSOV et al., 2007). PAs function as stress messengers in plant responses to different stress and play a important role on plant tolerance (GUPTA et al., 2013). Changes in PAs and ethylene levels were observed during senescence in plum (DE DIOS et al., 2006) and *Hibiscus syriacus* (SEO et al., 2007), and there is metabolic competition between ethylene and PAs under a biotic or abiotic high stress conditions (LI et al., 2004).

Biocides are used in pulsing and vase solutions in order to preserve the full postharvest longevity of cut flowers. The most widely held and effective biocides are esters of hydroxyquinoline (HQ) and their effectiveness as an biocide in cut flower handling solutions is widely know (DAMUNUPOLA and JOYCE, 2008). Sulphate (HQS) and citrate (HQC) forms of HQ are commonly used to increase cut flower longevity by acidifying the vase solution and acting as antitranspirant thus limiting water

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losses (DAMUNUPOLA and JOYCE, 2008). Chlorine (e.g. sodium hypochlorite, sodium dichloroisocyanuric acid; DICA) is an antimicrobial agent with a potential use as biocide in cut flower hydration solutions. Thus, chemical treatments as citrate compound 8-hydroxyquinoline (8-HQC) and chlorine when combined with cold storage, can modify the endogenous levels of PAs.

Bird of Paradise (*Strelitzia reginae*) is native to South Africa. Modified leaves, at the end of the stalk form canoe-like structures, four to eight inches long, from which flowers emerge forming a spectacular blossom. The petals are brilliant orange and contrasted with an arrow shaped tongue of blue (BURGESS, 2015). The vase life of bird paradize flowers varies from 6 to 15.5 days (JAROENKIT and PAULL, 2003). The longevity of bird of paradise flowers can be substantially increased by pulsing buds or flowers with a solution containing 10% sucrose, 250 ppm 8 hydroxyquinoline citrate (8-HQC) and 150 ppm citrate and storage at 6 to 7 °C (BMT, 2013).

This study aimed to analyze the endogenous levels of free PAs (Put, Spd and Spm) as possible senescence marker for bird-of-paradise cut flowers treated with biocides under cold storage.

2. MATERIALS AND METHODS

Experiment

Experiments were performed with fully opened bird-of-paradise flowers, collected in September from a cultivation field at São Manuel, São Paulo State, Brazil (22°43'52''S and 48°34'14''W), 750 m above sea level. After flowers harvest in the morning, flowers scapes were cut and standardized to 80 cm and hydrated approximately for 10 to 15 min.

The flowers were randomly transferred into containers (15-20 L) with 1.5 L of water. The base of flower scapes was immersed into pulse solution with 8-HQC (250 or 500 mg L^{-1}) and chlorine (100 mg L^{-1}) for 48 h, with pH ranging from 6.0 to 6.5. Flowers on treatment control were also pulsed in tap water. Solutions were changed every 48 h, to prevent the proliferation of microorganisms. The flowers were storaged at 10.5 °C with 90% relative humidity, for 12 days during the experiment. Floral sepals, petals, stems and bracts samples were collected at 0, 4, 8 and 12 days for biochemical characterization of free PAs.

Determination of polyamines

Polyamines were determined by thin layer chromatography following the method described by Flores and Galston (1982), and adapted by Lima et al. (2008). Fresh material of sepals, petals, stems and bracts (50 mg) were homogenized for 1 min in 5% (v/v) cold perchloric acid (Merck), using a food homogenizer. After centrifugation for 20 min at 4 °C, dansyl chloride (400 µL; Sigma 95%), satured sodium carbonate (200 µL) were added to the supernatant. Proline (100 mg L^{-1}; Sigma) was added to the supernatant after 1 h to stop the reaction and the solution was brought to 60 °C. The mixture was maintained in the dark for 30 min at ambient temperature. Toluene was used to extract dansylated PAs and aliquots were applied onto thin layer chromatography plates (glass plates coated with 60 G silica Gel - Merch; 20 × 20). Separation was carried out in laboratory bowls containing chloroform: triethylamine (Merck) (10:1, v/v). Put (Sigma), Spd (Sigma) and Spm (Sigma) standards were subjected to the same process. The entire procedure was monitored under UV light (254 nm). PAs were quantified by comparison against standards, which were also applied onto to the plates, by fluorescence emission spectroscopy (Physics 3600 - Advanced Physics Lab-1 - Summer, excitation at 350 nm and emission measurement at 495 nm) in a Video Documentation System, using the Image Master version 2.0 software program.

Statistical analysis

The experimental design was randomized, with four pulsing treatments (control, 100 mg L^{-1} chlorine, 250 mg L^{-1} 8-HQC or 500 mg L^{-1} 8-HQC), four tissues (sepals, petals, stems and bracts) and four harvest periods (0, 4, 8 and 12 days), consisting of seven replicates and five floral samples each. Analysis of variance was performed to detect differences between treatments means using SAS/STAT software (2008 version).

3. RESULTS

Put levels in sepals were constant on pulsing treatments (Figure 1A). High Put levels were observed in sepals after pulsed/treated with 100 mg L^{-1}. Put levels in sepals were above 10 µg g^{-1} and below 40 µg g^{-1}, independent of the application of biocides (Figure 1 A). Put levels in petals (Figure 1B) of treatment 100 mg L^{-1} chlorine had a sharp decline at the beginning of the experiment. Treatment 500 mg L^{-1} 8-HQC had higher Put levels on the fourth day in all plant tissues, when compared to day zero, when started to decline throughout the storage period. Petals treated with 250 mg L^{-1} 8-HQC showed higher Put levels on the eighth day when compared to previous periods and decreased until the twelfth day. Stems in the Control had higher Put levels during storage (Figure 1C). Put levels in stems treated with 100 mg L^{-1} chlorine remained constant until the eight day, and an increasing tendency was observed (Figure 1C). Bracts of chlorine treatment showed higher Put levels until the eighth day and then started to decline.

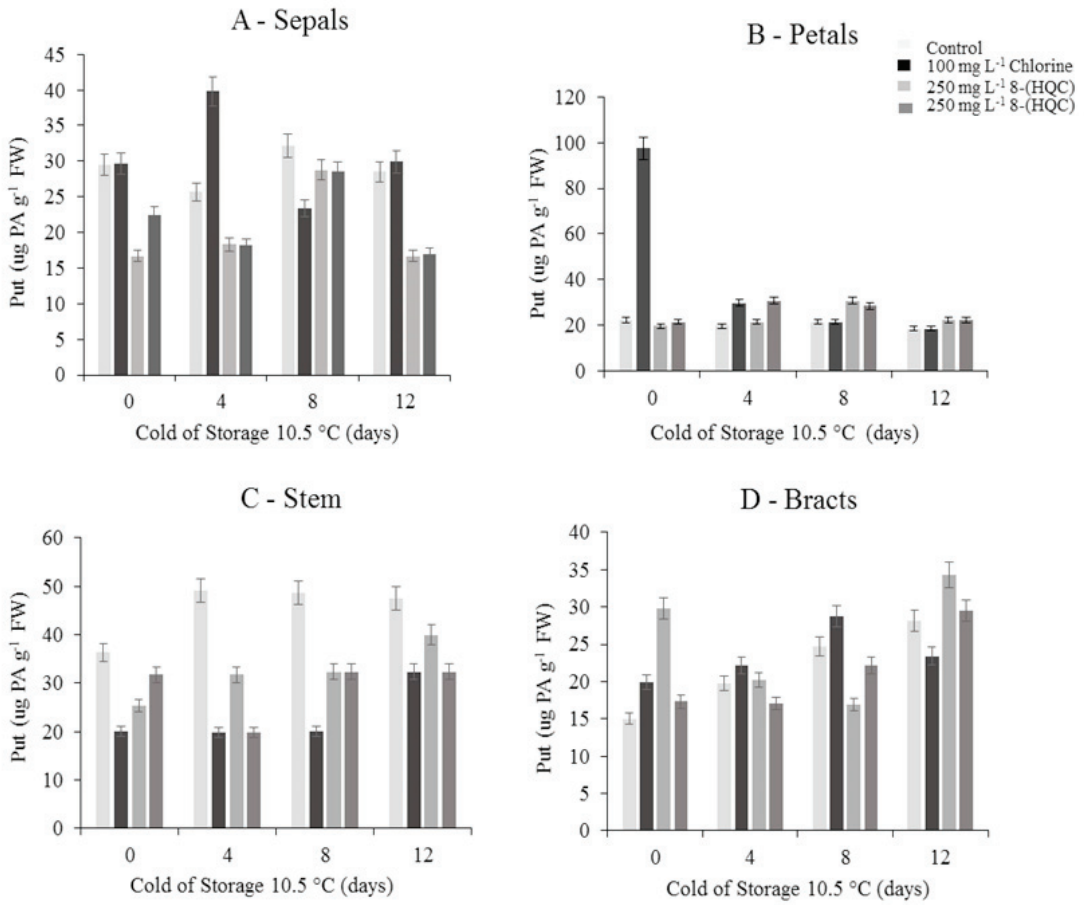

Figure 1. Putrescine (Put) level (μg g^{-1} fresh weight) in bird-of-paradise (sepals, petals, stem and bracts) submitted four pulsing treatments during storage at 10.5 °C for twelve days.

Spd levels in sepals were higher than Put levels (Figure 2A). Sepals showed Spd levels above 80 μg g^{-1} and below 40 μg g^{-1}. Petals (Figure 2B), likewise to Put levels, Spd levels in petals declined on the treatment 100 mg L^{-1}.

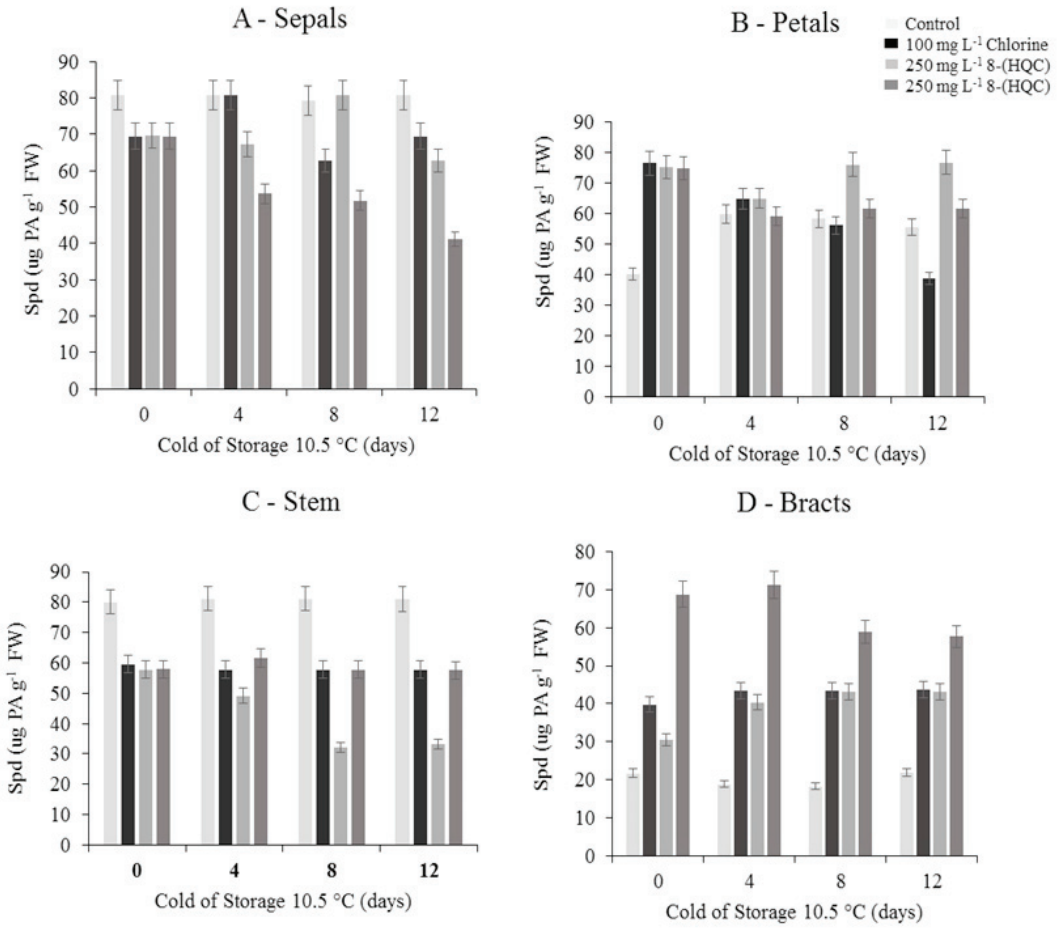

Figure 2. Spermidine (Spd) level (µg g^{-1} fresh weight) in bird-of-paradise (sepals, petals, stem and bracts) submitted four pulsing treatments during storage at 10.5 °C for twelve days.

Control showed higher Spd levels in relation to the starting point and a decline over the experiment period. Stems had higher Spd levels (Figure 2C) on Control. Bracts of treatment 100 mg L^{-1} chlorine had high Spd levels on day four when compared to day zero, remaining constant. Spd levels in stem of treatment 250 mg L^{-1} 8-HQC in the, a declined until eighth day, followed by an increase. Higher Spd levels in bracts of treatment with 500 mg L^{-1} 8-HQC was observed at fourth day (Figure 2D), from this point, there was a sharp drop in Spd levels. In contrast, Spd levels increased in bracts during the storage period in 250 mg L^{-1} 8-HQC treatment.

Spm levels in sepals on treatment 500 mg L^{-1} 8-HQC increase (Fig. 3A), except at twelfth day. Spm levels in sepals on treatment 250 mg L^{-1} 8-HQC decline until eighth day, increasing after this point.

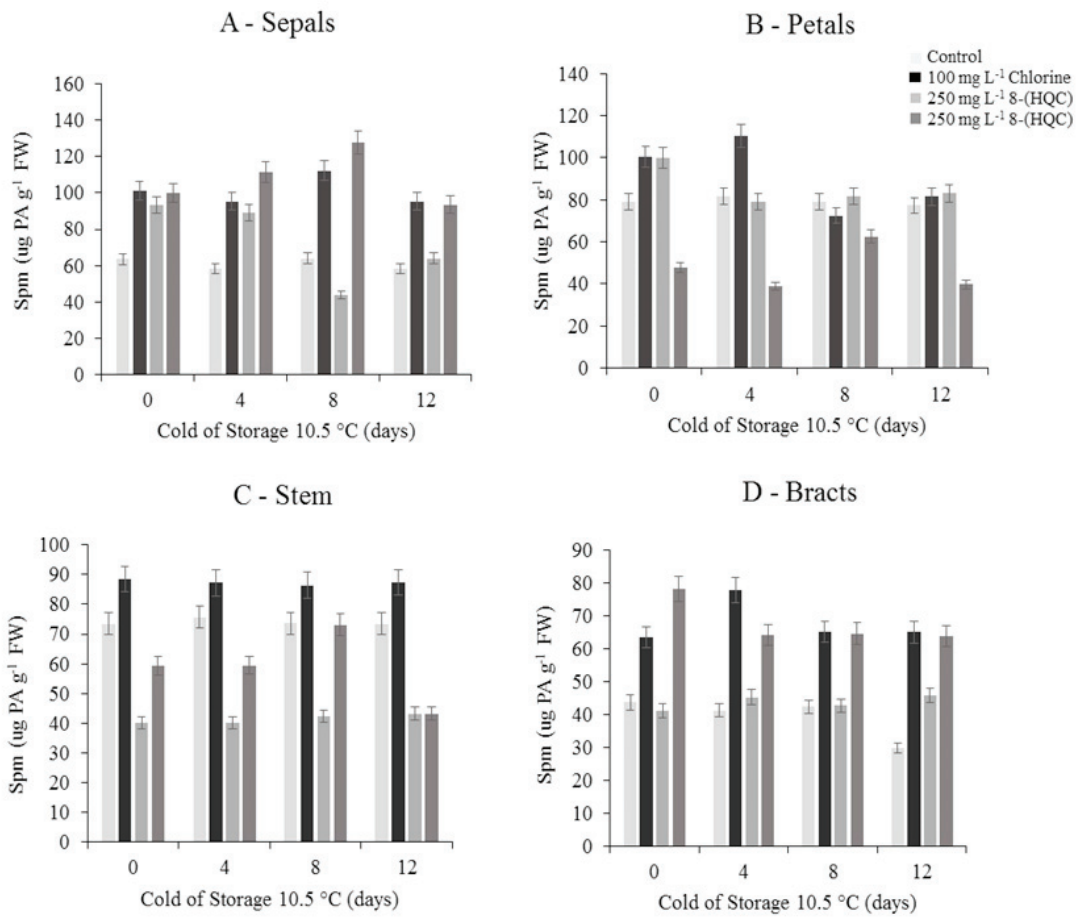

Figure 3. Spermine (Spm) level (μg g^{-1} fresh weight) in bird-of-paradise (sepals, petals, stem and bracts) submitted four pulsing treatments during storage at 10.5 °C for twelve days.

Petals in the chlorine treatment showed higher Spm levels from the fourth day, and decreased until the end (Figure 3B). Spm levels of the control decreased from the fourth day. Spm levels in stems on 250 mg L^{-1} 8-HQC increased (Figure 3C), while on 500 mg L^{-1} 8-HQC showed a decline at the end of the experimention. Stems from treatment 100 mg L^{-1} chlorine had the highest Spm levels. Bracts showed the highest Spm levels on treatements 100 mg L^{-1} chlorine and 500 mg L^{-1} 8-HQC (Figure 3D). Bracts of treatment 250 mg L^{-1} 8-HQC, increased levels at the end of the experiment, when compared with previous periods.

According to the average ratings (Table 1) of visual appearance of bird-of-paradise (sepals, petals and bracts) the longevity of floral scapes are between 6 and 8 days. Treatment 100 mg L^{-1} chlorine was more efficient to maintain bird-of-paradise for sepals, while for petals and bracts 100 mg L-1 chlorine and 250 mg L-1 8-HQC were more efficient (Table 1).

Table 1. Medium between the solutions interaction and bird-of-paradise in relation longevity

Treatments (10.5 ºC)	Sepals	Petals	Bracts
Water	2.05a	1.97 ab	1.95ab
100 mg L^{-1} cloro	2.05a	2.05ab	2.03ab
250 mg L-1 8-HQC	1.85 b	2.12 b	2.07 b
500 mg L-1 8-HQC	1.83 b	1.92a	1.92 a

Means followed by the same columns do not differ by Tukey test 5% probability

4. DISCUSSION

The highest Put levels was observed on treatment Control, except in the petals. 500 mg L^{-1} 8-HQC induced higher Spd levels in the sepals, stems and bracts compared with petals. Higher Spm levels were observed on chlorine treatment in the sepals when compared to stems and bracts. Pulsing x tissue x harvest periods interaction showed a decrease of Put levels during exposure to cold with exception of petals. Put levels in petals and stems of treatment 500 mg L^{-1} 8-HQC declined since the fourth day, probably due to the oxidation of such amine (BOUCHEREAU et al., 1999). These results are comparable with the data reported by Vieira et al. (2010) for chrysanthemum Faroe (*Dendranthema grandiflorium*), with lower Put levels during cold storage (10 ºC for 48 hours), but without the use of pulsing solution during the post-harvest. Diamine levels are not affected by treatments at lower temperatures, such as Groppa and Benavides (2008) studies, which reported that Put levels did not change significantly in cucumber cultivars. PAs can vary due to plant organ, degree of ripeness and postharvest treatments during cold exposure as reported for pepper, cucumber, zucchini and citrus (orange and lemon) (BARRACHINA et al., 2000; CHATTOPADHAYAY et al., 2002; NAYYAR and CHANDER, 2004). Changes in PAs levels may be a consequence of stress and/or environmental factors, pointing to their possible role as biochemical markers of metabolic events, such as senescence (YAMAGUCHI et al., 2007).

Spd levels in sepals and bracts treated with 500 mg L^{-1} 8-HQC and petals treated with 100 mg L^{-1} chlorine declined of during refrigerated storage. Our results are consistent with the literature reports, for exemple, when flower senescence starts there is a decrease of PAs levels and this effect is attribute to the competition between PAs and ethylene (BOUCHEREAU et al., 1999). The catabolism of Spd through the action of oxidase enzymes (produces pyrroline with diaminopropane and H_2O_2) would be the most important factor for lower PAs (SMITH, 1985). The PAs degradation occur by PAs oxidases, generating H_2O_2, substrate for the peroxidase (BOUCHEREAU et al., 1999; CONA et al., 2006).

Put levels increased in the bracts at eighth day when pulsed with 8-HQC and Control and this effect can be attributed to the senescence, as a reduction in Spm and Spd levels and accumulation of Put (BOUCHEREAU et al., 1999; CAPELL et al., 2004). However, the highest levels of amines were observed on Spd and Spm in sepals. Changes in PAs content can be described as protective responses intended to maintain the structural integrity of membrane and cell walls (EDREVA, 1997). The relationship between free endogenous polyamines (PAs) and ethylene during growth and ripening of pear fruit, was monitored and after harvest, Put levels were higher than Spd and Spd levels (MORA et al., 2005). An intimate connection between polyamines and floral development makes it possible to establish some physiological or biochemical markers using the individual polyamine, the ratios or the total polyamines (LIU et al., 2000). Polyamines have been reported to delay senescence in various tissues and species; however, spermine and putrescine did not have delay senescence in carnation flowers; indeed, on some treatments they advanced senescence (DOWNS and LOVELL, 1986).

The increase of PAs endogenous levels in several species are correlated with the reduction of injuries caused by lower temperatures (KRAMER and WANG, 1989), once these amines act to remove the reactive oxygen species and also support the stabilization of membranes (LARHER et al., 2003; GROPPA and BENAVIDES, 2008). Yamamoto et al. (2012) provided further evidence on the physiological importance of Spd in conveying chilling-stress tolerance when exposed rice seedlings to low temperature and observed that Put and Spd levels increased in leaf blades and a positive correlation between leaf chlorophyll fluorescence values and leaf Spd contents. As opposed to cold storage, under high temperatures there would be a reduction in PAs levels, affecting cell division (POLJAKOFF-MAYBER and LERNER, 1994). The variations in endogenous Put, Spd and Spm levels in our study were not related to 8-HQC and chlorine pulsing, but to the cold storage of floral scapes of bird-of-paradise at 10.5 °C, confirming data reported by Barrachina et al. (2000), Chattopadhayay et al. (2002) and Nayyar and Chander (2004) that PAs levels may vary with the post-harvest treatment, maturity stage and organ of the plant.

Deleterious effects on floral scapes of bird-of-paradise due to treatment 500 mg L^{-1} 8-HQC, showing lower floral scapes quality during the experimentation period. Similarly in *Achillea* flowers, treatment with pulse solution of 500 mg L^{-1} 8-HQC for 24 hours reduced vase life (REDMAN et al., 2002), may be due to excess acidification of the solution (SHIVA and BHATTACHARJEE, 2003).

5. CONCLUSIONS

The treatments with chlorine or HQC, concentration independent, become more unstable Put, Spm and Spd levels on the stem. The same instability occurred in the sepals for Spd. Differently it happened in the bracts, in which the treatments with Chlorine or HQC, concentration independent, induced high levels of Spd and Spm. The concentrations of 250 mg L^{-1} 8-HQC or 100 mg L^{-1} of chlorine provided greater longevity for sepals, petals and bracts in of bird-of-paradise flowers refrigerated. Pulsing treatments during cold storage at 10.5 °C in floral scapes of bird-of-paradise showed no clear relation vase life and endogenous levels of PAs. We observed that Spd and Spm levels were higher than that Put under low temperature storage.

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AUTHORS CONTRIBUTIONS

MRSV: conception and design of the research, obtaining data, analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **GPPL:** conception and design of the research, analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **LMSF:** analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **AVS:** analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **RCC:** analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **PAS:** analyze and interpretation of data, statistical analysis, write and critically analyses of manuscript. **ART:** analyze and interpretation of data, write and critically analyses of manuscript. **ANS:** analyze and interpretation of data, write and critically analyses of manuscript.

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