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Polyamine, peroxidase activity and total protein content during storage of bird-of-paradise

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Abstract

The present study was aimed to the determination of the effect of storage on some biochemical characteristics (polyamines content, peroxidase activity and total protein content) of flower tissue of bird-of-paradise. Flowers were selected and labeled, avoiding any mechanical damage, and randomly distributed in recipients for two postharvest trials. In the first experiment, flowers were placed in recipients containing tap water and stored in a cold room, at 7.5 ºC and 90 % RH, for a period of 12 days. In experiment 2, flowers were stored in room temperature for a period of 6 days. For biochemical characteristics, samples (sepals, petals, stems and bracts) from experiment 1 were collected at 0, 4, 8 and 12 days, and at 0, 2, 4 and 6 days for experiment 2. Results showed that flower bracts, stored at 7.5 °C, presented a lower content of polyamines (putrescine and spermidine), when compared to samples stored at room temperature. In both experiments, a high content of polyamines (spermine) was observed in flower tissues in relation to putrescine and spermidine. An increase of peroxidase activity was observed in sepals stored in the cold, and was related to polyamines degradation during senescence. No differences in total protein content were observed between the two treatments. The present study provides numeric results on changes in level of polyamines, peroxidase activity and total protein during postharvest of bird-of-paradise.

Keywords: postharvest; inflorescence; senescence; enzymes; polyamine.
Abbreviations: PAs_polyamines, Put_putrescine, Spd_spermidine, Spm_spermine, POX_peroxidase, H_2O_2_Hydrogen peroxide, ROS_reactive oxygen species, ACC_aminocyclopropane, aminocyclopropane acid, SAM_S-adenosylmethionine.

Introduction

The Strelitziaceae family, of the Zingiberales order, is represented by two genera, and the Strelitzia genre stands out, with a large number of species and natural hybrids. The Strelitzia reginae (*Strelitzia reginae* Banks ex. Aiton), popularly known as "bird of paradise", was mainly grown for cut flower production, due to its high postharvest longevity, stem size and strong inflorescence colors (Pivetta et al., 2007; Vieira et al., 2014). Flowers are generally classified as highly perishable products, due to the ephemeral nature of the different tissues, the high respiratory activity and the low carbohydrate content of reserves (Nowak and Rudnicki, 1990). After collection, biochemical, physiological and structural processes occur in flowers, leading to disorganization and breakdown of tissues and organs, which promote senescence. Diamines, such as putrescine (Put), and polyamines (PAs), namely spermidine (Spd) and spermine (Spm), are of common occurrence in higher plants. A wide literature reported on the increase of the activity of aminocyclopropane synthase 1 (ACC synthase) and aminocyclopropane oxidase (ACC oxidase), which, during the ageing process, convert S-adenosylmethionine (SAM) to ACC and ethylene, respectively. However, SAM is also a precursor in the synthesis of Spd and Spm, which are both related to the growth tissue ability (Tiburcio et al., 1997). According to Li et al. (2004), a competition between ethylene and PAs can be observed only under high metabolic stress conditions. Noteworthy, in some plants, namely iris flowers, as well as in Sandersonia (*Sandersonia aurantiaca*), Gladiolus (*Gladiolus hortulanus*), Roses (*Rosas* spp) and Bird-of-paradise (*Strelitzia reginae*), senescence is not stimulated by the presence of ethylene (Nowak and Rudnicki, 1990). Other substances may also be related to senescence, such as peroxidases (POX; EC 1. 11.1.7) which are enzymes catalyzing redox reactions using hydrogen peroxide (H_2O_2) as electron acceptor within different oxidative reactions (Blokhina et al., 2003), and proteins, whose synthesis is characterized by high and rapid physiological and molecular adaptability, in relation to environmental changes (Azeez et al., 2007). The application of some treatments, such as cold storage on post-harvest handling, improves flower longevity and, therefore, the marketing period (Nowak and Rudnicki, 1990; Vieira et al., 2014). Moreover, high temperatures during flower storage can cause a decrease in cell division, and this effect may be linked to PAs content (Poljakoff-Mayber and Lerner, 1994). Ferguson et al. (1990) reported that high temperatures can also directly or indirectly alter the protein content in vegetables, and lead to enzymes inactivation by structural denaturation and membrane disruption. On the other hand, low temperatures during storage may induce changes in the protein content (Thomashow, 2001) and POX activity, as they are related to stress conditions (El Hilari et al., 2003). Increased shelf-life of vegetables and horticultural commodities is extremely important, and much research has been undertaken to

determine the factors that influence the biochemical process (PAs content, POX activity and total protein content). Within this field, important advances have been made in the understanding of plant response to stress. Thus, the present work was aimed to study the effect of cold storage and temperature conditions on some biochemical characteristics (PAs content, POX activity and total protein content) in flower tissues of bird-of-paradise.

Results and Discussion

Polyamines (PAs: Put, Spd and Spm) content

Fig 1 shows a surface expressing the time course of Put content in flowers of bird-of-paradise under storage at 7.5° C (Fig 1A) and at room temperature (Fig 1B) in different tissues. Put content fluctuation occurred during cold storage (Fig 1A). During the experiment, petals showed low levels of Put, except in the 12th day. The sepal was the plant organ with the lowest content of Put throughout the experimental period. The highest Put content was observed in bracts at 4 days storage, while the levels of Put in the stems decreased under cold storage. Regarding flowers stored at room temperature, plants showed levels of Put above 24 mg g^{-1} and below 35 mg g^{-1} during the experimental period (Fig 1B). Bracts, followed by petals, were the organs with the highest content of Put. With the exception of sepals, no clear trend in Put content was observed at room temperature. Put content depended on storage temperature, but the greatest differences occurred among analyzed organs, and variations were observed within the organ with ageing. Thus, it can be stated that Put content decreased with plant organ ageing in bird-of-paradise. This was most evident in the stem under cold storage, probably due to the oxidation of this amine (Bouchereau et al., 1999). However, during 12-day storage, Put content increased in petals, probably this effect was due to cell senescence. Generally, a decrease in the levels of Spd and Spm and sometimes an accumulation of Put, was observed during senescence (Bouchereau et al., 1999), probably due to the reduction of substrate content (S-adenosylmethionine) for the formation of tri-and tetra-amines (Galston and Kaur-Sawnhey, 1987). Accumulation of Put was also noted in pepper, cucumber, zucchini and citrus (orange and lemon) during cold exposure (Martínez-Romero et al., 2003). Many studies showed that when Put/(Spd + Spm) ratio is low, it can be related to a protective effect on plants against some kind of stress (Capell et al., 2004). Some studies suggested that the levels of diamines are not affected by low temperature storage, as reported by Groppa and Benavides (2008), who found that the content of Put did not undergo significant changes in cucumber (*Cucumis sativus*) cultivars at low temperatures. As well, no changes in the levels of Put during storage at 1° C were observed in tangor "Murcott" fruits (*C. Reticulata* × *C. Sinensis*) (Edagi et al., 2010). However, these results were not comparable with the data reported by Rodriguez et al. (2001), who observed an increase in the levels of Put in eggplant (*Solanum melongena*) during storage at 3° C. Lower levels of Put were also observed in chrysanthemum Faroe during the exposure at room temperature, with respect to cold storage (Vieira et al., 2010). In the present study, a clear trend showing lower levels of Put was observed at room temperature, with the exception of sepals, probably due to ageing process in bird-of-paradise. In Fig 2, Spd content in flowers of bird-of-paradise conditioned at 7.5° C and room temperature is reported. The Spd levels also fluctuated under cold storage, except for bracts, which showed a decreased level of Spd at the last collection. Bracts showed Spd contents above 60 mg g^{-1} at time 0, and below 40 mg g^{-1} at day 12. Petals showed the lowest Spd concentrations under cold storage at day 4 (Fig 2A). Flowers stored for 6 days at room temperature showed Spd levels above 80 mg g^{-1} and below 40 mg g^{-1}, being values up to 60 mg g^{-1} observed with higher frequency in the yellow colored band of higher intensity. The highest and lowest levels of Spd were observed in bracts and petals, respectively. In general, the contents of Spd were higher in flower organs of bird-of-paradise stored at room temperature (Fig 2B). Regarding Spd content in bird-of-paradise flowers, a tendency for a decrease under cold storage in bracts can be noted. This is consistent with literature results, as a decrease in the levels of PAs occurs with senescence, and many authors attributed this effect to the competition with ethylene synthesis (Bouchereau et al., 1999). However, as bird-of-paradise flowers are insensitive to ethylene, the oxidative degradation of PAs by the action of oxidase enzymes would be the most important factor affecting PAs content (Smith, 1985). In contrast to this response, the level of Spd did not change in eggplants (*Solanum melongena*), remaining almost constant during storage at 3° C (Rodriguez et al., 2001). In tangor "Murcott" fruits (C. Reticulata × C. Sinensis), Edagi et al. (2010) observed distinct moments affecting the level of Spd during storage at 1° C. This did not occur in bird-of-paradise flowers stored at room temperature. The same effect was also observed by Santos (2007) in *Zingiber spectabile*, which did not show alteration in Spd content upon storage. In both experiments, results indicated that the variations of Spd content depended also on storage temperature, differently in different flower organs. Fig 3 shows a surface plot representing the time course of Spm content in bird-of-paradise flowers stored at 7.5° C and at room temperature. Among the studied PAs, Spm showed the highest concentration upon treatments. In the present study, the highest content of Spm, except for sepals, were found at day 4 in flower organs stored at 7.5° C. The lowest content of Spm was observed in petals at the 12th day storage (Fig 3A). Flowers stored at room temperature showed the highest levels of Spm in petals and bracts and the lowest level in sepals at the end of the experiment (Fig 3B). In general, petals, stems and bracts were the organs that showed the highest Spm content with both storage treatments. It was also noted, for both treatments, that the concentration of Spm decreased with ageing. Spm was the most represented PAs in bird-of-paradise and large variations of its content were observed throughout the experiment in both treatments. These results can be attributed to the presence of amino oxidases that catalyze the oxidative demolition of PAs, generating the corresponding aldehydes and hydrogen peroxide (Bouchereau et al., 1999). In accordance to previous studies, Spm levels showed the same trend (Vieira et al., 2010). However, post-harvest studies reported on increasing levels of Put in some species (Rodriguez et al., 2001). According to Chattopadhayay et al. (2002), PAs content may vary depending on the species and different environmental conditions, as observed for Put, Spd and Spm in the present study, showing production of PAs under water or low-temperature stress are related to protection against oxidative stress (Nayyar and Chander 2004). Our findings indicate that storing flowers of bird-of-paradise at room temperature and at 7.5° C contributes to changes in tissue metabolism, whose visual symptoms were the appearance of spots and loss of color in flower organs. The same evidences were attributed to the infection by the *Botrytis* fungus. Moreover, POX and polyphenol oxidases may lead to oxidative degradation of phenolic compounds with effects, on cell demolition,

analogous to those caused by pathogens. One of the most studied phenomenon is the appearance of dark substances

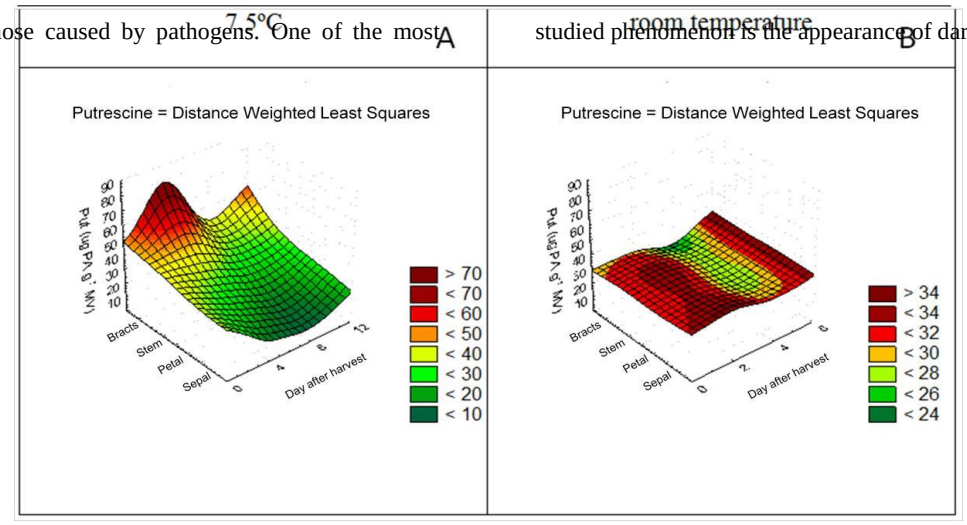

Fig 1. Surface plot representing putrescine (Put) content (mg g^{-1} fresh weight) in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored for 12 days at 7.5° C, 90% RH, (A) and at room temperature for 6 days (B).

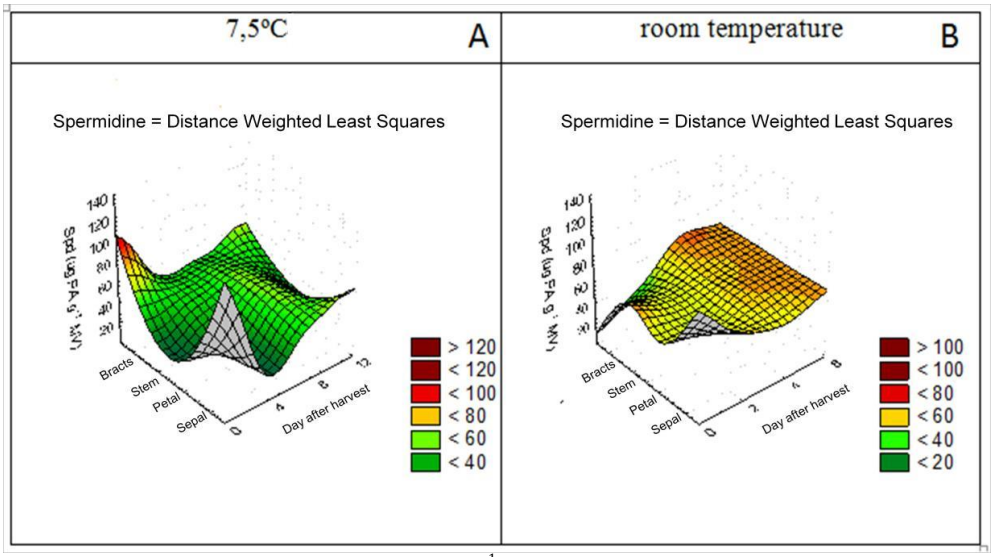

Fig 2. Surface plot representing spermidine (Spd) content (mg g^{-1} fresh weight) in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored for 12 days at 7.5° C, 90% RH, (A) and at room temperature for 6 days (B).

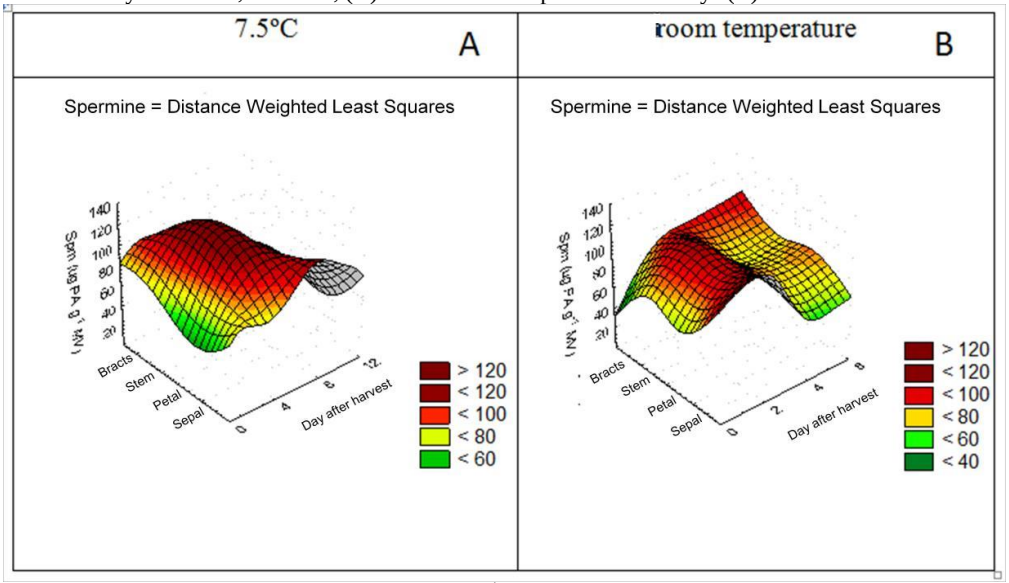

Fig 3. Surface plot representing spermine (Spm) content (mg g^{-1} fresh weight) in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored for 12 days at 7.5° C, 90% RH, (A) and at room temperature for 6 days (B).

from the oxidative polymerization of quinones, which can be produced by the activity of POX and polyphenol oxidases on phenolic substances (Bindschedler et al., 2002). There are also evidences that POX may be involved in the ageing process (Campos et al., 2004), and in particular, in the degradation of peroxides generated during biochemical processes, including the elimination of hydrogen peroxide produced from the oxidation of POX, as suggested in the present study. It is important to emphasize, the oxidation products cannot be converted to PAs and the role of PA oxidase in plant cell physiology may be a simple involvement with the terminal catabolism of PAs (Cervelli et al., 2000).

POX activity

Significant differences in POX activity were observed in sepals and stems in both experiments. Under cold storage, a decrease in enzyme activity was noted in bracts at the 12th day, while in petals, an increased activity was found at the 4th day, which remained almost constant with the following storage. Note also that POX activity was enhanced by cold storage in stems at the 12th day (Fig 4). The behavior observed in bracts and petals of bird-of-paradise can be explained by a defense response to stress caused by low temperature during storage, since cold induces oxidative stress in tissues (Purvis and Shewfelt, 1993), stimulating the development of tissue browning, as observed in the present study with the appearance of dark spots. In arracacha (*Arracacia xanthorriza*), Menolli et al. (2008) reported on tissue darkening with an increased activity of POX activity, during 7 day storage at 5° C. The increase in POX activity we observed in sepals up to the 12th day may be a consequence of senescence, due to the disintegration of the cell membranes and organelles and the formation of radicals and reactive species (ROS), such as O_2 and H_2O_2 (Costa et al., 2005). In petals of *gladiolus* L., Hossain et al. (2006) reported that an increased level of radicals and reactive species could be due to the regulation of programmed POX activity, which seems to be a prerequisite for the onset of senescence. Moreover, the response of POX activity in sepals under low temperature storage could be the result of PAs degradation by PAs oxidases, generating hydrogen peroxide, which would be metabolized by POX, thus stimulating an increase in the enzyme activity (Bouchereau et al., 1999). The results reported in the present work evidenced that flowers of bird-of-paradise, stored at room temperature, showed changes in POX activity only in stems (Fig 5). Bracts and sepals showed a gradual decrease of POX activity during the 6 day storage, while stems showed an activity increase from the 4th day, with a subsequent decline up to the 6th day. In this second experiment, a tendency to a decrease in POX activity in flower organs of bird-of-paradise was observed, with the exception of petals at the 6th day. These results confirm previously expressed considerations, indicating that POX is involved in the senescence process and in the control of cell wall stability, primarily by the elimination of ROS, and also by crosslinking components of the secondary cell wall (Passardi et al., 2004). Bartoli et al. (1997) also observed an increase in POX activity in petals of *Chrysanthemum morifolium* Ram during senescence and related this behavior to the defense response against oxidant molecules, which may promote membrane damage. According to Lima (2000), POX appears at low concentrations in green tissues and at high concentrations in mature tissues. However, other researchers did not observe any modification of POX activity during plant ageing (Vieira et al., 2010).

Total protein content

Throughout the present experiment, no significant changes were observed in relation to total protein content in flower organs of bird-of-paradise. Anyway, protein content tended to increase in sepals and petals at 4th day of cold storage, but decreased in other samples, except for petals collected at the 12th day. No variations in the protein content in stems were observed (Fig 6). A decrease in the protein content was observed in sepals during cold storage, indicating that senescence probably did not inhibit protein synthesis and/or increased protein degradation by proteases (Gietl and Schmid, 2001). This result confirms the findings of Sood et al. (2006), who noted that the protein content in rose was higher in young plants. In petals of *Sandersonia aurantiaca* (Eason et al., 2002) and *Dendrobium* cv. Khao Sanan (Lerslerwong et al., 2009) a decrease in protein content during senescence was also observed. As suggested by Woodson and Handa (1987), the knowledge of synthetic processes and of post-translational modifications regulating protein content and functions, have important implications for the successful control of flower longevity. In petals of bird-of-paradise, the increase of protein content can be considered as an adaptation for survival under low temperature stress. This may be the case observed in protein accumulation in petals at 12th day storage: this stress adaptation mechanism, ensures higher longevity of this flower organ, since, visually, injuries caused by cold stress were not observed. Similar results can be described for flowers stored at room temperature, that is, where no significant effect on total protein were observed (Fig 7). Under these conditions, only a slow protein content decrease with increasing senescence in sepals and petals were observed. Under conditions of high temperature, protein loss is accelerated as a result of increased protease activity and this, in turn, leads to accelerated leaf senescence (Al-Khatib and Paulsen, 1984; Zavaleta-Mancera et al., 1999; Ueda et al., 2000), however, this study did not evaluate leaves only other plant parts. In other flower organs, bract and stems, no significant variations were observed, and the protein content remained practically constant during the whole storage period at room temperature.

Materials and Methods

Experiment

Experiments were performed on opened flowers of bird-of-paradise, collected in September 2009 (average rainfall of 66.2 mm) in the cultivation field in San Manuel (Universidade Estadual Paulista), located at 22° 43' 52" South latitude and 48° 34' 14" West longitude, with an altitude of 750 meters. The climate is subtropical humid mesothermal with dry period during the winter season. After harvest, flowers were cut and deposited into a container, containing tap water to prevent air entry into the xylem vessels. Flower height was standardized at 80 cm and hydrated approximately for 10 to 15 minutes. Then, flowers were 90 randomly transferred into containers (15-20 L total capacity, in a water volume of 1.5 L), where they were followed for postharvest experiments. In experiment 1, flowers were placed in a cold room at 7.5° C and 90% RH for a period of 12 days (Vieira et al., 2014). In experiment 2, flowers were placed at room temperature for a period of 6 days. At room temperature conditions, plants quickly deteriorate, thus, in experiment 2,

an inevitably gradual physiological change of flowers occurred. The appearance of small light spots on petals and

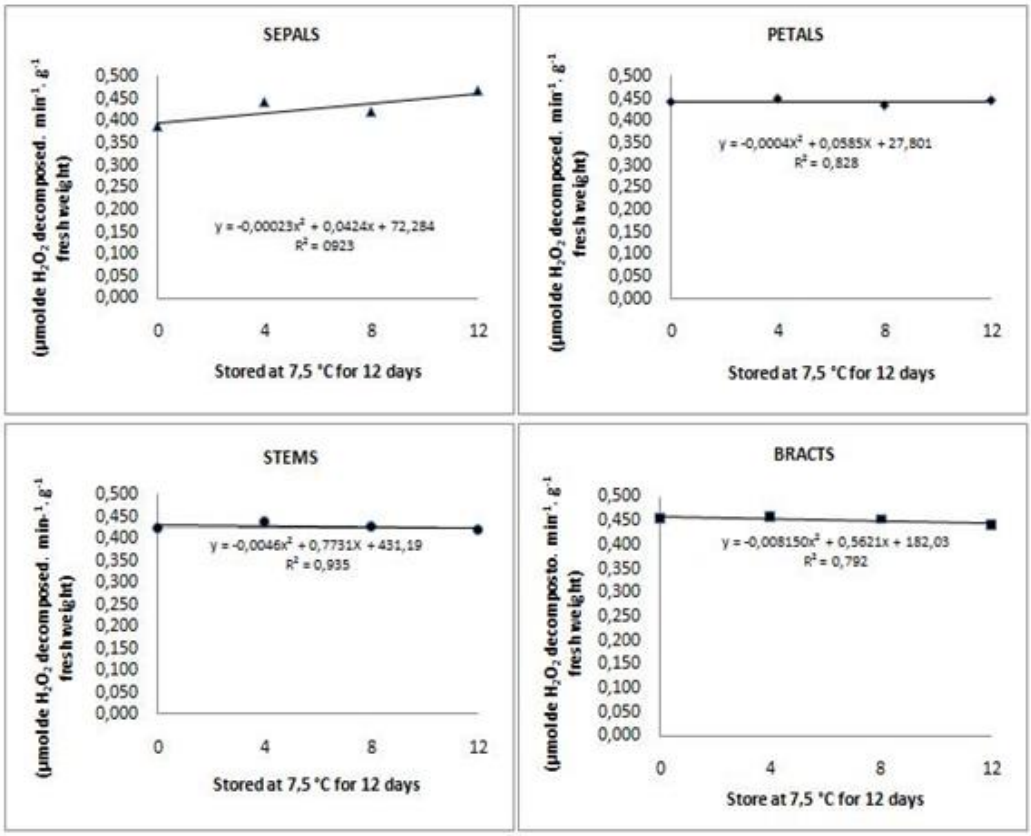

Fig 4. Peroxidase (POX) activity (□mol H_2O_2decomposed $min^{-1}g^{-1}$fresh weight) in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored at 7.5°C and 90 % RH for 12 days.

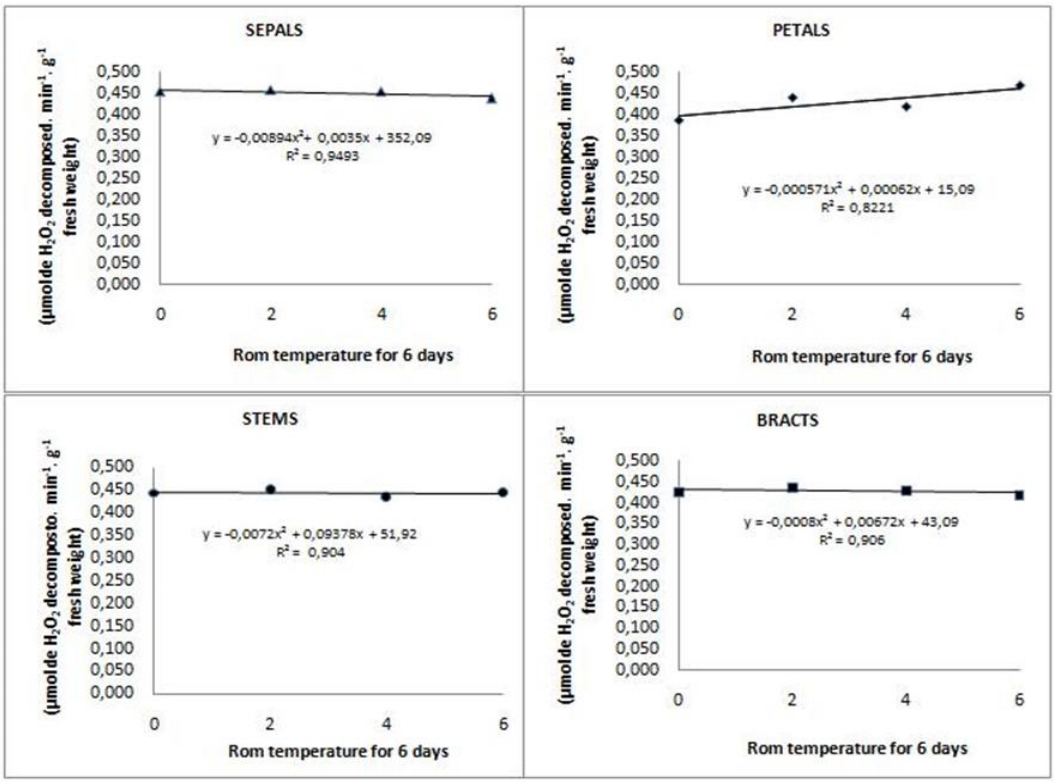

Fig 5. Peroxidase activity (POX) (□mol H_2O_2decomposed $min^{-1}g^{-1}$fresh weight) in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored at room temperature for 6 days.

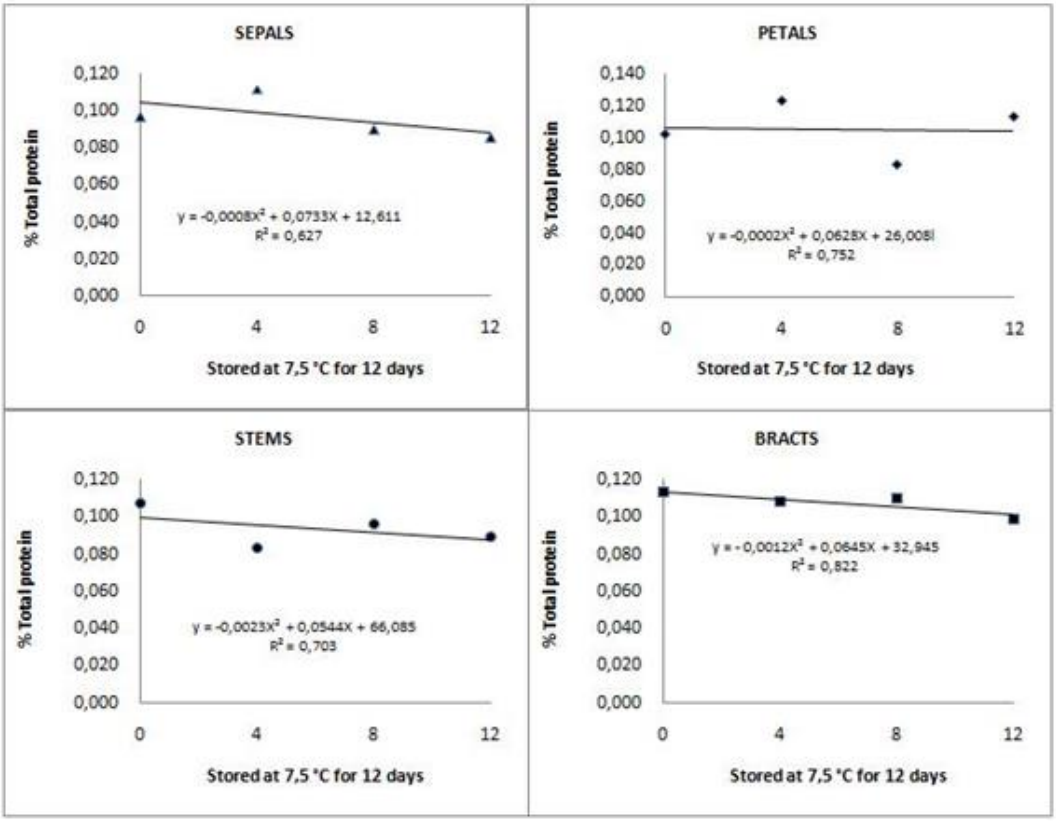

Fig 6. Total protein in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored at 7.5°C and 90 % RH for 12 days.

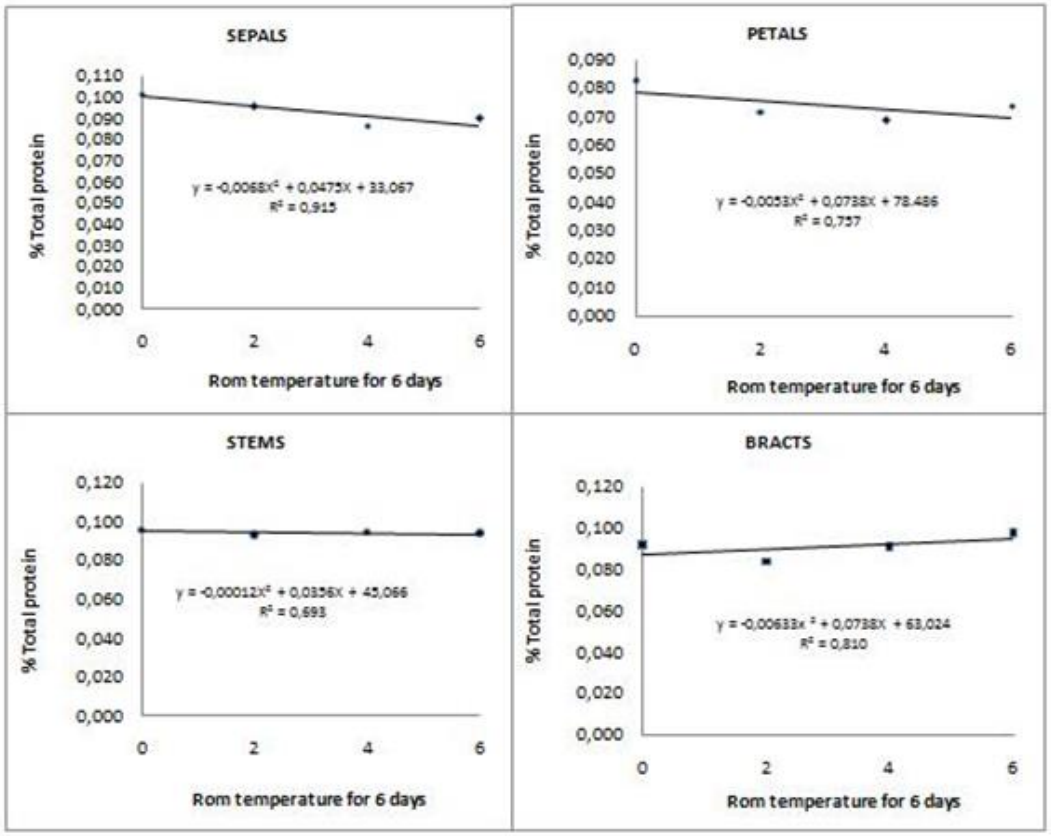

Fig 7. Total protein in flowers of bird-of-paradise (sepals, petals, stems and bracts) stored at room temperature for 6 days

sepals occurred, which will evolve into dark spots. In literature, this symptom was attributed to *Botrytis* fungus, the main cause of diseases of flowers (Pitta, 1990; Sewake and Uchida, 1995). Therefore, flowers from Strelitzia reginae were maintained only for six days at room temperature. However, these symptoms were also observed in flowers stored under cold storage, but at a lower Intensity. The experimental temperature and relative humidity were constantly recorded. Water was changed every 48 hours, with the aim of preventing the proliferation of microorganisms. For the two experiments, floral sepals, petals, stems and bracts were collected for biochemical characterization (PAs content, POX activity and total protein content). For stems, the middle of the stem was sampled and for bracts, the floral apex. For flowers stored in the cold, samples were collected at 0, 4, 8 and 12 days of storage. For flowers stored at room temperature, samples were collected every 2 days. After collection, samples were labeled, frozen in liquid nitrogen and stored in a freezer (- 80° C) for the analyzes of PAs content and POX activity, or oven dried under forced air circulation until constant weight for total protein analysis.

Determination of polyamines (PAs)

Polyamines were determined by thin layer chromatography following the method described by Flores and Galston (1982), adapted by Lima et al. (2008). The fresh material was homogenized for 1 min in 5% (v/v) cold perchloric acid (Merck), using a food homogenizer. After centrifugation for 20 min at 4ºC, dansyl chloride (Sigma, 95%) and satured sodium carbonate were added to the supernatant. Proline (100 mg L^{-1}) Sigma, min. 99%) was added to the supernatant. Proline (100 mg L^{-1}) (Sigma, min. 99%) was added after 1 h to stop the reaction and the solution was brought to 60 ºC. The mixture was maintained in the dark for 30 min, at room temperature. Toluene was used to extract the dansylated PAs and aliquots were applied onto thin layer chromatography plates (glass plates coated with 60G silica Gel – Merch; 20 x 20). Separation was carried out in laboratory bowls containing chloroform: triethylamine (Merck) (10:1). Put (Sigma, min. 98%), Spd (Sigma, min. 98%) and Spm (Sigma, min. 95% 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 paltes, by fluorescence emission spectroscopy (excitation at 350 nm and emission measurement at 495 nm), in a Video Documentation System, using the Image Master version 2.0 software program.

Determination of peroxidase activity (POX)

The activity of peroxides (POX, EC 1.11.1.7); umol of H_20_2 decomposed min^{-1} g^{-1} fresh weight was determined by the method described by the method described by Lima et al. (1999). Fresh material (leaves and flowers) was collected, weight in 50 mg aliquots and dissolved in 5 mL of 0,2 M potassium phosphate, pH 6,7, on ice. Samples were then centrifuged at 10000 x g for 10 min at 4 ºC and 1 mL of the supernatant was uses as the source of enzyme. Hydrogen peroxide solution (0,5 mL of 0.2 M potassium phosphate, pH 6,7, containing 4 mM 4-aminoantipyrine (4-amino-1,5-dimethyl-2-phenyl-4-pyrazolin-3-one; Sigma) and 10 mM dichlorophenol (Sigma). Samples were incubated for 5 min at 30 ºC and then the reactin was stopped with 2 mL of absolute ethanol (Merck). The spectrophotometric readings, corrected for blancks, were carried out at 505 nm.

Determination and total protein

Dried and finely ground samples were analyzed for crude protein (Kjedahl nitrogen x 6.25) following the procedures reported by AOAC (AOAC 1997).

Statistical analysis

The experimental design for the characterization of PAs content, POX activity and total protein content was randomized, involving two treatments (cold room 7.5 ºC x room temperature), four different tissues of bird-of-paradise (sepals, petals, stems and bracts) and four time collection consisted of seven replicates and five floral samples each. Analysis of variance (ANOVA) was performed to detect differences between treatment means, which were by response surface designs to PAs content and regression analysis to POX activity and total protein content.

Conclusions

Form the above reported results it can be concluded that the concentrations of Spd and Spm was found higher than that Put in flower organs of Strelitzia reginae, under low and room temperature storage, and a higher content of Spd was observed at room temperature. Moreover, an increase in POX activity in sepals stored at low temperature was observed, which might indicate that this enzyme is involved the senescence process of Strelitzia reginae flowers. At the same time, no significant variations of total protein content as a function of the storing period and storage temperature, was noted.

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References

Al-Khatib K, Paulsen GM (1984) Mode of high temperature injury to wheat during grain development. Physiol Plant. 61:363-368.

Aoac International (1997) Official methods of analysis. Aoac International, Gaitherburg. (Vol 2. 16th Ed, 3rd Revision).

Azeez A, Sane AP, Bhatnagar D, Nath P (2007) Enhanced expression of serine proteases during floral senescence in *Gladiolus*. Phytochem. 68:1352-1357.

Bindschedler LF, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. J Exp Bot. 53:1357-1376.

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann Bot. 91:179-194.

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. Plant Sci. 140:103-125.

Campos AD, Ferreira GA, Hampe MMV, Antunes IF, Brancão N, Silveira EP, Osório VA, Augustin E (2004)

Peroxidase and polyphenol oxidase activity in bean anthracnose resistance. Pesq Agrop Brasil. 39(7):637-643.

Capell T, Bassie L, Christou P (2004) Modulation of the polyamines biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc of the Natio Acade of Scien USA. 101:9909:9914.

Chattopadhayay MK, Tiwari BS, Chattopadhayay G, Bose A, Sengupta DN, Ghosh B (2002) Protective role of exogenous polyamines on salinity stressed rice (*Oryza sativa*) plants. Physiol Plant. 116:192-199.

Cervelli M, Tavladoraki P, Di Adostino S, Angelini R, Deferico R, Mariottini P (2000) Isolation and caracterization of three polyamines oxidase genes from *Zeamays*. Plant Physiol Biochemi. 38:667-677.

Costa LM, Civello MP, Chaves RA, Martinez AG (2005) Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20ºC. Postharves Biol Techn. 35:191-199.

Eason JR, Ryan JD, Pinkney TT, O'donoghue EM (2002) Programmed cell death during flowers senescence: isolation and characterization of cysteine proteinases from *Sandersonia aurantiaca*. Funct Plant Biolo. 29(6):1055-1064.

Edagi FK, Jomori MLL, Kluge RA, Lima GPP, Azevedo RA, Sestari I (2010) Inhibition of ethylene action delay chilling injury development in tangor 'Murcott'. Ciênc Rur. 40(7):1530-1536.

El-Hilari F, Ail-Oubahou A, Remah A, Akhayat O (2003) Chilling injury and peroxidase activity change in "Fortune" mandarin fruit during low temperature storage. Bulg J Plant Physiol. 29(1-2):44-54.

Ferguson DL, Guikema JA, Paulsen GM (1990) Ubiquitin pool modulation and protein degradation in wheat roots during high temperature stress. Plant Physiol. 92:740- 746.

Flores HE, Galston AW (1982) Analysis of polyamines in higher plants by high performance liquid chromatography. Plan Physiol. 69:701-706.

Galston AW, Kaur-Sawhney R (1987) Polyamines and senescence in plants. In: Thompson W Nothangel E Huffacker R. (Eds.). Plant senescence: Its biochemistry and physiology. Rockville, Ameri Societ Plant Physiol. pp.176-181.

Gietl C, Schmid M (2001) Ricinosmes: an organelle for development regulated cell death in senescence in plant tissues. Naturwissenschaften. 88:49-58.

Groppa MD, Benavides MP (2008) Polyamines and abiotic stress: recent advance. Amino Acids. 34:35-45.

Hossain Z, Mandal AKA, Datta SK, Biswas AK (2006) Decline in ascorbato peroxidase activity—a prerequisite factor for tepal senescence in gladiolus. J Plant Physiol. 163:186-194.

Lerslerwong L, Ketsa S, Van Doorn WG (2009) Protein degradation and peptidase activity petal senescence in *Dendrobium* cv. Khao Sanan. Postharvest Biol Technol. 52:84-90.

Li C, Jiao J, Wang G (2004) The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress. Plant Sci. 166:303-315.

Lima GPP, Brasil GO, Oliveira MA (1999) Polyamines and peroxidase activity in bean (*phaseolus vulgaris* l.) grown under saline stress. Scient Agríc. 56:21-25.

Lima GPP (2000) Marcadores bioquímicos de injúrias pelo frio e de maturação em bananas. 103p. Tese (Livre Docência) - Instituto de Biociências de Botucatu, Universidade Estadual Paulista, Botucatu.

Martínez-Romero D, Serrano M, Valero D (2003) Physiological changes in pepino (*Solanum muricatum* Ait.) fruit stored at chilling and nonchilling temperatures. Postharvest Biol Technol. 30:177-186.

Menolli LN, Finger FL, Puiatti M, Barbosa MJ, Barros RS (2008) Role of oxidative enzymes on the darkening induced by chilling of arracacha roots. Act Scient Agron. 30(1):57-63.

Nayyar H, Chander S (2004) Protective effects of polyamines against oxidative stress induced by water and Cold stress in chickpea. J Agron Crop Sci. 190: 355-365.

Nowak J, Rudnicki RM (1990) Postharvest handling and storage of cut flowers, florist greens and potted plant. Portland: Timber Press. 210p.

Passardi F, Penel C, Dunand C (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. Tren Plant Sci. 65:1879-1893.

Pivetta KFL, Barbosa JG, Araújo EF (2007) Propagação de palmeiras e strelitzia. In: Barbosa JG Lopes LC (Ed). Propagação de Plantas Ornamentais. Viçosa: UFV, 43-70.

Poljakoff-Mayber A, Lerner HR (1994) Plants in saline environments. In- Handbook of plant and crop stress. Pessarakli, M. (Ed.). Marcel Dekker, New York. pp. 65-96.

Purvis AC, Shewfelt RL (1993) Does the alternative pathway ameliorate chilling injury in the sensitive plant tissues. Plant Physiol. 88:712-718.

Rodriguez SC, Lopez B, Chaves AR (2001) Effect of different treatments on the evolution of polyamines during refrigerated storage of eggplants. J Agric Food Chem. 49:4700-4705.

Santos MHLC (2007) Fisiologia pós-colheita de sorvetão *(Zingiber spectabile Griff.) cultivado no submédio São Francisco*. 93p. Tese (Doutorado em Agronomia/Horticultura) - Universidade Estadual de Paulista, Botucatu.

Smith TA (1985) Polyamines. Ann Rev Plan Physiol Plan Mol Biol. 36:117-143.

Sood S, Vyas D, Nagar PK (2006) Physiological and biochemical studies during flower development in two rose species. Sci Hortic. 108:390-396.

Thomashow MF (2006) So what's new in the field of plant cold acclimation. Plant Physiol. 125:89-93.

Tiburcio AF, Altabella T, Borrelli A, Masgrau C (1997) Polyamine metabolism and its regulation. Physiol Plant. 100:664-674.

Ueda T, Seo S, Ohashi Y, Hashimoto J (2000). Circadian and senescence enhanced expression of a tobacco cysteine protease gene. Plant Mol Biol. 44:649-657.

Vieira MRS, Teixeira da Silva JÁ, Lima GPP, Vianello F (2010) Changes in polyamine, total protein and total carbohydrate content and peroxidase activity during the lifetime of chrysanthemum 'Faroe'. Floricul Ornam Biotech. 4:148-52.

Vieira MRS, Simões AN, Souza PA (2014). Recommended temperature and relative humidity for storage of Brazilian tropical flowers. Afric J Biotech. 13:1198-1201.

Vieira MRS, Lima GPP, Vianello F, Souza AV, Oliveira NG, Simões AN (2014) Influence of storage temperature on quality of inflorescences strelitzia (*Strelitzia reginae banks* ex Aiton). Biosc J. 30(2):783-790.

Woodson WR, Handa AK (1987) Changes in protein patterns and in vivo proteins synthesis during presenescence and senescence of hibiscus petals. J Plant Physiol. 128:67-75.

Zavaleta–Mancera HA, Franklin KA, Ougham HJ, Thomas H, Scott IM (1999) Regreening of senescent Nicotiana leaves I. Reappearance of NADPH-protochlorophyllide oxidoreductase and light harvesting chlorophyll a/b binding protein. J Exp Bot. 50:1677-1682.