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Precooling and cold storage effects on antioxidant system in calla lily postharvest

Efeitos do pré-resfriamento e armazenamento a frio no sistema antioxidante na pós-colheita de copo-de-leite

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ABSTRACT

Low-temperature storage is one of the most effective techniques to maintain the quality and durability of cut flowers. Both quality maintenance and durability are dependent on plant metabolism factors such as antioxidant system. The objective was to evaluate the effect of low-temperature on precooling and storage after harvest, on the metabolism of calla lily [*Zantedeschia aethiopica* (L.) K. Spreng] concerning the antioxidant system, levels of hydrogen peroxide (H_2O_2), and malondialdehyde (MDA). Therefore, different storage temperatures (4 °C and 21 °C) were tested as well as the effect of precooling in different periods (0 to 24 hours). It was observed that the activity of catalase (CAT) and superoxide dismutase (SOD) was increased at 4 °C, as well as inflorescence durability in the commercial standard (around 12 days). At 21 °C, the content of H_2O_2 and MDA was higher and the visual quality reduction in the inflorescence was accelerated. Low-temperature leads to a higher activity of the enzymes of the antioxidant system (CAT and SOD) and increases postharvest longevity for calla lily. Precooling did not influence the visual quality and longevity of calla lily. Long-term exposure periods at low temperatures, with 12 and 24 hours of precooling caused stress, evidenced higher MDA levels and higher CAT activity. SOD activity was related to low temperature and shorter precooling periods resulted in better antioxidant system activity and lower MDA levels.

Index terms: Durability; low-temperature; malondialdehyde; superoxide dismutase*; Zantedeschia aethiopica*.

RESUMO

O armazenamento em baixa temperatura é uma das técnicas mais eficazes para manter a qualidade e a durabilidade das flores cortadas. Tanto a manutenção da qualidade quanto a durabilidade são dependentes de fatores do metabolismo da planta como o sistema antioxidante. O objetivo foi avaliar o efeito da baixa temperatura no pré-resfriamento e armazenamento pós-colheita, no metabolismo do copo-de-leite [Zantedeschia aethiopica (L.) K. Spreng] quanto ao sistema antioxidante, níveis de peróxido de hidrogênio (H_2O_2) e malondialdeído (MDA). Portanto, diferentes temperaturas de armazenamento (4 °C e 21 °C) foram testadas assim como o efeito do pré-resfriamento em diferentes períodos (0 a 24 horas). Foi observado que a atividade da catalase (CAT) e da superóxido dismutase (SOD) aumentou no armazenamento à 4 °C, assim como a durabilidade da inflorescência no padrão comercial (aproximadamente 12 dias). À 21 °C, o teor de H_2O_2 e MDA foi maior e a redução da qualidade visual na inflorescência foi acelerada. A baixa temperatura leva a uma maior atividade das enzimas do sistema antioxidante (CAT e SOD) e aumenta a longevidade pós-colheita do copo-de-leite. O pré-resfriamento não influenciou na qualidade visual e longevidade do copo-de-leite. Períodos de exposição de longa duração a baixas temperaturas, com 12 e 24 horas de pré-resfriamento causaram estresse, evidenciados por maiores níveis de MDA e maior atividade de CAT. A atividade da SOD foi relacionada à baixa temperatura e períodos mais curtos de pré-resfriamento resultaram em melhor atividade do sistema antioxidante e menores níveis de MDA.

Termos para indexação: Durabilidade; baixa temperatura; malondialdeído; superóxido dismutase *Zantedeschia aethiopica*.

INTRODUCTION

The maintenance of flower quality and durability after harvest may be accomplished with the use of techniques such as low-temperature at storage, which results in a decrease in plant metabolism (Castricini et al., 2017; França et al., 2019; Mansouri, 2012; Sales et al., 2021; Verma; Singh, 2021). The cellular oxidation process, which culminates in senescence, is slowed down and postharvest quality and longevity are maintained (Valenzuela et al., 2017).

Cellular oxidation is a result of the imbalance between reactive oxygen species (ROS) production and the ability of enzymes in the antioxidant system to metabolize these toxic substances (Munõz; Munné-Bosch, 2018). This imbalance can occur during the postharvest stage due to stress factors such as ethylene (Bayanati et al., 2019), salinity, heavy metals, temperature (Darras, 2020; Overmyer; Brosche; Kangasja, 2003), and reaction to the incidence of pathogens (Buron-Moles et al., 2015).

High levels of ROS are related to senescence and programmed cell death, and hydrogen peroxide (H_2O_2) is directly related to these processes, with peaks in its production (Bayanati et al., 2019; Wang et al., 2016). ROS accumulation causes a series of damages to biomolecules such as proteins, lipids and nucleic acids, which can result in permanent damage to cells and finally cell death (Barbosa et al., 2014; He; He; Ding, 2018; Raja et al., 2017).

It is possible to verify the peroxidation of the lipid membrane by the presence of malondialdehyde (MDA), an indicator of the occurrence of oxidative stress (Blokhina; Virolainen; Fagerstedt, 2003; He; He; Ding, 2018). Some published works reported that low temperature storage also affects MDA content, as observed in roses (Pompodakis et al., 2010) and anthurium (Aghdam et al., 2016).

Understanding how the enzymes of the antioxidant system act on ROS production under low-temperature conditions is essential to understand the relationship between metabolism and flower postharvest quality. The stress resulting from temperature change produces ROS that may cause cell damage. As a reaction, plants have a complex antioxidant system that includes enzymatic substances, as superoxide dismutase-SOD, catalase-CAT, ascorbate peroxidase-APX, and peroxidase-POD, and non-enzymatic such as phenols and flavonoids (Hasanuzzaman et al., 2012). The antioxidant system is able to inhibit or delay cellular damage caused by ROS, acting on its oxidation reactions (Dumanović et al., 2021). Thus, enhancing antioxidant system in postharvest could scavenge ROS and contribute to increase vase life of cut flowers (Zheng; Guo, 2019). Studies carried out by Hassan et al. (2020) and Mazrou et al. (2022) with cut roses demonstrated the alleviated of H_2O_2 and MDA levels by enhancing the scavenge capacity of antioxidant system.

Adequate temperature control in the postharvest stage is essential (Verma; Singh, 2021). However, the effect of low-temperature on oxidative stress has not been elucidated for many ornamental species such as calla lily, which has reduced postharvest durability.

In order to better understand this process, the objective was to evaluate the behavior and production of ROS during storage at low-temperatures, relating to longevity and maintenance of calla lily inflorescence quality after harvest. Therefore, we would like to clarify some questions: Is the use of precooling an efficient procedure to improve calla lily postharvest quality and durability? How long after low-temperature exposure may some enzymes be detected? May the term of exposure influence that? Since the effect of low-temperature on oxidative stress has not been elucidated for calla lily and this species has reduced postharvest durability, which enzymes of the antioxidant system act on ROS production under low-temperature conditions?

MATERIAL AND METHODS

For the two experiments described below, one about two different storage temperatures and other about precooling, Calla lily (*Zantedeschia aethiopica* (L.) K. Spreng) stems were harvested fully open (Castro et al., 2014; Sales et al., 2015) on a commercial farm and transported dry, for approximately 20 minutes, to the postharvest laboratory. A cut was made at the base of the stem, and all were 0.5 m long. Inflorescences were selected and classified according to the calla lily standard for commercialization established by Almeida et al. (2008): A1 - turgid inflorescences, with the tip of the spathe tilted and absence of wrinkles or necrosis; A2 - turgid inflorescences, with the tip of the spathe slightly curled down and absence of wrinkles or necrosis; B - Turgid inflorescences, spathe tip slightly curled down, presence of wrinkles, absence of necrosis; C - Withered inflorescences, with the tip of the spathe totally curled down, presence of necrosis, at the point to be discarded.

For both experiments, three trained evaluators, following the protocol described by Almeida et al. (2008), classified flower quality standards daily.

In the experiment about two different storage temperatures, the underside part of the flower stems were placed in transparent plastic containers containing 0.6 L distilled water and stored for 13 days at two temperatures, 4 ºC (cold chamber) and 21 ºC (constant room temperature simulated with air conditioner). Both experiment were kept at 85% ± 5% relative humidity. The experiment was finished when more than 50% of the flower stems reached senescence (C classification). For each storage temperature, 4 ºC and 21 ºC, 7 replications (7 biological replicates) were used, each consisting of 2 stems. The experiment followed a completely randomized design. For biochemical analyses,

3 flower stems (3 biological replicates) from each tested temperature were removed daily.

In the experiment about precooling, the effects of the method on metabolism and visual quality of calla lily inflorescences were evaluated. Floral stems were maintained at 4 °C during 2, 6, 12, and 24 hours, in addition to a control treatment, without precooling (placed directly in the room at 21 ºC). Afterwards, the precooled stems were maintained at 21ºC (constant room temperature simulated with air conditioner) with 85% ± 5% relative humidity, until senescence reached 50% of the stems (C classification). For biochemical analyses, samples were extracted from spathes, at 0, 2, 6, 12, and 24 hours after the end of the period at low temperature, disposed in liquid nitrogen (NL) (-196 °C), and transferred to an ultra-low freezer (-80 ºC). This procedure was repeated after 6, 12, 24, 48, 96, 144, 192, and 240 hours. Biochemical analyses were then carried out to determine the activity of the enzymes from the antioxidant system, CAT and SOD, as well as the quantification of H_2O_2 and lipid peroxidation - malondialdehyde (MDA).

Biochemical Analyses

H_2O_2 and lipid peroxidation

For the quantification of H_2O_2 and lipid peroxidation, 0.2 g of calla lily spathe from 3 biological replicates (3 flower stems) were macerated in NL with Polyvinylpolypyrolidone (PVPP). The macerate was then homogenized in 0.0015 L of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 G for 15 minutes, at 4 °C. The supernatant was collected and stored in an ultra-freezer (-80 ºC).

H_2O_2 was quantified according to Velikova, Yordano and Edreva (2000) by adding an aliquot of the sample to the reaction medium containing 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide. Quantification was obtained by measuring the absorbance at 390 nm based on a standard curve with known concentrations of H_2O_2. The quantification was performed with 3 technical replicates to each biological replicate. The results were expressed in mol Kg^{-1} FW (fresh weight).

Lipid peroxidation was quantified according to Buege and Aust (1978) by adding sample aliquots to the reaction medium containing 0.5% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA), followed by incubation at 90 ºC for 30 minutes. The reaction was stopped by rapid cooling on ice, and readings were taken using a spectrophotometer, at 535 nm and 600 nm. TBA form complexes in red color with low molecular weight aldehydes, such as malondialdehyde (MDA), a secondary product of the peroxidation process. Lipid peroxidation was expressed in mol MDA per Kg^{-1} FW (fresh weight).

Antioxidant enzymes

After sample storage, enzyme (CAT and SOD) extracts from 3 biological replicates (3 flower stems) were obtained by cold maceration of 0.2 g of the spathe in liquid nitrogen (NL) added with PVPP (Polyvinylpolypyrolidone). The macerate was homogenized in 0.0015 L of the extraction buffer solution (400 mM potassium phosphate - pH 7.8; 10 mM EDTA, 200 mM ascorbic acid and water) (Biemelt; Keetman; Albrecht, 1998) and then centrifuged at 13,000 G for 600 s at 4 °C. The supernatant was collected and stored in an ultra-freezer (-80 ºC).

CAT activity was determined according to Havir and McHale (1987). Aliquots of the enzymatic extract were added to the incubation medium containing 200 mM potassium phosphate (pH 7.0), water and 250 mM hydrogen peroxide. Enzyme activity was determined by the decrease in absorbance at 240 nm, every 15 s for 180 s, monitored by the consumption of hydrogen peroxide. Enzyme activity was determined with 3 technical replicates to each biological replicate. The molar extinction coefficient used was 36 mM^{-1} cm^{-1} (Azevedo et al., 1998). The results were expressed in mol H_2O_2 min^{-1} kg^{-1} FW (fresh weight).

SOD activity was determined by the ability of the enzyme to inhibit the photo-reduction of nitrotetrazolium blue (NBT) (Giannopolitis; Ries, 1977). Readings to determine enzyme activity were taken at 560 nm. One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under the test conditions. The results were expressed in U min^{-1} kg^{-1} $protein^{-1}$.

Statistical analysis

The data obtained from the both experiments were subjected to analysis of variance using the statistical software SISVAR 5.6 (Ferreira, 2014) and means were compared using the Scott-Knott test ($p < 0.05$).

RESULTS AND DISCUSSION

Two different storage temperatures e xperiment

Storage temperature influenced the visual quality of calla lily inflorescences over time, varying the number of days the inflorescences remained in each quality class as A1, A2, B, or C (Figures 1 and 2).

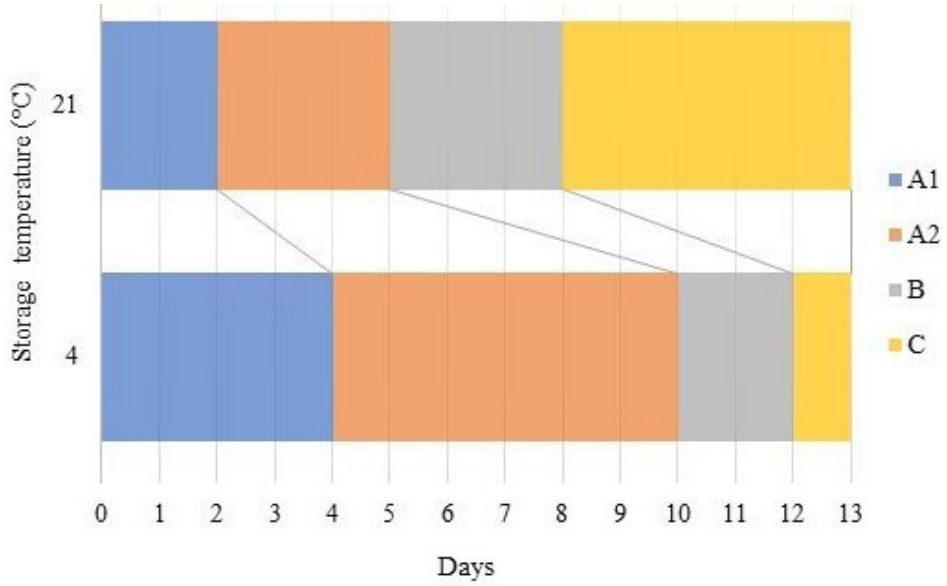

Figure 1: Postharvest classification of calla lily inflorescences as a function of the evaluation days. A1 - Turgid inflorescences, the tip of the spathe inclined, absence of wrinkles or necrosis; A2 - Turgid inflorescences, the base of the spathe slightly curled down, absence of wrinkles or necrosis; B - Turgid inflorescences, spatula tip slightly curled down, presence of wrinkles, absence of necrosis; C - Withered inflorescences, spatula tip curled down, presence of necrosis.

Floral stems stored at a low temperature, 4 °C, presented better durability, approximately twice the time in higher standards in the initial days when compared to those maintained at room temperature (21 °C), as also observed by Almeida et al. (2008).

In addition, the visual quality was related to the peroxide and lipid peroxidation contents as well as with the activity of antioxidant enzymes.

The quantification of H_2O_2 and lipid peroxidation in the spathes may be related both to the storage temperature and to the period during which the calla lily flower stems remained stored (Figure 3A and B). In floral stems stored at 4 °C, the H_2O_2 concentration showed slight changes (Figure 3A).

Differently, in inflorescences disposed at 21 °C, there was an increase in H_2O_2 concentration, presenting the highest values at the end of the storage period. The variation is mainly due to the temperatures and not to the storage period. MDA concentrations increased during the storage period for both temperatures, reaching the highest concentration at the end of the durability period, for inflorescences stored at 21 ºC (Figure 3B). For floral stems maintained at 4 °C, MDA contents were approximately constant.

Stress conditions can induce the production of ROS, which are toxic molecules that can lead to oxidative stress in cells. Excessive production of ROS in cells can alter the redox state resulting in cell damage, increasing the level of lipid peroxidation (Pourzarnegar; Hashemabadi; Kaviani, 2020). However, plants have a cellular antioxidant apparatus, including enzymatic and non-enzymatic antioxidants that control ROS levels and maintain homeostasis (Menegazzo et al., 2021).

Among ROS, $O_2^{\cdot}$- and H_2O_2 stand out as the main products resulting from the dismutation of the superoxide radical $O_2^{\cdot}$- into H_2O_2 and molecular oxygen (O_2) (Caverzan; Casassola; Brammer, 2016; Fernando-Santos et al., 2021; Liu et al., 2018) which, through oxidation, destroys the cytoplasmic membrane that loses its semi-permeability (Cao et al., 2009). Due to exposure at different temperatures, the content of H_2O_2 tends to be higher at room temperature when compared to cold temperatures, which, by reducing metabolism, also reduces the H_2O_2 content, maintaining this behavior throughout the days (Cao et al., 2009; Yang et al., 2012).

The behavior described above was also evidenced in calla lily floral stems, since in the storage carried out at low temperature (4 °C), the observed H_2O_2 content was lower than that of floral stems kept at 21 °C (Figure 3A). It is also interesting to observe that the production of H_2O_2 is not continuous, occurring in cycles of approximately 48 hours. A rapid elevation occurs after 11 days when the

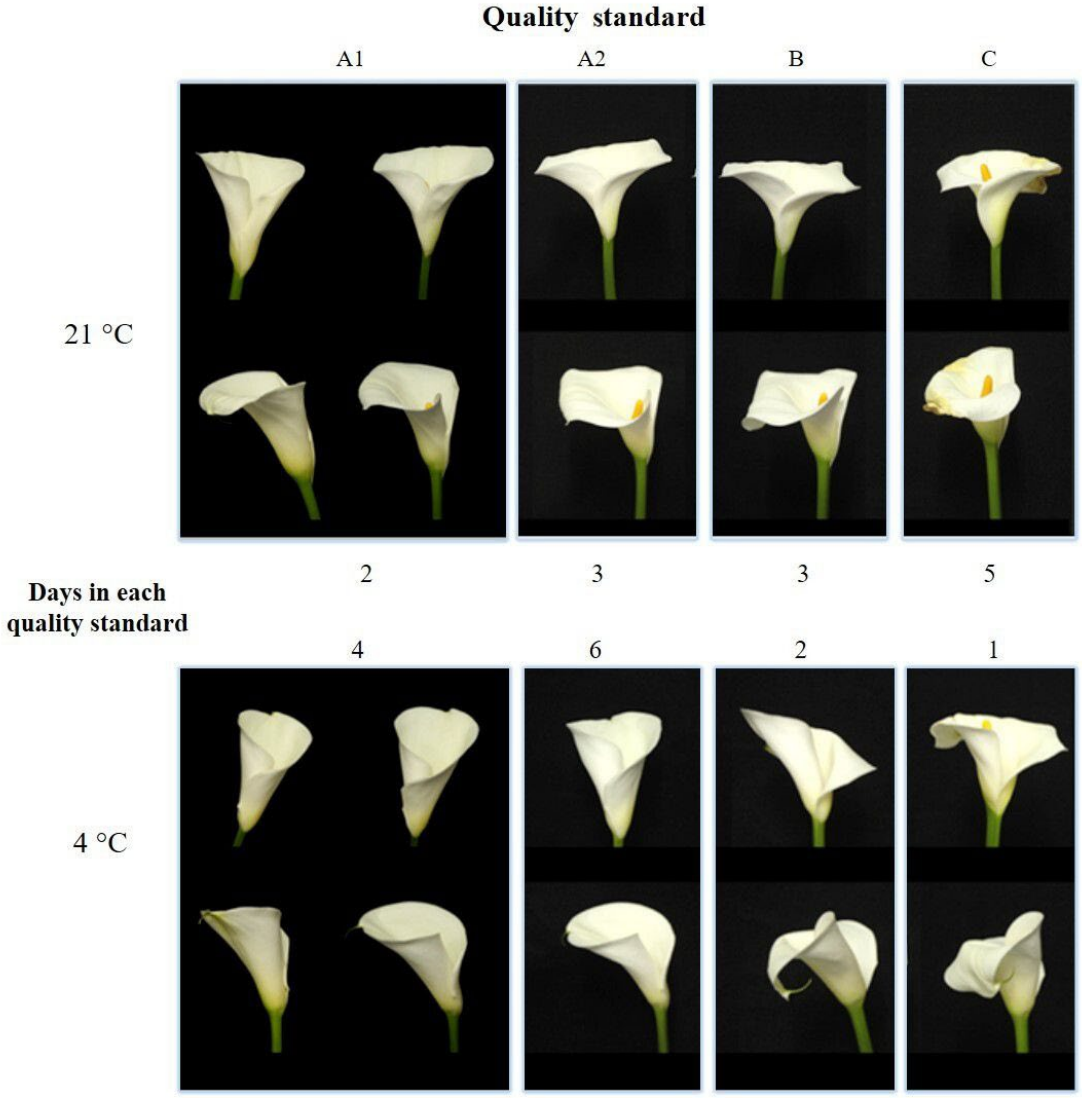

Figure 2: Number of days in each quality standard.

floral stems are already classified as C (Figures 1 and 2) and lose their commercial quality, which is directly linked to senescence. In floral stems stored at 4 °C, the content of H_2O_2 shows a slight increase after the 8th day (Figure 3A), when the floral stems have already changed from quality standard B to C (Figures 1 and 2). Comparing the H_2O_2 content and the quality standard, it was observed that the production of H_2O_2 did not indicate that it was a substance whose production reflects on visual quality, but rather one of the products present during senescence.

Analyzing the content of MDA in spathe of calla lily inflorescences, it was not affected by low temperature (Figure 3B), in contrast to daylily, rose and anthurium, in which the low temperature induced a rapid reduction in MDA content (Aghdam et al., 2016; Cao et al., 2009; Pompodakis et al., 2010). The pattern of MDA production was similar when comparing inflorescences kept at low temperature (4 °C) with room temperature (21 °C) over the days of storage (Figure 3A and B). It was observed that, after harvesting, the content is reduced until the 3rd day, returning to initial levels after another 2 days. For floral stems kept at room temperature (21 ºC), after the 9th day (Figure 3B), there was a rapid increase in the content of this enzyme, coinciding with the loss of quality (Figure 1 and 2).

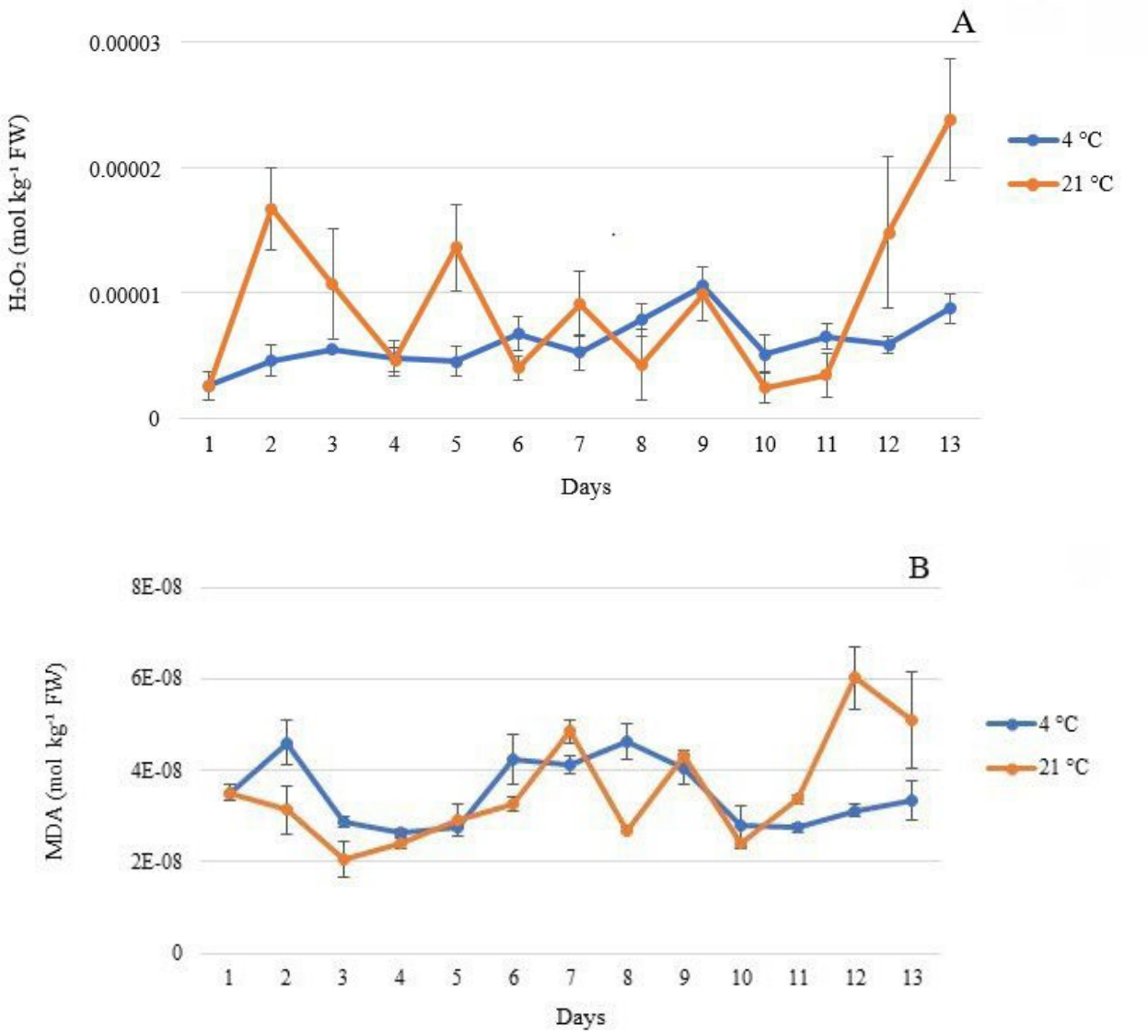

Figure 3: A) H_2O_2 and B) MDA levels, in calla lily spathes, according to storage period (days) and temperature (4 °C and 21 °C). FW = fresh weight.

MDA is one of the products of lipid peroxidation, used as an analytical parameter to determine lipid peroxidation and indicate the occurrence of oxidative stress (Blokhina; Virolainen; Fagerstedt., 2003; Cao et al., 2009; Fernando-Santos et al., 2021; Jyothsna; Murthy, 2016). Low temperature may have reduced or delayed oxidative stress, so that there was no variation in MDA in the period evaluated, indicating that low temperature reduced lipid peroxidation.

ROS are produced by plants under stress conditions such as physical damage caused by cutting the flower stem. From the cutting of the stem, these can also produce ROS due to the senescence process and also for injuries that can occur during storage. ROS act as signaling molecules, changing the expression of several genes, including those of antioxidant enzymes, in order to reduce their damage (Pereira et al., 2017).

H_2O_2, as well as other ROS, is produced at high levels in situations of oxidative stress and, among the factors that trigger this stress, are storage temperature and senescence (Hodges et al., 2004). In addition to these factors, the temperature at harvest

and the harvesting action also cause physiological and biochemical reactions that can lead to stress.

About the activity of antioxidant enzymes, CAT activity in spathes is also related both to the storage temperature and period (Figure 4A). The pattern of SOD activity was different from that of CAT (Figure 4B).

At a low temperature (4 °C), CAT activity reduced up to the 9th day of storage, which coincides with the period of commercial quality (Figures 1 and 2). From the 10th day onwards, CAT activity increased as a result of the senescence process and with loss of commercial quality (Figures 1 and 2). Subsequently, on the 13th-day, when inflorescences were in C classification, CAT activity decreases, which may be a consequence of cell death, with no activity of this enzyme. For floral stems maintained at 21 °C (Figure 4A), there was no CAT activity, showing that stress due to low temperature is a stimulus for its action. At a low temperature (4 °C), there was no change in concentrations, with a small reduction after the 12th day (period of disposal - C classification), which may indicate cell death. For floral stems maintained at 21 ºC, there is a reduction in the content just after harvest, and the values remain constant until the end of the senescence period, indicating that this enzyme was not influenced by temperature or harvest stress.

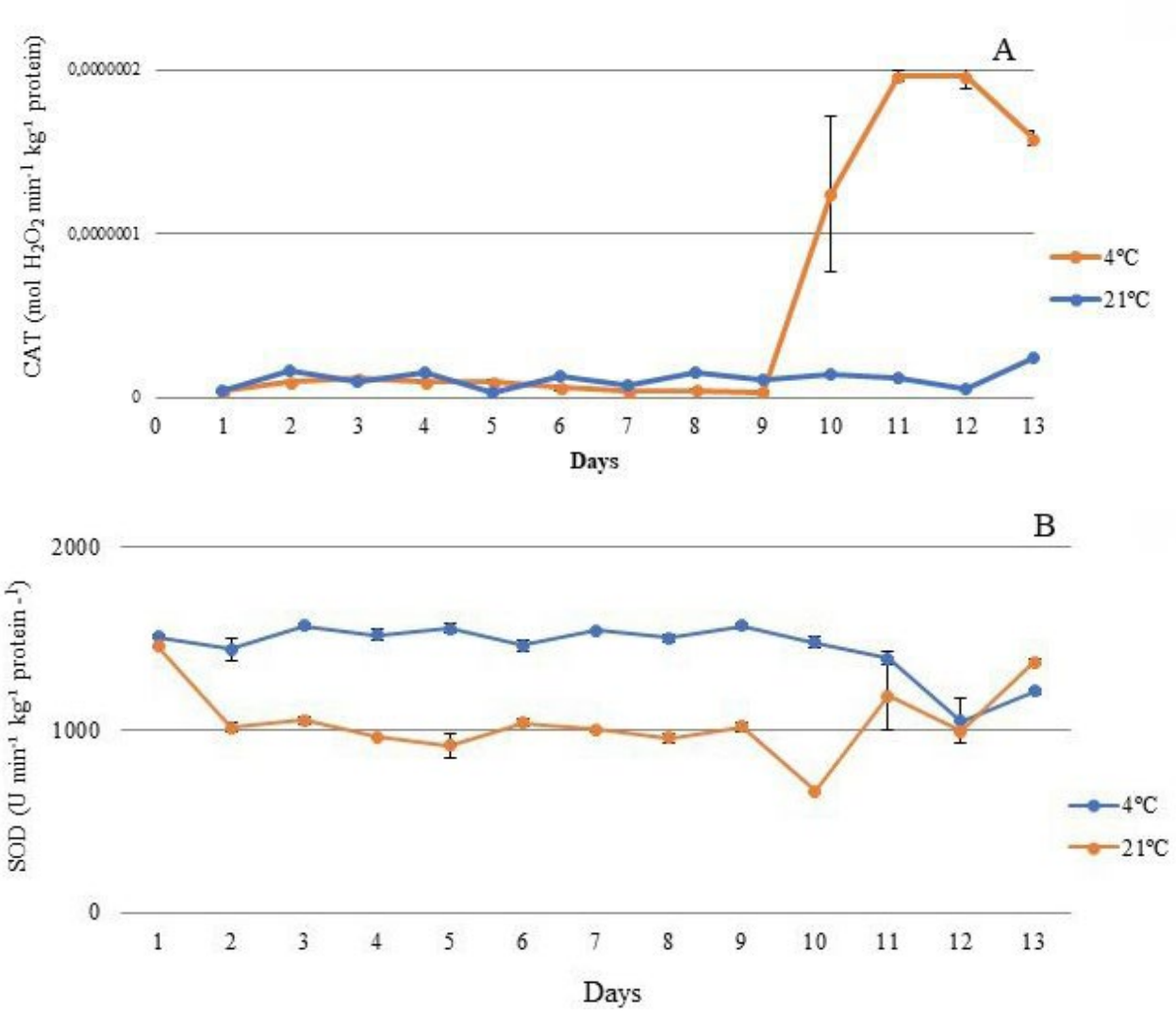

Figure 4: A) CAT and B) SOD activity in calla lily spathes, as a function of storage period and temperature (4 ºC and 21 °C).

The enzymatic antioxidant system involves a sequential operation and simultaneous actions of different enzymes, including SOD and CAT. SOD is present in several cell compartments and catalyzes the dismutation of the superoxide anion into H_2O_2 and O_2. H_2O_2, in turn, is removed by several other antioxidant enzymes such as CAT (Menegazzo et al., 2021).

CAT is responsible for converting H_2O_2 into O_2 during cut flower senescence while SOD, commonly found in peroxisomes, mitochondria, the cytosol, and the apoplast, simultaneously reduces and oxidizes the superoxide anion ($O_2^{\cdot-}$) to produce H_2O_2 and O_2 according to the reaction: $2O_2^{\cdot-} + 2H + \rightarrow O_2 + H_2O_2$ (Fernando-Santos et al., 2021).

SOD activity is observed throughout the storage period, at both temperatures tested for calla lily stems (Figure 4B). However, SOD activity was higher in floral stems kept at a low temperature, 4 °C (Figure 4B), indicating a possible relationship of ROS production with storage at low temperatures and the effectiveness of the antioxidant system, since it is active (Hodges et al., 2004; Liu et al., 2018).

In contrast to some authors (Caverzan; Casassola; Brammer., 2016), higher levels of H_2O_2 observed in storage at 21 °C (Figure 3A) are not related to SOD activity, which showed higher activity at low temperatures (Figure 4B). However, the presence of H_2O_2 in floral stems stored at low temperature suggests the presence of superoxide radicals in a sufficient amount to induce the production of SOD. Althought, the production of SOD proved to be directly related to low temperatures once, even with a higher production of H_2O_2, it did not lead to higher levels of SOD in inflorescences kept at 21 ºC when compared to those kept at a low temperature (4 ºC).

Furthermore, the presence and observed levels of H_2O_2, mainly in inflorescences stored at 21 ºC, showed an increase, but not related to a proportional increase in SOD activity. Exception for the end of the storage period, indicating that one of the main sources of H_2O_2 is $O_2^{\cdot-}$-dismutase. H_2O_2 can be the result of an O_2 dismutation reaction catalyzed by SOD or due to a non-enzymatic reaction in which $O_2^{\cdot-}$ accepts one electron and two protons (Sharma et al., 2012). Thus, storage at a low temperature (4 ºC) provided greater durability (Figures 1 and 2) and greater SOD activity (Figure 4B) after harvest, indicating the beneficial effect of this enzyme in preserving quality.

As for the production of CAT (Figure 4A), the levels observed were close to 0 during the storage period, rising rapidly from the 9th day in spathes of floral stems kept at a low temperature. The production occurred only at low temperatures. However, only at the end of the storage period, the inflorescences lost their commercial quality (Figures 1 and 2) and there was a decrease in SOD content (Figure 4B). CAT is an important antioxidant enzyme that acts in defense against ROS, catalyzing the decomposition of H_2O_2 into $O_2 + H_2O$ (Yang et al., 2012). These observations indicated that the production of H_2O_2 occurred at the end of the storage period when ROS were more actively produced, which may be directly related to the senescence process.

Precooling experiment

There was no influence of precooling on the visual quality of calla lily inflorescences. Thus, inflorescences remained, on average, the same number of days in each class of visual quality (A1, A2, B, and C), regardless of the use or not of precooling.

H_2O_2 contents of calla lily spathes were not influenced by precooling (Figure 5A), in contrast to the levels of MDA (Figure 5B).

The cold stress due to the precooling applied immediately after harvest did not influence the production of H_2O_2, which was a consequence of the natural senescence process of the floral stem. The presence of H_2O_2 was detected, and the values remained constant 144 hours after precooling, with a rapid increase afterwards.

Regarding the production of MDA, there was a drastic reduction soon after harvest, especially in the period of 6 to 12 hours. In the low-temperature period, the minimum MDA value was reached around 24 hours after harvest, when it started to increase, with an increase 96 hours after harvest, and also 240 hours later. This behavior, regardless of precooling, indicates that MDA production is related to the senescence process without the influence of temperature.

Concerning the activity of CAT, precooling affected enzyme activity (Figure 6A).

CAT activity was high in the control treatment, when precooling was not applied, or when floral stems were subjected to low temperatures for a long-term (12 and 24 hours), indicating that low temperatures affect the production of this enzyme, as well as the stress caused by harvest, observed in inflorescences of the control treatment. In these treatments, some activity increases were observed, mainly at 6, 48, and 96 hours, indicating that the production is not continuous, but in alternate cycles. Precooling in short periods (2 to 6 hours) has no stress effect, with no CAT production. The production of SOD was not altered as a result of precooling, but due to harvest stress (Figure 6B). An increase in the concentration of SOD occurred 12 hours after harvest, extending until 96 hours, followed by a reduction in concentration. This behavior was observed in all treatments.

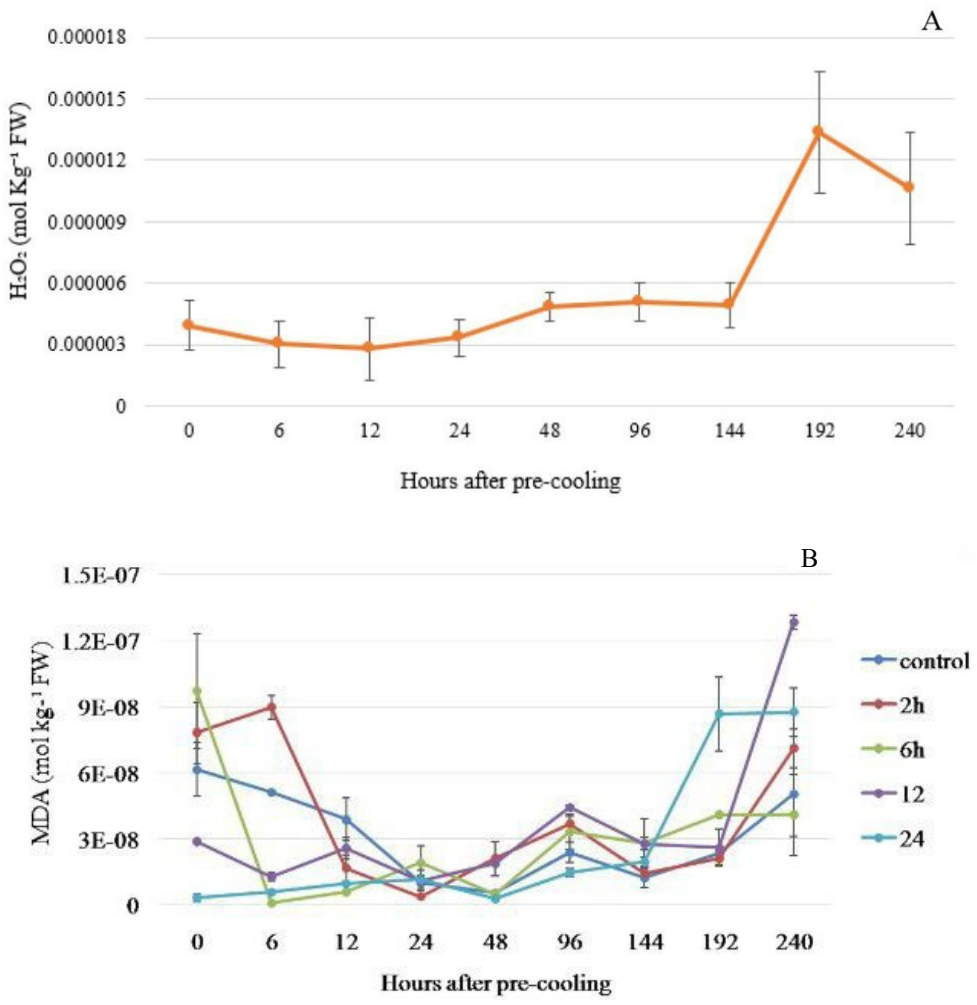

Figure 5: A) H_2O_2 levels in calla lily spathes, as a function of time intervals (hours) after precooling, and B) MDA levels on the control treatment (without precooling and direct storage at 21 ºC) and precooling periods (hours) at 4 °C.

Early in the senescence period, an increase in antioxidant components was observed as the first reaction to ROS, followed by a decline in the content of these components in the advanced senescence period (Cavaiuolo; Cocetta; Ferrante, 2013; Pourzarnegar; Hashemabadi; Kaviani, 2020). As a reaction, there is an increase in H_2O_2 levels, which is therefore characteristic of the advance of natural senescence (Bayanati et al., 2019; Hodges et al., 2004; Liu et al., 2018). Treatments for at low temperature, the H_2O_2 content tends to decrease (Liu et al., 2018). The presence of H_2O_2 occurred in Calla lily spathes, regardless of precooling time (Figure 5A). The increase in the H_2O_2 content was observed after 144 hours, when the inflorescences were classified as C and no longer met the commercial quality parameters (Figures 1 and 2).

Higher levels of H_2O_2 are related to lipid peroxidation, which is a process detectable by the concentration of MDA (Aghdam; Bodbodak, 2013; Liu et al., 2018). MDA levels were influenced by precooling and time after this treatment (Figure 5B). Furthermore, the contents were reduced right after harvesting and increased as a consequence of senescence and oxidative process. In floral stems that were not pre-cooled for short periods (2 or 6 hours), MDA levels increased after 24 to 48 hours of treatment. On the other hand, in spathes of floral stems that received precooling, MDA production is delayed, starting after 96 to 144 hours, indicating that these floral stems can react to oxidative stress, maintaining an external visual quality for a longer period. Under all conditions, MDA values were higher

after 192 to 240 hours, when the floral stems were already being discarded (Figure 5B). Senescence causes an increase in lipid peroxidation, which is indicative of the prevalence of ROS in tissues and also induces the production of toxic products for cells such as MDA (Jyothsna; Murthy, 2016).

The temperature at which the vegetable arrives from the field, called 'field heat', can be a post-harvest stressor by triggering reactions that result in deterioration. However, with the application of precooling, there is a deceleration of biochemical reactions and better maintenance of product quality and longevity (Sindhuja; Jena, 2017; Makule; Dimoso; Tassou, 2022). Thus, it was possible to verify the beneficial effect of precooling on calla lily floral stems, especially in periods of 12 to 24 hours, confirmed by the reduction in the initial levels of MDA.

Given the above, CAT maintained its highest activity in the hours following harvest, in reaction to this stress. It was observed that, 24 hours after the removal of the precooling treatment, regardless of the length of stay, there was a trend towards a reduction in CAT concentrations (Figure 6A), indicating the effectiveness of this treatment in controlling the oxidative process. It was also observed that, during this period, there was a lower production of MDA (Figure 5B), an indicator of the oxidative process. Thereafter, concentrations were higher, as well as a reaction to increasing MDA levels. The reduction occurred after 240 hours, indicating cell death and the absence of substrate for the reaction.

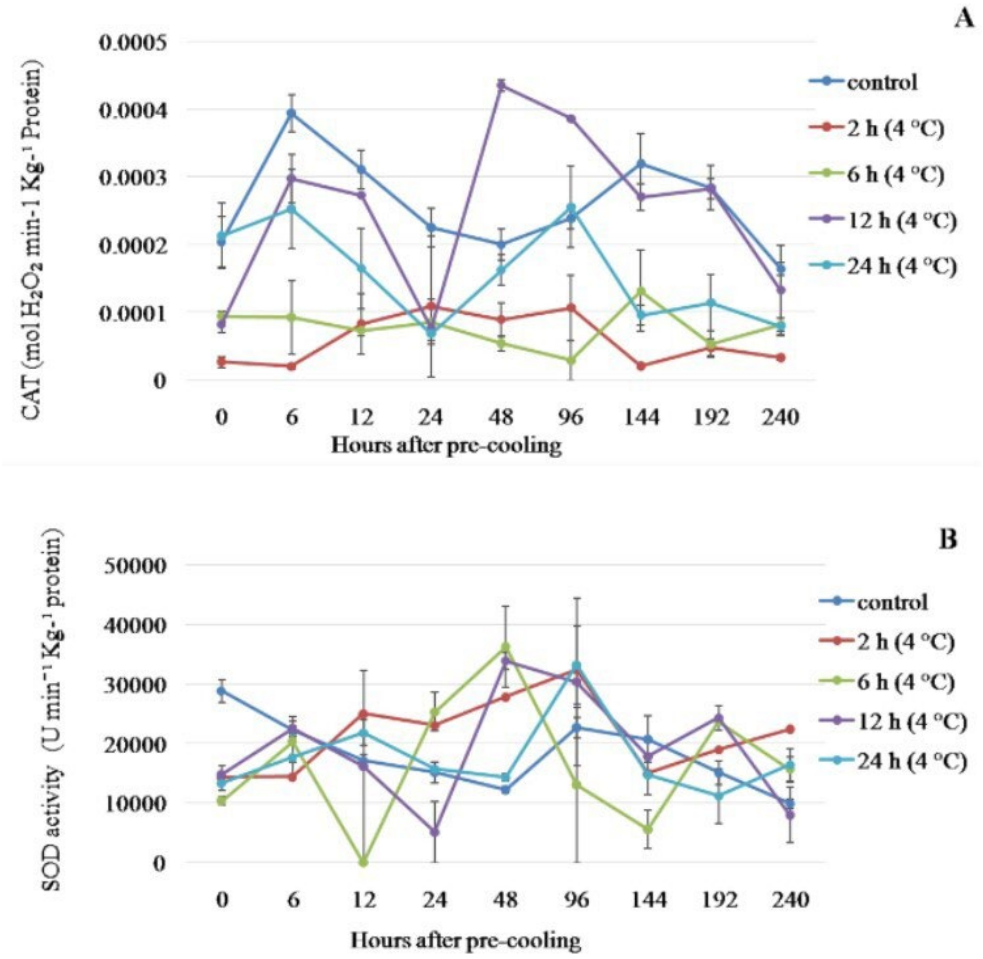

Figure 6: A) CAT and B) SOD activity in calla lily spathes. Control (without precooling, with direct storage) at 21 ºC and precooling periods (hours) at 4 ºC, according to time intervals for the sample (hours) after precooling.

However, there was an increase in CAT activity after 12 and 24 hours of precooling, as well as in the control treatment, in which the inflorescences did not receive the cold treatment. Thus, the production of this enzyme was a reaction to stress and the effects had different causes. The increase in CAT contents in the floral stems of the control treatment was due to the stress caused by the cutting process of the stems during harvest. For those stems submitted to precooling for 6 and 12 hours, the oxidative process was inhibited, thus reducing the production of CAT. In floral stems submitted to precooling for 12 to 24 hours, a reaction to cold was observed through the increase in CAT content and reduction in MDA.

In general, the increase in MDA concentration suggests an increase in H_2O_2, due to the occurrence of a lower concentration of CAT. The pre-treatment times of 12 and 24 hours initially induced low concentrations of MDA, indicating that the long-term precooling favored the reduction in ROS formation, providing greater oxidative stress and decreased CAT production. For the other temperatures, including the control, there was initially a higher concentration of MDA, which was controlled by the increase in the production of CAT. Even at the end of the storage time, the increase in MDA was smaller, with a reduction in the concentration of CAT. Thus, although long-term precooling has been shown to be more efficient in controlling lipid peroxidation, in the end, this peroxidation was superior in shorter precooling times.

SOD activity has also been associated in response to low temperature situations (Liu et al., 2018; Zhu et al., 2008). An increase in SOD activity was observed in pre-cooled peaches, demonstrating a positive correlation between low temperature and the activity of this enzyme (Zhang et al., 2022). SOD is the first defense front of the antioxidant system, showing activity even in the absence of stress, since cells breathe continuously and can produce ROS (Alscher; Erturk; Heath, 2002; Cao et al., 2009). A distinct pattern was observed between stems submitted to precooling and control, indicating that the reaction corresponds to tensions from different sources: harvest and cold. For floral stems that were not subjected to precooling, the presence of SOD was observed; however, this does not show an increase with time after harvest.

Precooling time inhibits the initial SOD production, delaying its larger increases. In inflorescences kept in precooling for 6 hours, the production of SOD was stimulated after 12 hours of removal from low temperature, while for the 12 and 24 hours precooling treatments, this onset was delayed to 24 and 48 hours, indicating that the occurrence of stress was also delayed. The highest SOD level was recorded 48 to 96 hours after low-temperature removal.

In the floral stems that remained in precooling for a long term, the production pattern of SOD and CAT is similar, indicating the effect of low temperature on the production of these enzymes (Figure 6A and B). In contrast, exposure for a short period induced a different behavior when analyzing the production of SOD and CAT. The oxidative process characterized by H_2O_2 content in temperature changes can be positively correlated with SOD activity and negatively correlated with CAT activity (Zhu et al., 2008). SOD activity can be verified during storage senescence, which may present greater activity than CAT, reflecting an imbalance in the antioxidant system and, as a consequence, the inability to slow down or stimulate senescence (Starzyńska; Leja, Mareczek, 2003), altering the SOD/CAT ratio, in the later stages of natural senescence (Kanazawa et al., 2000).

CONCLUSIONS

SOD activity is directly related to low temperature. Precooling treatment did not influence the visual quality and durability of calla lily inflorescences. Long-term exposure at low temperature, with 12 and 24 hours precooling, besides the temperature difference in storage at 21 ºC, contributed to the stress, evidenced by the higher levels of MDA and higher activity of CAT. Thus, shorter periods of exposure to low-temperatures, during precooling, provide the best activity of the antioxidant system and lower levels of MDA.

AUTHOR CONTRIBUTIONS

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