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Methyl jasmonate and salicylic acid on postharvest physiology of Bird of Paradise

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ABSTRACT

Bird of Paradise (*Strelitzia reginae* Banks) has great market potential due to its intrinsic beauty and longer vase life. However, storage and transportation conditions are not always adequate and may lead to negative consequences on postharvest physiology and the quality of these flowers. Thus, this study determined the effect of pulsing treatment containing methyl jasmonate (MeJA) or salicylic acid (SA) in association with sucrose on the postharvest physiology of Bird of Paradise. Two independent experiments were carried out. In the first one, floral stems were treated with 100, 250 and 400 µmol L^{-1} of MeJA plus 200 g L^{-1} of sucrose for 24 h. In the second experiment, the treatments consisted of 2, 4 and 6 mmol L^{-1} of SA in association with 200 g L^{-1} sucrose for 24 h. After the pulsing treatment, the floral stems of both experiments were kept in containers with water at room temperature. The analyses were performed on days 0 and 7 and the assessment comprised: water uptake rate, transpiration rate, fresh mass loss, electrolyte leakage, peroxidase activity (POD) and phenolic compounds of the bracts and sepals. MeJA and SA reduced the water uptake rate by the stems. SA reduced transpiration rate, loss of fresh mass and phenolic compounds of sepals on day 0. On day 7, MeJA treatment reduced electrolyte leakage and increased POD activity. Otherwise, SA had no effect on electrolyte leakage and reduced POD activity. These results suggest that MeJA and AS act antagonistically on the electrolyte leakage and POD activity which might promote different effects on the postharvest physiology of Bird of Paradise stems, such as adverse patterns of the transpiration rate.

Key words: peroxidase; plant growth regulators; *Strelitzia reginae*; transpiration

Metil jasmonato e ácido salicílico na fisiologia pós-colheita de Ave do Paraíso

RESUMO

A Ave do Paraíso (*Strelitzia reginae* Banks) tem grande potencial de mercado devido à sua beleza intrínseca e longa vida de vaso. Contudo, as condições de armazenamento e transporte nem sempre são adequadas e podem levar a consequências negativas sobre a fisiologia e qualidade pós-colheita dessas flores. Portanto, este estudo determinou o efeito do pulsing de metil jasmonato (MeJA) ou ácido salicílico (AS) em associação com a sacarose na fisiologia pós-colheita de flores de Ave do Paraíso. Para isso, dois experimentos independentes foram realizados. No primeiro experimento, as hastes florais foram tratadas com 100, 250 e 400 µmol L^{-1} de MeJA juntamente com 200 g L^{-1} de sacarose por 24 h. No segundo experimento, os tratamentos consistiram de 2, 4 e 6 mmol L^{-1} de AS em associação com 200 g L^{-1} de sacarose por 24 h. Após a aplicação do pulsing, as hastes florais de ambos os experimentos foram armazenadas em recipientes com água e conduzidos a temperatura ambiente. As análises foram realizadas nos dias 0 e 7 e a avaliação compreendeu: taxa de absorção de água, taxa de transpiração, perda de massa fresca, extravasamento de eletrólito, atividade de peroxidase (POD) e compostos fenólicos das brácteas e sépalas. MeJA e SA reduziram a taxa de absorção de água pelas hastes. SA reduziu a taxa de transpiração, perda de massa fresca e compostos fenólicos de sépalas no dia 0. No dia 7, o tratamento com MeJA reduziu o extravasamento de eletrólitos e aumentou a atividade da POD. Por outro lado, SA não teve efeito algum sobre o extravasamento de eletrólitos e reduziu significativamente a atividade da POD. Estes resultados sugerem que MeJA e AS atuam de forma antagônica sobre o extravasamento de eletrólitos bem como atividade da POD, o que pode promover efeitos diferentes sobre a fisiologia pós-colheita de hastes de Ave do Paraíso, tais como padrões adversos da taxa transpiratória.

Palavras-chave: peroxidase; reguladores vegetais; *Strelitzia reginae*; transpiração

Introduction

Floriculture is growing steadily, especially for tropical flower species, due to the increased interest in exotic flowers. Bird of Paradise (*Strelitzia reginae* Banks) presents great market potential due to its rusticity, stem length and beauty (Dias et al., 2013). One major setback of the flower market are the high postharvest losses (Dias-Tagliacozzo & Castro, 2002), caused by the flower decay that can be intensified low shipping and storage conditions and often conducted without the aid of refrigeration and simple postharvest treatments (Dias et al., 2013).

In this context, methyl jasmonate (MeJA) and salicylic acid (AS) aid in extending postharvest life of several vegetables (Asghari & Aghdam, 2010), reducing their losses. MeJA is a plant hormone that acts on the growth, development, and responses to biotic and abiotic stresses, playing roles in intracellular signaling and acting in the induction of plant defense (Mueller et al., 1993). Also, MeJA acts delaying the senescence, inducing the flowering as well as in the stomata closure modulation and floral opening regulation (Avanci et al., 2010). Likewise, as observed in experiments with strawberry (Babalar al., 2007), pomegranate (Sayyari et al., 2009), orange (Hung et al., 2007), apple (Mo et al., 2008) and kiwifruit (Aghdam et al., 2010), the use of SA showed positive results in the reduction of ethylene production, induction of resistance to diseases, prevention of oxidative stress, decrease in respiratory rate, maturation and senescence (Asghari & Aghdam, 2010).

Furthermore, the different opening of inflorescences has also been a major problem in the post-harvest of Bird of Paradise flowers (Macnish et al., 2009). For this purpose, pulsing with sucrose has been widely used to improve the floral opening by acting on the osmotic regulation of the tissues, reducing the water potential of the stem, improving the water uptake and providing carbohydrates for maintenance of the respiratory process (Costa et al., 2015).

Therefore, due to the lack of studies on the use of MeJa and AS in combination with other postharvest preservation techniques, such as pulsing on Bird of Paradise flowers, the objective of this study was to determine the effect of pulsing treatment containing methyl jasmonate (MeJA) or salicylic acid (SA) associated with sucrose on the postharvest physiology of Bird of Paradise.

Material and Methods

The stems were collected in the commercial harvest point (when the flower buds showed the color of the cultivar) at experimental field of the Universidade Federal de Viçosa (UFV), located in Viçosa-MG, 20 ° 45 ‘S, 42 ° 51’ W, 651 m altitude. The rainfall regime allows the establishment of a tropical rain forest, providing an excellent environmental condition for the establishment of Strelitziaceae family members. Subsequently, the stems were transported to UFV’s Postharvest Physiology laboratory, where they were selected and standardized to a length of 25 cm.

Two independent experiments were carried out. In the first one, floral stems were treated with 100, 250 and 400 μmol L^{-1} of MeJA (Methyl Jasmonate, Sigma-Aldrich, 95%) plus 200 g L^{-1} of sucrose for 24 h. In the second experiment, treatments consisted of 2, 4 and 6 mmol L^{-1} of SA (Salicylic Acid, Sigma Aldrich, 99%) in association with 200 g L^{-1} sucrose for 24 h. After that, the stems of both experiments were kept in containers with water at room temperature of 25±4 °C in the first experiment and 27±2°C in the second experiment. In both experiments, the water was exchanged daily. The temperature was determined by Data-logger instrument (HTR-157, Instrutherm). The analyses were performed on days 0 and 7 and the assessment comprised: water uptake rate, transpiration rate, fresh mass loss, electrolyte leakage, peroxidase activity (POD) and phenolic compounds of the bracts and sepals.

Water uptake and transpiration rates were determined according to the methodology described by Van Doorn et al. (2002). The stems were laid in individual tubes previously weighed and contained 200 g of deionized water. The tubes were weighed on days 0 and 7 with and without the stems. To reduce the effects of evaporation, the upper end of the tubes were wrapped with four layers of PVC film. Water uptake rate and transpiration rate were obtained in mg g^{-1} of fresh mass day^{-1}. Water uptake was calculated using the equation: $V = (MS_i\text{-}MS_f)/MH_f$. In addition, the estimated transpiration rate was calculated using the equation: $T = V - (MH_f - MH_i)$, where V is absorbed solution volume, MS_i is mass of solution on day 0, MS_f is mass of solution on day 7, MH_f is mass of stems on day 7, T is transpiration rate, and MH_i is mass of stems on day 0. To determine the loss of fresh mass, flower stems were weighed on days 0 and 7, and the data expressed as a percentage.

Electrolyte leakage (EE) was determined with a conductivity meter (LFT 613T, Schott Geratie). Four 10-mm diameter disks were removed from the bracts, soaked in 20 mL of distilled water in a closed container for six h, to assess first the free conductivity (FC) reading. Afterward, the containers were placed capped in an oven at 90 ºC for two h. After cooling, total conductivity (CT) readings were performed, where $EE = (CL/CT) \times 100$ (Lima et al., 2002).

Peroxidase (POD) was determined according to Marques et al. (2011), with modifications. In the extraction, 5 g of bracts and frozen sepals were ground with 25 mL of extraction buffer (0.1 mol L^{-1} phosphate buffer, pH 6.0, added with 0.1% sodium bisulfite and 0.15 mol L^{-1} of sodium chloride). The homogenate was filtered and centrifuged at 17,000 g for 30 min at 4°C. In the enzymatic activity assay, an aliquot of the filtered homogenate was added to the reaction medium containing 0.5 mL of guaiacol (1.7%), 1.5 mL of extraction buffer (pH 6.0) and 0.5 mL of hydrogen peroxide (1.8%). Readings were performed using a spectrophotometer at 470 nm and enzymatic activity expressed in μmol min^{-1} $mg^{-1}$of protein. The same filtered homogenate used in the enzymatic assay was used for protein quantification by the Bradford method (1976), using bovine serum albumin (BSA) as standard.

The phenolic compounds were determined according to Folin-Denis, described by Kubota (1995), with modifications. Fifty grams of bracts and sepals were macerated with 10 mL of methanol. The homogenate was centrifuged, a 0.5 mL aliquot was mixed with 2.5 mL of 1:3 Folin-Denis reagent and two

mL of 10% $NaCO_3$. After one h in the dark, the samples had their absorbance read in a spectrophotometer at 700 nm, using D-catechin as standard.

The experiment was carried out in a complete randomized block design, in a split-plot scheme, where evaluation days were in the plots and the doses in the split-plots. With four blocks and experimental unit of two floral stems. Data was subjected to analysis of variance and the means compared by the Dunnett test at the level of 5% of significance, using the System of Statistical Analysis and Genetics of the UFV (Cruz, 2006).

Results and Discussion

MeJA and SA pulsing treatment reduced significantly the stems water uptake rate (Figure 1A and 1B). On day 7, water uptake of the control without MeJA was 54.2; 50.0 and 66.7% higher than the treatments containing100, 250 and 400 µmol L^{-1} of MeJA, respectively, while floral stems without SA absorbed about 75 to 82% more water than SA treatments. The reduction in water uptake occurs due to obstruction of the vascular bundles of the stems, leading to the interruption of water conductance. Blockage of conducting vessels are a

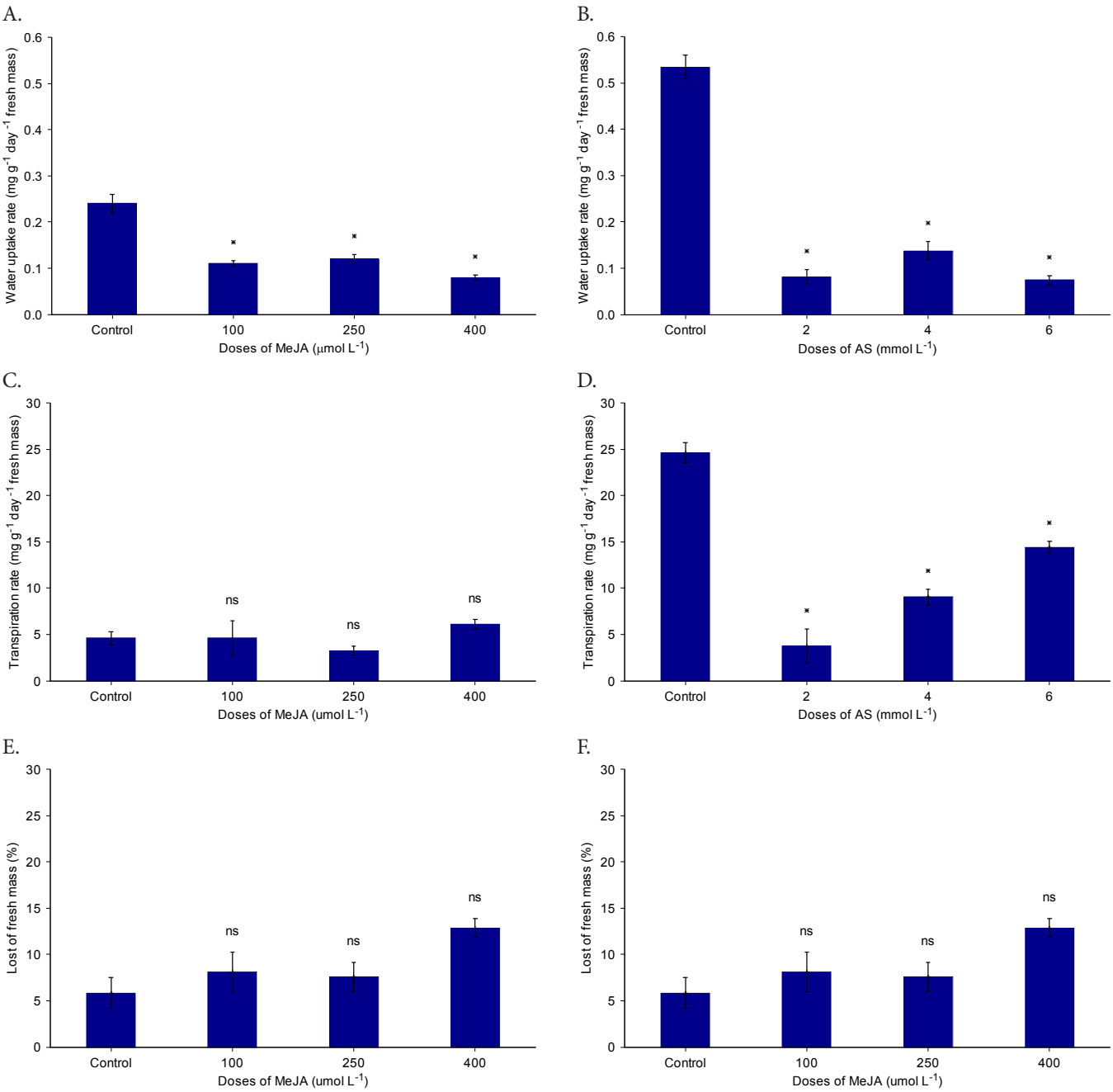

Figure 1. A - Water uptake rate of the pulsing stem with sucrose and methyl jasmonate (Control, 100, 250 and 400 µmol L^{-1}), B - Water uptake rate of the pulsing stem with sucrose and salicylic acid (Control, 2, 4 and 6 mmol L^{-1}), C - Transpiration rate of the pulsing stem with sucrose and methyl jasmonate (Control, 100, 250 and 400 µmol L-1), D - Transpiration rate of the pulsing stem with sucrose and salicylic acid (Control, 2, 4 and 6 mmol L^{-1}), E - Loss of fresh mass of submitted stems to pulsing with sucrose and methyl jasmonate (Control, 100, 250 and 400 µmol L^{-1}, F - Loss of fresh mass of submitted stems to pulsing with sucrose and salicylic acid (Control, 2, 4, 6 mmol L^{-1}). The averages followed by * differ from control while the averages followed by ns do not differ from the control by the Dunnett test ($p \leq 0.05$). The bars represent the standard error of 8 floral stems.

physiological response due to microbial growth or embolism (Marques et al., 2011). In stems of Bird of Paradise, the blocking of conducting vessels can be accelerated by the action of peroxidase (POD) and polyphenol oxidase (PPO) enzymes induced by the cutting of the base of the stems and bacterial growth (Marques et al., 2011). These enzymes block the conducting vessels through the oxidation of the ρ-coumaril, coniferyl, and synapyl alcohols, which are lignin precursors (Boerjan et al., 2003). The application of MeJA and SA may have led to an increase in the activity of these enzymes in the stems, intensifying the vascular blockage. Since these plant regulators act on the plant's defense system against oxidative stress, generated by stem cutting.

No difference was found in the transpiration rates between MeJA and control treatments (Figure 1C). However, the presence of 2, 4 and 6 mmol L^{-1} of SA in the pulsing solution resulted in the reduction of 88.2; 70.4 and 52.0% of transpiration compared to the control treatment with no SA (Figure 1D).

Asghari & Aghdam (2010) reported that transpiration could be reduced with SA application, and its effect depends on concentration and species.

MeJA has no effects on fresh mass loss of the flowers (Figure 1E). The control, 100, 250 and 400 µmol L^{-1} of MeJA presented loss of fresh mass of 7.29; 9.51; 9.03 and 12.06%, respectively. Because the water uptake of the stems was reduced with the use of MeJA and no difference in the transpiratory rate was found, the treatments with MeJA presented the largest fresh mass decrease. Differently from that observed with the use of MeJA, control treatments, 2, 4 and 6 mmol L^{-1} of SA showed a loss of fresh mass of 36.51; 5.28; 11.69 and 17.35%, respectively, yet not statistically significant (Figure 1 F).

MeJA had an effect on electrolyte leakage only on day 7, in which the electrolyte leakage was reduced when compared with control treatment (Figure 2A and 2B). This response is closely linked with the antioxidative enzymes that remove reactive oxygen species (ROS) which are triggered by MeJA. Otherwise, no effect in the electrolyte extravasation was observed with the use of SA (Figure 2B).

Only the dose of 100 µmol L^{-1} of MeJA increased POD activity in the bracts and sepals (Table 1), while doses of 4 and 6 mmol L^{-1} of SA reduced the enzymatic activity of POD in the sepals on day 7 (Table 1). Studies on sweet cherries treated with SA (Yao & Tian, 2005) and strawberry after 8 days of MeJa and AS application AS (Asghari & Hasanlooe, 2015) showed an increase in POD. Peroxidase is an antioxidant enzyme, with the increase in its activity related to the accumulation of hydrogen peroxide. Such enzyme uses hydrogen peroxide as oxidant and phenolic compounds as electron donors (Barbosa et al., 2014). Reactive oxygen species are produced by plants under stress conditions, such as physical damage caused by the cut of the flower, to act as signaling molecules, altering the expression of several genes, among them those of antioxidant

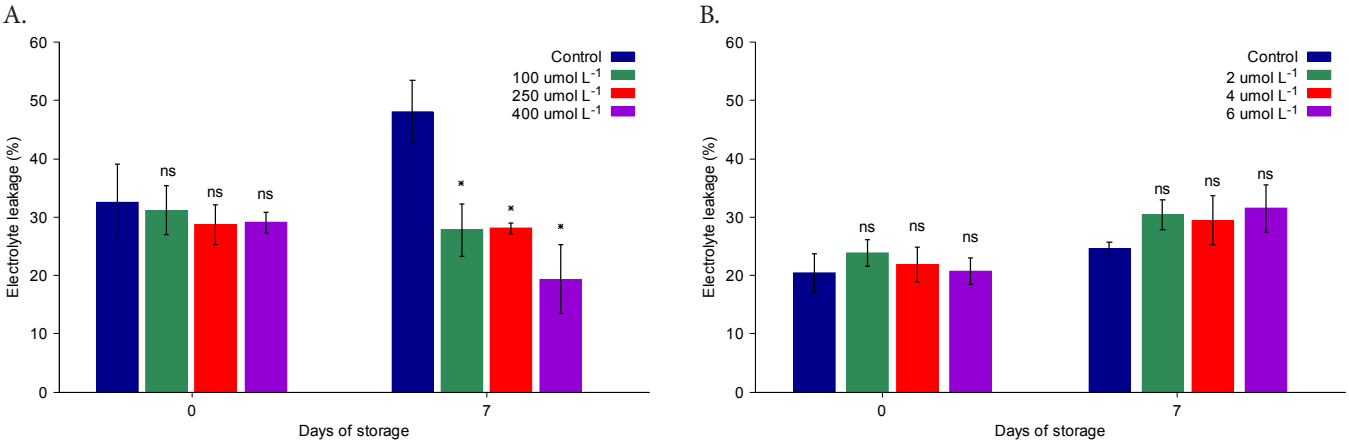

Figure 2. Electrolyte leakage of Bird of Paradise bracts on storage days 0 and 7 after being submitted to pulsing with: A. methyl jasmonate (Control, 100, 250 and 400 µmol L^{-1}) or B. salicylic acid (Control, 2, 4, 6 mmol L^{-1}). The averages followed by * differ from control while the averages followed by ns do not differ from the control by the Dunnett test ($p \leq 0.05$). The bars represent the standard error of 8 floral stems.

Table 1. Activity of peroxidase (POD) and phenolic compounds of the bracts and sepals of Bird of Paradise on days 0 and 7 of storage after being submitted to pulsing with methyl jasmonate (Control, 100, 250 and 400 µmol L^{-1}) or salicylic acid (Control , 2, 4, 6 mmol L^{-1}).

MeJA	POD Activity (µmol min^{-1} mg^{-1} of protein)				Phenolic compounds (mg g^{-1} D-catechin)			
	Bract		Sepals		Bract		Sepals	
	0 day	7 day	0 day	7 day	0 day	7 day	0 day	7 day
Control	0,80	1,80	1,89	5,72	0,19	0,44	0,15	0,63
100 µmol L^{-1}	2,73 *	7,62 *	4,72 *	7,40 *	0,13 ns	0,62 ns	0,14 ns	0,88 ns
250 µmol L^{-1}	0,43 ns	2,85 ns	3,87 ns	5,11 ns	0,08 ns	0,58 ns	0,19 ns	0,53 ns
400 µmol L^{-1}	0,86 ns	1,85 ns	2,78 ns	5,32 ns	0,04 ns	0,61 ns	0,15 ns	0,63 ns
AS								
Control	0,91	6,37	1,26	11,15	0,21	0,23	2,48	2,6
2 mmol L^{-1}	1,18 ns	5,28 ns	1,80 ns	11,69 ns	0,08 ns	0,39 ns	1,07 *	2,29 ns
4 mmol L^{-1}	1,00 ns	6,03 ns	1,86 ns	4,12 *	0,21 ns	0,22 ns	0,42 *	2,66 ns
6 mmol L^{-1}	1,85 ns	4,49 ns	2,55 ns	4,75 *	0,09 ns	0,24 ns	1,85 *	2,23 ns

The averages followed by * differ from control while the averages followed by ns do not differ from the control by the Dunnett test ($p \geq 0.05$).

enzymes, aiming at reducing their damages (Barbosa et al., 2014). MeJA and SA act differently in different parts of the antioxidant system (Asghari & Hasanlooe, 2015).

Only SA showed an effect on the phenolic compounds, reducing their contents in the sepals on day 0 (Table 1). Phenolic compounds are substances that occur naturally in plants and act as antibacterial agents and receiver of most oxidant molecules (Ali et al., 2007). Pérez-Tortosa et al. (2012) observed an increase in phenols with the application of SA, which also occurred in cherries (Dokhanieh et al., 2013) and roots of *Panax ginseng* with the application of MeJA and SA (Ali et al., 2007). These plant regulators present action under the enzymes of the synthesis of phenolic compounds pathway (Ali et al., 2007). Under stress, as an injury, plants induce defense responses and increase levels of secondary metabolites (Ali et al., 2007), which act as a signaling molecule, inducing enzymes from the secondary metabolic pathway to form defense compounds, such as phenols (Ali et al., 2007). An increase was found in the activity of enzymes of the biosynthetic route of phenolic compounds, Glucose-6-phosphate dehydrogenase and chiquimate dehydrogenase in roots of *Panax ginseng* with the application of MeJA and SA (Ali et al., 2007), in which the highest increase was that with the use of SA. No increase of the phenolic compounds was observed in this study with the application of MeJA and SA, but an increase was found with storage time.

Conclusion

MeJA and AS act antagonistically on the electrolyte leakage and POD activity which might promote different effects on the postharvest physiology of Bird of Paradise stems, such as adverse patterns of the transpiration rate. Hence, in further studies, these data might be utilized in the integration of effects triggered by MeJa or AS on postharvest physiology with quality characteristics in the Bird of Paradise vase life.

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