

## Salicylic acid reduces chilling injury in post-harvest of Bird-of-Paradise

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**ABSTRACT:** The irregular opening of the florets and susceptibility to chilling damages are the major issues of cut Bird-of-Paradise flowers (*Strelitzia reginae*). The salicylic acid (SA) has shown to be a promising action in reducing chilling injury in many crops; however, its effect is not well elucidated in cut flowers. The objective of this study was to determine the action of SA on the development of reduction of chilling injury during storage of Bird-of-Paradise flowers. The stems were pulsed with 200 g L<sup>-1</sup> of sucrose in added with three concentrations of SA (2, 4 and 6 mmol L<sup>-1</sup>) and control with only 200 g L<sup>-1</sup> of sucrose for 24 hours. Subsequently, the stems were transferred to containers with water and conditioned at 5 °C for chilling injury induction for 28 days. Water uptake rate, transpiration rate, fresh mass, electrolyte leakage, activity of peroxidase and phenolic compounds of bracts and sepals were determined weekly. The SA reduces the transpiration rate and keeps the mass fresh until 21 days. A reduction was observed in electrolyte leakage up to 14 days. All doses of SA increased POD activity of the bracts while water uptake rate, POD activity of the sepals and phenolic compound content of bracts and sepals are dependent on dose and storage period. It is concluded that the SA applied via pulsing reduces the chilling injury on Bird-of-Paradise cut flowers. In addition, it reduces the transpiration rate and maintains the fresh mass of the floral stems until 21 days. No correlation was found between POD activity and phenolic compounds.

**Key words:** electrolyte leakage; peroxidase; *Strelitzia reginae*; vase life

## O ácido salicílico reduz a injúria por frio na pós-colheita de Ave do Paraíso

**RESUMO:** A abertura irregular das inflorescências e susceptibilidade a danos pelo frio são os maiores problemas em flores de corte de Ave-do-Paraíso (*Strelitzia reginae*). O ácido salicílico (AS) demonstrou ser promissor na redução do chilling em muitas culturas, no entanto, o seu efeito não é elucidado em flores. O objetivo deste estudo foi determinar a ação do AS na redução da injúria por frio na pós-colheita de flores de ave do paraíso. As hastes foram colocadas imersas em soluções com 200 g L<sup>-1</sup> de sacarose, acrescidas de três concentrações de AS (2, 4 e 6 mmol L<sup>-1</sup>) e do controle somente com 200 g L<sup>-1</sup> de sacarose por 24 h. Posteriormente, as hastes foram transferidas para recipientes com água e acondicionadas a 5 °C para indução da injúria por frio, durante 28 dias. A taxa de absorção de água, taxa transpiratória, massa fresca, extravasamento de eletrólitos, atividade da peroxidase e compostos fenólicos das brácteas e sépalas foram determinadas semanalmente. O AS reduz a taxa transpiratória e mantém a massa fresca até os 21 dias. Houve uma redução no extravasamento de eletrólitos até 14 dias. Todas as doses de AS aumentaram a atividade POD das brácteas, enquanto a taxa de absorção de água, a atividade de POD das sépalas e o conteúdo de compostos fenólicos das brácteas e sépalas são dependentes da dose e do período de armazenamento. Conclui-se que o AS aplicada via pulsing reduz a injúria por frio em flores de corte de Ave-do-Paraíso. Além disso, reduz a taxa transpiratória e mantém a massa fresca das hastes florais até os 21 dias. Não houve correlação entre a atividade do POD e os compostos fenólicos.

**Palavras-chave:** extravasamento de eletrólitos; peroxidase; *Strelitzia reginae*; vida de vaso

## Introduction

The market for exotic crops has been increasing since the last decade. Bird-of-Paradise (*Strelitzia reginae*) flower presents favorable production characteristics due to its rusticity, relative high postharvest durability, long stems and attractive flowers (Wood, 1995).

Bird-of-Paradise has a variable shelf life, ranging from six to 16 days, depending on the pre- and postharvest treatments (Bayogan et al., 2008). This difference is determined by environmental and soil conditions during growth, maturity at harvest and storage as well as handling and transport conditions (Bayogan et al., 2008). However, the irregular opening of florets and susceptibility to damages caused by cold weather are the two major obstacles to the production of this species (Macnish et al., 2009). Temperatures between 10 and 13°C promote the development of physiological chilling (Jaroenkit & Paull, 2003), resulting in discoloration, accelerated loss of water, necrotic lesions, cell leakage, delay on floral opening and increase on the susceptibility to attacks by pathogens (Finger et al., 2003).

Salicylic acid (SA) may be an alternative in reducing chilling by altering the expression of resistance genes to stress, particularly abiotic (light, drought, salinity, cold, UV radiation and heat shock) by inducing the synthesis of heat shock proteins (HSPs) (Asghari & Aghdam, 2010), as observed in wheat (Tasgin et al., 2006), peach (Cao et al., 2010), watermelon (Jing-Hua et al., 2008), pomegranate (Sayyari et al., 2009) and Anthurium (Promyoua, 2012). However, there are no studies that determine the action of this substance in combination with other postharvest preservative techniques, such as pulsing, which favors the opening of florets and promotes a positive water balance.

The objective of this study was to determine the action of SA in reducing chilling injury in cut flowers of Bird-of-Paradise.

## Material and Methods

The stems were collected at the marketable harvest point (when the flower buds showed the color of the cultivar) at the experimental field of the Post-Harvest Physiology Laboratory located at the Federal University of Viçosa (UFV), Viçosa-MG, 20°45'S, 42°51'W, at 651 m above sea level. Afterwards, they were submitted to pulsing solutions with 200 g L<sup>-1</sup> sucrose and 2, 4 and 6 mmol L<sup>-1</sup> of SA, and control with 200 g L<sup>-1</sup> of sucrose for 24 h. Subsequently, the stalks were transferred to containers filled with water water and stored at 5°C for induction of chilling injury for 28 days. The evaluations were carried out weekly until the appearance of necrotic spots on the sepals, on the petals and on the end bracts.

Water uptake rate and transpiration were determined according to the methodology described by Van Doorn et al. (2002). The stems were placed in individual weighed tubes, initially containing approximately 200 g of deionized water. The tubes were weighed daily with and without stems.

To undo the effects of evaporation, the upper ends of the tubes were wrapped with PVC film in four layers. The water absorption rate and perspiration was obtained in mg g<sup>-1</sup> fresh mass and the water absorption calculated using the formula:  $V = (PS_i - PS_f) / PH_f$ . V: volume of solution absorbed; PS<sub>i</sub>: initial weight of the solution; PS<sub>f</sub>: final weight of the solution; PH<sub>f</sub>: final mass of the stem. The transpiration rate was estimated by subtracting the variation of the fresh weight of stems by volume of solution absorbed by the formula:  $T = Vc - [PH_i - PH_f]$  where T: transpiration rate; Vc: volume consumed solution; PH<sub>i</sub>: initial mass of the stem; PH<sub>f</sub>: final mass of the stem.

In order to determine the variation of fresh mass, the flower stems were weighed daily and the data were expressed in percentages related with the initial weight of the stems, which was regarded as 100 %.

The electrolyte leakage was determined by means of conductivity (LFT 613T, Schott Geratie). Four discs of 10 mm in diameter had the bract removed, then immersed in 20 ml of distilled water in a closed container for 6 hours to carry out the first free conductivity reading. Then, the containers were placed in an oven at 90°C for 2 hours. After cooling, the total conductivity reading was performed using the equation: electrolyte leakage = (free conductivity / total conductivity) x 100 (Lima et al., 2002).

For the extraction of peroxidase, 5 g of plant material was grinded in 25 ml of extraction buffer (phosphate buffer 0.1 mol L<sup>-1</sup>, pH 6.0 plus 0.1 % sodium bisulphite and 0.15 mol L<sup>-1</sup> sodium chloride). The homogenized was filtered and centrifuged at 17,000 rpm for 30 minutes at 4°C. Enzymatic activity of peroxidase was measured in a reaction media containing 0.5 mL of guaiacol (1.7 %), 1.5 mL of 0.1 mol L<sup>-1</sup> phosphate buffer (pH 6.0) and 0.5 ml of hydrogen peroxide (1.8 %). Absorbance change rate was determined in spectrophotometer at 470 nm. Activity was expressed in  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  of protein (Marques et al., 2011). The same crude extract used in the determination of the enzymatic activity was used for quantification of the protein by the method of Bradford (1976) using BSA as standard.

Total phenolics content was measured by the Folin-Denis method described by Kubota (1995) with modifications. About 50 g of plant material was macerated with 10 ml of methanol. The homogenized material was centrifuged, then a 0.5-mL aliquot was removed and mixed with 2.5 ml of Folin-Denis 1:3 and with 2 ml of 10 % NaHCO<sub>3</sub>. After 1 hour in the dark, the samples were read in spectrophotometer absorbance at 700 nm using D-catechin as a standard.

The experimental design used in this study was randomized blocks in split plots, where the doses were the subdivided parcels and the sub-plots were the daily assessments. The experiment was composed of four blocks and the experimental unit consisted of two flower stems. Data were analyzed using analysis of variance and deployment of interactions and regressions were done using the System of Statistical Analysis and Genetics used at the Federal University of Viçosa (Cruz, 2006). Regression model

was chosen based on the significance of the regression coefficients using the t test at  $p > 0.05$  of probability, the coefficient of determination ( $R^2 = \text{SQreg} / \text{SQtrat}$ ) and the biological behavior in the study. For the analysis of water absorption and fresh mass, descriptive analyses were performed.

## Results and Discussion

The SA 6-mmol L<sup>-1</sup> dose increased water uptake rate at 7 and 14 days of storage, while at 28 days, doses of 4 mmol L<sup>-1</sup> and 6 mmol L<sup>-1</sup> promoted higher water uptake (Figure 1). The increment in water uptake with the use of SA is related to its effect on stomatal conductance, transpiration and water uptake (Asghari & Aghdam, 2010), as observed in this work, according to SA concentration.

The lowest water uptake rate did not depend on the treatment for the 21 days. Reduction of water absorption rate is attributable to the blocking of xylem vessels and may involve the action of peroxidase and polyphenol oxidase (Marques et al., 2011). The same authors suggest that the mechanical damage generated by the cutting of the stems may increase the expression of genes involved in wound healing, including the peroxidase and polyphenol oxidase. These enzymes are involved in vascular blockage through oxidation of p-coumaril alcohol, coniferyl and sinapil, which are the precursors of lignin (Boerjan et al., 2003). Lignin is part of the secondary metabolism of plants and in case of stress it functions as protective mechanism but at the cost of reduction or interruption of water flow in the vessels (Loubaud & Van Doorn, 2004).

All evaluated SA doses reduced the transpiration rate on days 7, 14 and 21. However, at 28 days, an increase was observed in transpiration with SA. Such increment was directly proportional to the dose used (Figure 1). In all treatments, the transpiration rate was higher than the water uptake rate, leading the floral stems to a negative water balance.

An increment was found in the fresh mass in all treatments at 7 days of storage in relation to day 0 (Figure 1). The increase in the control indicates that the application of pulsing allowed a greater absorption of water. Such behavior may be justified due to the action of sucrose, which induces

stomatal closure and reduces osmotic potential, promoting the water absorption (Marousky, 1972). Nevertheless, pulsing with SA in this period is associated to a greater fresh mass of floral stems when compared to the control which is attributable to the reduction in transpiration rate with the application of SA. The SA reduces respiration rate by affecting the activity of the alternative oxidase pathway (Raskin et al., 1989) and by showing a negative effect on cellulases and antioxidant enzymes, maintaining the fresh mass. From day 7, a reduction was found in the fresh mass of the flower stems in relation to day 0, where the smallest reduction was observed in the SA treatments at 14 and 21 days of storage (Figure 1). This is also associated with the effect of SA on plant transpiration as 28 days, a greater reduction was found in the fresh mass of the plants (Figure 1).

The variation in stem fresh mass with the storage is caused by respiration, reduction of water absorption due to obstruction of the stem or genetically determined factors (Ichimura et al., 2006). Floral longevity is directly related to the maintenance of a high level of water, or at least constant, of fresh mass flowers during the postharvest life (Ichimura et al., 2006). A reduction of 10 to 15 % of fresh mass may compromise the quality and durability of flowers (Nowak & Rudnicki, 1990). In the present study, the reduction of the fresh mass remained below 8.4 %.

The application of SA reduced the leakage rate only at 7 and 14 days of storage. In this period, as SA doses increased, cell leakage rate gradually reduced. The cooling of the flowers causes damage to cell membranes and electrolyte leakage is the main parameter used to assess the integrity of cell membranes, showing the impact of chilling injury in many products. The damage to the membranes is the initial event in the development of injuries caused by cold and the symptoms result, in part, by the generation of reactive oxygen species (ROS) and their removal is due to the capacity of antioxidant enzymes present. Lipid molecules pass from gel state to the crystalline, resulting in changes in the metabolism such as leakage of ions, loss of mitochondrial activity, changes in the production of ethylene and enzyme systems associated with the membrane and in the toxic metabolites accumulation.

The reduction in electrolyte leakage by using SA may be related to the induction into defense gene expression, resulting in the decrease in lipid peroxidation in the membrane

**Table 1.** Regression models adjusted for analysis with *Strelitzia reginae* applied via pulsing increased by 2, 4 and 6 mmol L<sup>-1</sup> of SA and control (without SA), stored at 5 °C for 28 days.

	Water absorption rate (mg g <sup>-1</sup> day <sup>-1</sup> fresh mass)		Transpiration rate (mg g <sup>-1</sup> day <sup>-1</sup> fresh mass)	
Day 0	$\hat{y} = 0.20 - 0.03 x + 0.006 x^2$	R <sup>2</sup> = 0.99	$\hat{y} = 3.38^{**} - 0.08 x$	R <sup>2</sup> = 0.70
Day 7	$\hat{y} = 0.26 - 0.01 x + 0.002 x^2$	R <sup>2</sup> = 0.30	$\hat{y} = 1.20 - 0.30 x + 0.04 x^2$	R <sup>2</sup> = 0.58
Day 14	$\hat{y} = 0.06 + 0.01 x - 0.002 x^2$	R <sup>2</sup> = 0.98	$\hat{y} = 1.20 - 0.31 x + 0.04 x^2$	R <sup>2</sup> = 0,58
Day 28	$\hat{y} = 0.10 - 0.005 x + 0.003 x^2$	R <sup>2</sup> = 0.94	$\hat{y} = 0.96 + 2.12 x + 0,05 x^2$	R <sup>2</sup> = 0.89
	Fresh mass (%)		Leakage of electrolytes (%)	
Day 0	$\hat{y} = 104.17^{**} + 2.83 x - 0.36 x^2$	R <sup>2</sup> = 0.78	$\hat{y} = 27.56^{***} - 0.74 x$	R <sup>2</sup> = 0.93
Day 7	$\hat{y} = 98.69^{***} + 0.49 x - 0.06 x^2$	R <sup>2</sup> = 0.57	$\hat{y} = 33.16^{***} - 0.99 x$	R <sup>2</sup> = 0.83
Day 14	$\hat{y} = 98.68^{***} + 0.08 x - 0.02 x^2$	R <sup>2</sup> = 0.49	$\hat{y} = 30.51^{***} + 0.86 x$	R <sup>2</sup> = 0.87
Day 28	$\hat{y} = 97.28^{**} - 2.78 x + 0.34 x^2$	R <sup>2</sup> = 0.89	$\hat{y} = 31.70 + 8.28 x - 1.28 x^2$	R <sup>2</sup> = 0.74

**Table 2.** Regression models adjusted for analysis with *Strelitzia reginae* applied via pulsing increased by 2, 4 and 6 mmol L<sup>-1</sup> of SA and control (without SA), stored at 5 °C for 28 days.

	Activity of peroxidase of bracts ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ of protein)		Activity of peroxidase of sepals ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ of protein)	
Day 0	$\hat{y} = 4.63 + 0.55x - 0.06x^2$	$R^2 = 0.57$	$\hat{y} = 2.73 + 0.46x - 0.03x^2$	$R^2 = 0.77$
Day 7	$\hat{y} = 2.75 - 0.30x + 0.11x^2$	$R^2 = 0.65$	$\hat{y} = 2.69 + 1.61x - 0.10x^2$	$R^2 = 0.67$
Day 14	$\hat{y} = 2.56 + 0.54x + 0.005x^2$	$R^2 = 0.97$	$\hat{y} = 3.58 + 0.22x - 0.08x^2$	$R^2 = 0.75$
Day 21	$\hat{y} = 3.06 - 0.03x + 0.09x^2$	$R^2 = 0.93$	$\hat{y} = 3.42 - 0.50x + 0.12x^2$	$R^2 = 0.65$
Day 28	$\hat{y} = 3.12 + 1.47x - 0.22x^2$	$R^2 = 0.73$	$\hat{y} = 7.02 - 1.44x + 0.18x^2$	$R^2 = 0.79$
	Phenolic compounds of bracts (mg D - catechin g <sup>-1</sup> )		Phenolic compounds of sepals (mg D - catechin g <sup>-1</sup> )	
Day 0	$\hat{y} = 0.03 + 0.005x$	$R^2 = 0.67$	$\hat{y} = 0.73 + 0.29x - 0.04x^2$	$R^2 = 0.73$
Day 7	$\hat{y} = 0.17 + 0.009x$	$R^2 = 0.37$	$\hat{y} = 1.84 - 0.13x + 0.02x^2$	$R^2 = 0.39$
Day 14	$\hat{y} = 0.49 + 1.20x - 0.20x^2$	$R^2 = 0.77$	$\hat{y} = 1.29 + 0.02x + 0.003x^2$	$R^2 = 0.57$
Day 21	$\hat{y} = 1.66 - 0.62x + 0.06x^2$	$R^2 = 0.97$	$\hat{y} = 1.65 - 0.16x + 0.06x^2$	$R^2 = 0.87$
Day 28	$\hat{y} = 1.61 - 0.45x + 0.11x^2$	$R^2 = 0.94$	$\hat{y} = 1.98 - 0.79x + 0.17x^2$	$R^2 = 0.99$

(Kazemi et al., 2011). Salicylic acid induces the expression of alternative oxidase and enlarge the cell antioxidant capacity by reducing oxidative stress and the incidence of injuries caused by the cold. Its split defense system is divided in two forms, including the alternative oxidase genes and the expression of SOD, catalase, ascorbate/cycle glutathione, glutathione peroxidase system and thioredoxin system (Buchanan et al., 2001). The enhancement in the levels of alternative oxidase transcript by SA before cold treatment reduced the incidence of chilling injury in green peppers (Fung et al., 2004). In addition, it acts in the biosynthesis of heat shock proteins (HSPs), which allows the storage of products at low temperatures without the development of chilling injury (Asghari & Aghdam, 2010). It was verified in studies carried out with tomato and peach, the reduction in chilling injury with the use of SA by inducing HSPs biosynthesis (Ding et al., 2001). An improvement occurred in cold storage with use of SA in many types of fruits and vegetables such as peaches (Khademi & Ershadi, 2013), sweet cherry (Yao & Tian, 2005) and asparagus (Wei et al., 2011).

All the evaluated doses of SA increased POD activity in the bracts. The highest activity was observed with the application of 4 and 6 mmol L<sup>-1</sup>. In sepals, the increase occurred only on storage days 0 and 7 and at the dose of 6 mmol L<sup>-1</sup> at 21 days (Figure 2). As POD activity increased, a reduction in phenolic compounds was expected since POD is one of the enzymes that perform the oxidation of phenols (Zhang & Hua, 2008). However, a reduction was found only with the dose of 6 mmol L<sup>-1</sup> at 14 days. At 21 days, all doses reduced phenol content and at 28 days, only the doses of 4 and 6 mmol L<sup>-1</sup> were reduced (Figure 2) while in the sepals, the reduction in phenol content occurred at 7 days at the 2 mmol L<sup>-1</sup> dose at 21 days and at doses of 2 and 6 mmol L<sup>-1</sup> at 28 days (Figure 2). No correlation was found between the increase in the peroxidase and reduction in the phenolic compounds. The reduction in the phenolic compounds is desirable, so, this compound is associated with the appearance of spots darkened by enzymatic action. In addition, the peroxidase activity of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as electron acceptor in donating electrons to phenolic compounds (Passardi et

al., 2005) leads to darkening by oxidative polymerization of quinones (Bindschedler et al., 2002). The increase in phenolic compounds may be associated with the appearance of darkened spots due to enzymatic action. POD is one of the enzymes that carries out the oxidation of phenols (Zhang & Hua, 2008) and it is responsible for stress-induced darkening in different plant species for the oxidative polymerization of quinones (Bindschedler et al., 2002).

## Conclusion

The SA applied via pulsing reduces the chilling injury on Bird-of-Paradise cut flowers. In addition, it reduces the transpiration rate and maintains the fresh mass of the floral stems until 21 days. No correlation was found between POD activity and phenolic compounds.

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