





Alleviation of postharvest chilling injury in anthurium cut flowers by salicylic acid treatment

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Highlights

- Salicylic acid alleviated chilling injury of anthurium cut flowers.
- Salicylic acid reduced electrolyte leakage and MDA content and enhanced proline and glycine betaine accumulation.
- Salicylic acid enhanced antioxidant enzymes SOD, CAT, APX and GR activity.

- Chilling injury alleviation was associated with increased PAL and decreased PPO activities.

Abstract

Due to the sensitivity of anthurium flowers to chilling injury, its optimum temperature storage is 12.5–20°C. Reactive oxygen species (ROS) accumulation during postharvest chilling stress leads to loss of membrane integrity which can be coincided with phenols oxidation by polyphenol oxidase (PPO) activity, the enzyme responsible for browning. Higher phenylalanine ammonia-lyase (PAL) activity, the enzyme responsible for phenols accumulation, under chilling stress, can act as a defense mechanism overcome chilling stress. In this experiment, the impact of 0, 1, 2 and 4mM salicylic acid (SA) treatment applied by postharvest stem-end dipping (15 min at 20°C) on chilling injury of *Anthurium andraeanum* cv. Sirion cut flowers storage at 4°C for 21 days were investigated. SA treatment delayed spathe browning and retards electrolyte leakage and malondialdehyde (MDA) increase. The SA treated anthurium cut flowers exhibited significantly higher PAL enzyme activity, associated with lower PPO activity, which were coincided with higher total phenol accumulation and higher DPPH scavenging activity during storage at 4°C for 21 days. Also, higher DPPH scavenging activity in anthurium cut flowers treated with SA can be results from higher antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) activity. Also, SA treatment enhanced endogenous proline and glycine betaine (GB) accumulation in anthurium cut flowers during storage at 4°C, which was coincided with higher spathe relative water content (RWC). These results suggested that SA treatment can be used as a useful technology for the alleviation of postharvest chilling injury in anthurium cut flowers by enhancing total phenol, proline, and GB accumulation coincide with enhancing antioxidant system activity leading to higher membrane integrity showed by lower electrolyte leakage and MDA content.

Introduction

When anthurium cut flowers, endemic to tropical climates, are stored at 4°C, (1) phenylalanine ammonia-lyase (PAL) enzyme activity increased due to the chilling lead to accumulation of phenols in vacuoles; (2) electrolyte leakage and malondialdehyde (MDA)

content increased, which indicated membrane integrity and selective permeability declined; (3) polyphenol oxidase (PPO) enzyme activity as responsible for spathe browning increased; (4) phenols accumulated in vacuoles leak to cytoplasm due to loss of membrane integrity and contribute to spathe browning, observable symptom of chilling injury (Aghdam et al., 2015). Aghdam et al. (2016) suggested that the declining anthurium cut flowers membrane integrity was due to higher phospholipase D (PLD) enzyme activity leads to releasing unsaturated fatty acids, which under peroxidation by lipoxygenase (LOX) results to declining membrane fluidity due to decreasing unsaturated/saturated fatty acids (unSFA/SFA) ratio, ultimately manifesting spathe browning. Also, declining anthurium cut flowers membrane integrity may also result from H_2O_2 accumulation, which triggers membranes lipid peroxidation, interrupt cellular compartmentalization, which accompanied with MDA accumulation, lessen economic marketability of anthurium cut flowers (Aghdam et al., 2016).

Promyou and Ketsa (2014) suggested that chilling injury in anthurium flowers stored at $4^\circ C$ was accompanied with higher membrane lipid peroxidation which hastens anthurium flower senescence leading to short vase life. Aghdam et al. (2015) reported that γ -aminobutyric acid (GABA) treatments retarded electrolyte leakage and MDA content increase and enhanced proline accumulation in anthurium cut flowers during storage at $4^\circ C$. Also, anthurium cut flowers treated with GABA exhibited higher phenols accumulation, which was associated with higher PAL activity coincided with lower PPO enzyme activity. Also, anthurium cut flowers treated with GABA exhibited higher DPPH \cdot scavenging capacity during storage at $4^\circ C$. Also, Aghdam et al. (2016) suggested that the spathe browning of anthurium cut flowers storage at $4^\circ C$ was accompanied with H_2O_2 accumulation, and the reduction of anthurium cut flowers spathe browning by GABA treatments was due to maintenance of membrane integrity by higher superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) enzymes activity coincided with lower H_2O_2 accumulation associated with lower PLD and LOX enzymes activity coincided with higher unSFA/SFA ratio.

Gao et al. (2016) reported that the brassinolide treatment alleviated peach fruit mesocarp browning as chilling symptom. Alleviation of peach fruit mesocarp browning was associated with lower H_2O_2 and O_2^- accumulation, which led to maintaining membrane integrity, showed by lower MDA accumulation. Also, brassinolide treatment enhanced shikimate dehydrogenase enzyme activity, which is responsible for phenylalanine biosynthesis, leading to phenylalanine accumulation. Also, brassinolide treatment enhanced PAL, cinnamate-4-hydroxylase and 4-coumarate:coenzyme A ligase enzyme activities, which are responsible for

phenols biosynthesis from phenylalanine by phenylpropanoids pathway. Phenols accumulation in peach fruits treated with brassinolide coincided with lower PPO enzyme activity, which lead to alleviation mesocarp browning. It can be suggested that higher PAL/PPO enzymes activity coincided with higher phenols accumulation, accompanied by higher antioxidant system activity result in enhancing membrane integrity due to declining lipid peroxidation, and, ultimately, to the escape of reactive oxygen species (ROS) accumulation, ultimately improve chilling tolerance. Therefore, a postharvest strategy with the ability for enhancing the antioxidant system activity coincided with enhancing PAL/PPO enzymes activity can be used for lessening spathe browning of anthurium cut flowers during storage at 4°C.

Salicylic acid (SA), as natural and safe phenolic molecule, at non-toxic concentrations can be commercially used in alleviating chilling injury in fruits, vegetables, and flowers (Asghari and Aghdam, 2010). Promyou et al. (2012) reported that postharvest SA treatment alleviated chilling injury in anthurium cut flowers by increasing CAT and SOD activities and decreasing LOX activity, was associated with decreasing electrolyte leakage and MDA content, led to lessening spathe browning. Cai et al. (2014) reported that the postharvest SA treatment alleviated chilling injury in grape berries, which was manifested by lowering weight loss, softening, and electrolyte leakage. Cai et al. (2014) suggested that the alleviation of chilling injury in grape berries by postharvest SA treatment may be attributed to (1) enhancing sucrose consumption by increasing glycolysis and tricarboxylic acid cycle activity, (2) enhancing energy, ATP, supply, (3) enhancing antioxidant system activity such as APX and glutathione-S-transferase (GST) and (4) enhancing heat shock proteins (HSPs) accumulation. Thus, SA has the high commercial potential for use at low concentrations in alleviating chilling injury in fruits, vegetables, and flowers.

In this work, the effects of postharvest SA treatment on the spathe browning as chilling injury symptoms, electrolyte leakage and MDA content as membrane integrity indicators, PAL and PPO activities associated with total phenols content and DPPH• scavenging capacity, antioxidant enzymes CAT, APX, SOD, GR activity associated with proline and glycine betaine contents and relative water content (RWC) of anthurium cut flowers were evaluated. We propose that maintenance of membrane integrity, lower electrolyte leakage and MDA content, associated with higher antioxidant enzymes CAT, APX, SOD, GR activity, higher proline and glycine betaine accumulation, and lower PPO enzyme activity coinciding with higher PAL enzyme activity leading to higher total phenolic accumulation and total antioxidant capacity,

is proposed as possible mechanism for the alleviation impact of SA on chilling injury of anthurium cut flowers.

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Flowers and treatment

For postharvest salicylic acid treatment, 360 flowers of *Anthurium andraeanum* cv. Sirion with pink spathe were harvested in the morning when 40–50% of the true flowers on the spadix had fully opened and placed in water at the growers' property and transported at 12°C in water to the laboratory. Then, the flower stems were recut to 30cm length and divided into 6 lots of 90 flowers for the following treatments in triplicate (30 flowers per replicate) by dipping of individual flower stems at 0...

Chilling injury symptoms

Spathe browning increased during storage at 4°C and the increase was delayed by postharvest SA treatment ($P<0.01$; Fig. 1, Fig. 2). Treatment with postharvest SA at 2mM resulted in a lower chilling injury score (Fig. 1; $P<0.01$) and browning index (Fig. 2; $P<0.01$), while postharvest treatment with SA at 4mM resulted in higher chilling injury scores. Thus, SA effects on the chilling injury of anthurium cut flowers are concentration dependent. Based on these results, 2mM SA for postharvest...

Discussion

During storage at 4°C for 21 days, exogenous 2mM SA treatment ameliorated the chilling injury of anthurium cut flowers. Anthurium cut flowers treated with SA showed higher

chilling tolerance within longest vase life, 27 days at 4°C, and exhibited higher ability to maintain membrane integrity under chilling temperature. As regards chilling injury shorten anthurium cut flowers vase life by triggering flower senescence (Promyou and Ketsa, 2014), higher vase life in anthurium cut flower treated...

Conclusion

In conclusion, the present study sheds light on beneficial impact of SA treatment on reducing chilling injury in anthurium cut flowers during low temperature storage. Our results suggest that the reduction of chilling injury in anthurium cut flowers by SA treatment can be due to (1) enhancing PAL enzyme activity associated with decreasing PPO enzyme activity which led to accumulation total phenols, (2) enhancing antioxidant CAT, APX, SOD, GR enzymes activity, (3) enhancing proline and GB...

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