





Amelioration of postharvest chilling injury in anthurium cut flowers by γ -aminobutyric acid (GABA) treatments

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Highlights

- γ -Aminobutyric acid (GABA) ameliorated chilling injury of anthurium cut flowers.
- GABA reduced electrolyte leakage and MDA content and enhanced proline accumulation.
- Chilling injury mitigation was associated with increased PAL and decreased PPO activities.

Abstract

The optimum temperature storage of anthurium flowers is 12.5–20°C because they are very sensitive to chilling injury (CI). CI is associated with the loss of membrane integrity which can be aligned to phenolic oxidation due to polyphenol oxidase (PPO) activity, the enzyme responsible for tissue browning. The increment of phenylalanine ammonia-lyase (PAL) activity, the enzyme responsible for phenols accumulation, in response to chilling stress has been considered as defense mechanism to chilling stress. In this study, the effects of 0, 1, 5, 10, 15 and 20mM γ -aminobutyric acid (GABA) treatment applied by preharvest spraying or postharvest stem-end dipping (15 min at 20°C) on CI of anthurium flowers (cv. Sirion) stored at 4°C for 21 days was investigated. CI symptoms were accompanied by spathe browning and increase in electrolyte leakage as well as malondialdehyde (MDA) content. GABA treatment at 1 and 5mM by pre and postharvest treatment, respectively, delayed spathe browning and increases in electrolyte leakage and MDA accumulation. The GABA treated anthurium cut flowers exhibited significantly higher PAL enzyme activity, associated with lower PPO activity. Higher PAL enzyme activity in anthurium cut flowers treated with GABA coincided with higher total phenol accumulation and higher DPPH scavenging activity than control flowers during storage at 4°C for 21 days. Also, proline content in anthurium cut flowers treated with GABA was significantly higher than control flowers during storage. These results suggest that GABA treatment can be used as a useful technology for enhancing tolerance of anthurium cut flowers to postharvest chilling injury by increasing total phenol and proline accumulation and decreasing MDA content, and thus maintaining membrane integrity.

Introduction

Low temperature storage is widely used as a postharvest treatment to delay senescence in vegetables and ornamentals, and ripening in fruits, and thereby maintaining their postharvest quality. However, tropical and subtropical crops such as anthurium (*Anthurium andraeanum* L.) flowers are sensitive to chilling injury (CI), a physiopathy affecting these crops when subjected to temperatures below 12°C but above the freezing point (Aghdam and Bodbodak, 2013). The recommended optimum temperature for storage of anthurium cut flowers is 12.5–20°C (Promyou et al., 2012). The main symptom of CI in anthurium flowers under low temperature storage is spadix wilting and spathe browning (Paull, 1987, Promyou et al., 2012). There is a tremendous need to apply economical and convenient techniques to reduce CI

while prolonging maximal shelf life of anthurium cut flowers, in order to potentiate their possibilities of long distances transport, a producers demand for their introduction in the global markets (Aghdam et al., 2013). Promyou and Ketsa (2014) suggested that CI in anthurium flowers stored at 4°C is associated with increase in the membrane lipid peroxidation and loss of membrane semi-permeability, which triggers anthurium flower senescence leading to short vase life. Recently, Promyou et al. (2012) reported that postharvest treatment with salicylic acid (2mM for 15 min) alleviated CI in anthurium cut flowers, an effect associated with decreasing electrolyte leakage, MDA content and lipoxygenase (LOX) activity, and increasing catalase (CAT) and superoxide dismutase (SOD) activities, which led to a diminution of spathe browning and fresh weight loss, two detrimental effects of CI on anthurium cut flowers.

When horticultural crops are stored under chilling temperature: (1) PAL activity increases due to the CI effect, inducing increase of total phenols (TP) that accumulates in vacuoles; (2) a membrane selective permeability loss occurs; (3) PPO activity increases in cytoplasm that is responsible for browning; (4) phenols accumulated in vacuoles leak to cytoplasm due to loss of vacuole membrane (tonoplast) selective permeability and contribute to browning incidence, an effect influenced by PAL activity (Sevillano et al., 2009). PAL as a key enzyme in the phenylpropanoid pathway catalyzing the conversion of phenylalanine to trans-cinnamic acid. PAL connects primary metabolism (shikimic acid pathway) with secondary metabolism (phenylpropanoids pathway) (Dixon and Paiva, 1995). Meng et al. (2009) reported that the phenolics have dual function, firstly phenolics can be oxidized by PPO, which leads to browning, as the main CI symptom in horticultural crops and secondly phenols, which accumulate in horticultural crops in response to chilling stress, have antioxidant capacity. Chen et al. (2008) reported that heat pretreatment (38°C for 2 days) mitigated CI in banana fruit during storage at 8°C. Mitigation of CI was determined by a reduction of electrolyte leakage and MDA content and heat treatment increased PAL gene expression and enzyme activity, increasing TP content. It was suggested that higher TP might enhance CI tolerance in banana fruit. Aghdam et al. (2012) reported that the brassinosteroids (BRs) treatment mitigated CI in tomato fruit, which was accompanied with the reduction of electrolyte leakage and MDA content and the increase of proline content. Aghdam et al. (2012) showed that BRs treatment enhanced PAL enzyme activity which led to total phenol accumulation.

γ -Aminobutyric acid (GABA), a four carbon non-protein amino acid with an amino group on the γ -carbon, is widely distributed in bacteria, plants and animals (Shelp et al., 1995, Fait et

al., 2007). Abiotic stresses such as chilling, heat, drought, UV irradiation, and low O₂ cause GABA to accumulate in plants (Shelp et al., 1999). GABA is believed to function in regulation of cytosolic pH (Shelp et al., 1999), mitigation of oxidative stress (Bouche et al., 2003), induction of nitrate transport (Beuvé et al., 2004), regulation of pollen tube growth and guidance (Palanivelu et al., 2003), and cell elongation (Renault et al., 2011). GABA have anti-chilling function in horticultural crops and exogenous GABA treatment has the ability to enhance resistance to postharvest CI in fruits and vegetables such as banana (Wang et al., 2014) and peach fruit (Shang et al., 2011, Yang et al., 2011).

To our knowledge, no information exists on the effect of exogenous GABA treatment on CI in anthurium cut flowers. In this work, the effects of pre and postharvest GABA treatment on the spathe browning as CI symptoms, electrolyte leakage and MDA content as membrane integrity indicators, PAL and PPO activities associated with total phenols content, proline content and DPPH• scavenging capacity of anthurium cut flowers were evaluated. We propose that maintenance of membrane integrity associated with enhanced total antioxidant capacity and proline accumulation, and reduced PPO enzyme activity coinciding with increasing PAL enzyme activity leading to total phenolic accumulation, is proposed as possible mechanism for the mitigation impact of exogenous GABA on CI of cut flowers.

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Flowers and treatments

GABA at 1, 5, 10, 15 and 20mM was sprayed on anthurium (*A. andraeanum* L.) flowers cv. Sirion at commercial greenhouse by using a hand-sprayer until flowers were wet to runoff. Additional flowers were also sprayed with distilled water as the control. The sprays were applied three times at 7-day intervals before harvest, when 40–50% of the true flowers on the

spadix had fully opened (Promyou et al., 2012). Flowers were cut in the morning, placed in water at the growers' property and transported...

Chilling injury symptoms and vase life

CI score increased during the whole storage at 4°C and the increase was delayed by pre and postharvest GABA treatment ($P<0.01$; Figs. 1 and 3). CI symptoms of anthurium cut flowers cv. Sirion were visible within 7 days of storage. Treatment with preharvest GABA at 1 mM and postharvest GABA at 5 mM resulted in a lower CI score ($P<0.01$) as well as browning index ($P<0.05$) (Fig. 1, Fig. 2, Fig. 3, Fig. 4), while pre and postharvest treatment with GABA at 20 mM resulted in higher CI scores and browning...

Discussion

During storage at 4°C for 21 days, exogenous pre and postharvest GABA treatment ameliorated the chilling injury symptoms of anthurium cut flowers indicated by the delay in CI score and browning index increase. Electrolyte leakage is an effective parameter to assess membrane permeability and therefore is used as an indicator of membrane integrity (Marangoni et al., 1996, Lyons, 1973). Also, lipid peroxidation by activation of LOX, which can be evaluated by MDA, is responsible for loss of cell...

Conclusion

Electrolyte leakage and MDA increase were retarded by exogenous GABA treatment. GABA treatment increased the accumulation of proline. Higher content of total phenols was also observed in the GABA-treated anthurium cut flowers which accompanied by an increase in PAL activity associated with decreasing PPO enzyme activity. DPPH radical scavenging activity was significantly promoted in GABA treated anthurium cut flowers during the storage. CI mitigation of anthurium cut flowers under exogenous...

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