





Enhancement of postharvest chilling tolerance of anthurium cut flowers by γ -aminobutyric acid (GABA) treatments

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Highlights

- γ -Aminobutyric acid (GABA) alleviated chilling injury of anthurium cut flowers.
- GABA treatments reduced PLD and LOX activities, which coincided with higher unSFA/SFA ratio.
- GABA treatments enhanced antioxidant enzymes activity, which coincided with lower H₂O₂ accumulation.

Abstract

Anthurium cut flowers, originally a tropical flower, cannot easily be stored at low temperatures, due to the risk of chilling injury (CI). In response to chilling stress, increment of phospholipase D (PLD) enzyme activity leads to releasing unsaturated fatty acids, which under peroxidation by lipoxygenase (LOX) results to declining membrane fluidity and manifesting chilling symptoms. In this study, the effects of γ -aminobutyric acid (GABA) treatment applied by preharvest spraying (1 mM) or postharvest stem-end dipping (5 mM, 15 min at 20°C) on CI of anthurium cut flowers (cv. Sirion) stored at 4°C for 21 days were investigated. GABA treatment at 1 and 5 mM by pre and postharvest treatment, respectively, significantly delayed spathe browning. The GABA treated *anthurium* cut flowers exhibited significantly lower PLD and LOX activities, which coincided with a higher unsaturated fatty acids/saturated fatty acids (unSFA/SFA) ratio. Also, *anthurium* cut flowers treated with GABA showed lower H₂O₂ accumulation, which results from higher antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activity. These results suggest that GABA treatment can be used as a useful procedure for enhancing tolerance of *anthurium* cut flowers to postharvest chilling injury by decreasing PLD and LOX activities coincide with enhancing antioxidant system activity, which lead to lowering ROS accumulation and promoting higher unSFA/SFA ratio and ultimately maintaining membrane integrity.

Introduction

Anthurium cut flowers, endemic to tropical climates, cannot easily be stored at low temperatures, due to the risk of chilling injury (CI) (Promyou et al., 2012). Spadix wilting and spathe browning are considered as observable symptom of CI in *anthurium* cut flowers under low temperature storage (Paull, 1987, Promyou et al., 2012). There is a great demand to exert economical and convenient strategies for diminishing CI in *anthurium* cut flowers, which lead to longer shelf life, until potentiate their probability for long distance transport, as producers request for their introduction in the global markets (Aghdam et al., 2013).

Dysfunction of membrane and accumulation of reactive oxygen species (ROS) are adverse impact of chilling stress in horticultural crops (Aghdam et al., 2013). Under chilling temperature, transition of cell membrane phase from a fluid liquid-crystalline to a rigid solid-gel lead to diminishing membrane selective permeability, which can be assayed by electrolyte

leakage (Aghdam et al., 2013). Phospholipase D (PLD) starts the membrane phospholipid degradation by removing the head group of phospholipids, and generating the second messenger phosphatidic acid (PA). Accumulation of PA in membrane can result to membrane damage by enhancing PLD and PLC enzymes activity, which are responsible for membrane degradation and or by enhancing ROS accumulation via binding to membranous NADPH oxidase, which is responsible for $O_2^{\bullet-}$ generation, this scenario is coincide with decreasing membrane fluidity under chilling temperature (Zhou et al., 2014, Testerink and Munnik, 2011). Participation of PLD with phosphatidate phosphatase and lipolytic acyl hydrolase (LAH) enzymes lead to releasing unsaturated fatty acids, which can undergo peroxidation by lipoxygenase (LOX) leading to the formation of rigid solid-gel phase (Sirikesorn et al., 2015). Increase in membrane unsaturation fatty acids such as linoleic and linolenic acids lead to maintaining membrane integrity and fluidity (Nishida and Murata, 1996). An increase in membrane fluidity diminishes the tendency for phase transition from fluid liquid-crystalline to rigid solid-gel phase resulting in improved CI tolerance (Los and Murata, 2004).

Accumulation of damaging ROS agitates oxidation of cells fundamental molecules such as membrane lipids, proteins, and, damage to DNA and RNA and ultimately triggers oxidative stress. In plant cells, ROS accumulation may be due to the unavoidable leakage of electrons onto O_2 from the electron transport system in chloroplasts and mitochondria, and or by activation of membranous NADPH oxidases (Bhattacharjee, 2012). Declining membrane integrity may also result from oxidative stress, since chilling stress increases the ROS accumulation. ROS accumulation triggers membranes lipid peroxidation, which can be assayed by malondialdehyde (MDA) content (Aghdam and Bodbodak, 2013). Lipid peroxidation could be occurs by enzymatic oxidation of unsaturated fatty acids by LOX or by non-enzymatic oxidation due to ROS accumulation. In addition to peroxidation of membrane unsaturated fatty acids, LOX is responsible for the generation of $O_2^{\bullet-}$ (Apel and Hirt, 2004).

γ -Aminobutyric acid (GABA), four carbon non-protein amino acid, accumulate in plants in response to abiotic stresses such as chilling, heat, drought, UV irradiation and high CO_2/O_2 (Shelp et al., 1995, Shelp et al., 1999, Fait et al., 2007). Alleviation of postharvest CI in horticultural crops such as banana (Wang et al., 2014), peach (Shang et al., 2011, Yang et al., 2011) and anthurium cut flower (Aghdam et al., 2015) achieved by exogenous GABA treatments. Browning of anthurium cut flowers spathe has been attributed to chilling injury, result in ROS accumulation, which might provoke membrane lipid peroxidation (Aghdam et al., 2013, Aghdam et al., 2015). ROS accumulation might interrupt cellular

compartmentalization leading to contacting of polyphenol oxidase (PPO) from plastid with phenols from vacuole to manifesting browning, which in turn, might reduce the storage quality and marketability of anthurium cut flowers (Aghdam et al., 2015). Promyou and Ketsa (2014) reported that the CI in anthurium cut flowers was associated with peroxidation of membrane unsaturation fatty acids, which lead to declining membrane integrity, assayed by increase electrolyte leakage and MDA accumulation. Promyou and Ketsa (2014) also suggested that short vase life of anthurium flowers stored at 4°C can be attributed to CI, which trigger flower senescence. Promyou et al. (2012) reported that anthurium cut flowers treated with salicylic acid exhibited lower electrolyte leakage and MDA content, which results from lower LOX activity coincided with higher antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activity, ultimately lead to delayed spathe browning. Recently, Aghdam et al. (2015) reported that pre and postharvest GABA treatments retarded electrolyte leakage and an increase in MDA content along with enhanced accumulation of proline in cut flowers of anthurium during storage at 4°C. As well, cut flowers treated with GABA exhibited an increased accumulation of total phenols. The accumulation of phenols was associated with an increase in phenylalanine ammonia-lyase (PAL) activity, and decreased PPO enzyme activity. Also, DPPH• scavenging capacity was significantly enhanced in GABA treated anthurium cut flowers during cold storage (Aghdam et al., 2015).

It can be speculated that a lower PLD and LOX enzymes activity coincided with higher unsaturated fatty acids/saturated fatty acids (unSFA/SFA) ratio, accompanied by higher antioxidant system activity such as SOD, CAT, ascorbate peroxidase (APX) and glutathione reductase (GR) result in enhancing membrane integrity due to declining lipid peroxidation, and, ultimately, to the avoidance of ROS accumulation, all of which has an positive effects on chilling tolerance. Therefore, a postharvest treatment to enhancing the capacity of antioxidant system activity along with reducing PLD and LOX enzymes activity may be have potential for minimizing spathe browning and extending the vase life of anthurium cut flowers.

In this study, the impact of pre and postharvest GABA treatments on the spathe browning as CI symptoms, antioxidant enzymes CAT, APX, SOD, GR activity associated with H₂O₂ content, and PLD and LOX enzymes activity associated with unSFA/SFA ratio of anthurium cut flowers were evaluated. We propose that maintenance of membrane integrity is achieved by (1) higher antioxidant enzymes CAT, APX, SOD, GR activity, which led to lower H₂O₂ accumulation (2) lower PLD and LOX enzymes activity which coincided with higher

unSFA/SFA ratio. This is proposed as a possible mechanism for the alleviating impact of GABA on CI of anthurium cut flowers.

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Section snippets

Flowers and treatments

Based on our previous study (Aghdam et al., 2015), 1 mM GABA for preharvest treatment and 5 mM GABA for postharvest treatment were used. GABA at 1 mM was sprayed on anthurium (*Anthurium andraeanum* L.) flowers cv. Sirion at commercial greenhouse by using a hand-sprayer until flowers were wet resulting in runoff. Additional flowers were sprayed with distilled water as the control. Sprays were applied 3 times at 7-day intervals before harvest, when 40–50% of the true flowers on the spadix had fully...

Chilling injury symptoms

CI score increased during the whole storage at 4°C and the increase was delayed by pre and postharvest GABA treatment ($P < 0.01$; Fig. 1, Fig. 2). 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 ($P < 0.01$). On day 21, the CI score of anthurium cut flowers treated with preharvest 1 mM GABA and postharvest 5 mM GABA were 55.5% lower than control....

PLD and LOX enzymes activity

As shown in Table 1, Table ...

Discussion

Accompanying the development of CI in anthurium cut flowers, we observed a significant increase in PLD and LOX activities in flowers, indicating that activation of PLD and LOX might cause irreversible membrane damage and finally the occurrence of CI. GABA treatments significantly reduced the development of CI symptoms and decreased the activities of PLD and LOX during storage at 4°C. These results suggest that PLD and LOX might be involved in the induction of CI in anthurium cut flowers. Since...

Conclusion

In conclusion, the present study sheds light on beneficial impact of GABA treatment on reducing CI in anthurium cut flowers during low temperature storage. Our results suggest that the loss of membrane integrity, decrease in membrane lipid unsaturation, and increases in PLD and LOX activities may be involved in the development of CI in anthurium cut flowers. The reduction of CI in anthurium cut flowers by GABA treatment might due to maintenance of membrane integrity by (1) higher antioxidant...

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