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Hydrogen sulfide alleviates chilling injury of banana fruit by enhanced antioxidant system and proline content

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ABSTRACT

Hydrogen sulfide (H_2S) has been proven to act as an antioxidant in prolonging storage of postharvest fruits and vegetables, indicating that H_2S might have the potential to ameliorate chilling injury (CI). Effect of H_2S fumigation released from 0.5 mM H_2S donor (NaHS) on banana fruit during chilling storage followed by a ripening period was evaluated. Results demonstrated that H_2S fumigation maintained higher values of lightness, peel firmness and reduced accumulation of malondialdehyde. It was also found that H_2S promoted the phenylalanine ammonia lyase activity, total phenolics content and antioxidant capacity. Moreover, H_2O_2 and superoxide anion accumulation were reduced by H_2S with up-regulated activities of guaiacol peroxidase, superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase. Further investigation on proline metabolism showed that elevated proline content which resulted from increased Δ^1-pyrroline-5-carboxylate synthetase activity and decreased proline dehydrogenase activity, might be related to CI tolerance improvement. These data indicate that H_2S alleviates CI of banana fruit may through the enhancement of antioxidant system and proline content.

1. Introduction

Chilling injury (CI) occurs at low non-freezing temperatures, decreasing the quality and limiting the shelf-life of many plants and fruits. Dysfunction of cell membrane and excess production of reactive oxygen species (ROS) are two primary events involved in CI development (Ben-Amor et al., 1999; Chongchatuporn et al., 2013). Many applications were proposed to alleviate CI symptoms, such as methyl jasmonate and salicylic acid (Wang et al., 2006; Cao et al., 2010). These researches support us to speculate that approaches which will reduce ROS accumulation or enhance antioxidant enzyme activity or sustain the integrity of cell membrane might be applied to ameliorate CI.

Hydrogen sulfide (H_2S), traditionally thought as a phytotoxin, is now considered as the third major gaseous transmitter besides nitric oxide and carbon monoxide (Christou et al., 2013). It has been proven for many years that H_2S can be generated via enzymatic reactions in many plant species and it plays versatile roles in various physiological processes (Wilson et al., 1978; Winner et al., 1981). Moreover, H_2S acts as an antioxidative signaling molecule to cope with abiotic stress through reduction of ROS biosynthesis and promotion of antioxidative enzyme activity (Zhang et al., 2010; Christou et al., 2013). Interestingly, H_2S is also found as an antioxidant to maintain quality of postharvest fruits by elevating activities of antioxidative enzymes, like catalase (CAT) and ascorbate peroxidase (APX) (Hu et al., 2012, 2014). It seems that H_2S might have a comprehensive antioxidative role in plants and fruits. As Hu et al. (2014) pointed out that it was safe to treat fresh-cut pears with H_2S at low concentration. Thus, we speculated that H_2S could be applied to alleviate CI since oxidative damage is one of the main reasons stimulating CI development.

Proline accumulates under diverse stress in plants (Verbruggen and Hermans, 2008). As we know, proline can be synthesized from glutamate by Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and catalyzed by proline dehydrogenase (PDH). Recently, role of proline involved in CI occurrence has been evaluated and it is suggested that higher accumulation of proline content might enhance CI tolerance (Shang et al., 2011; Li et al., 2014a). Interestingly, it was also reported that, under salt stress, different H_2S donor had significantly different effects on proline content (Lisjak et al., 2013). However, effects of H_2S on proline metabolism during CI have not been illustrated. Therefore, an investigation of H_2S on changes of proline content associated with activities of P5CS and PDH in fruit during chilling storage may help us to better understand the physiological role of proline participating in CI.

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Banana fruit is highly susceptible to cold temperature and easy to develop CI symptoms including rapid peel browning, pulp rigidity and pitting (Chen et al., 2008). Several methods such as hot water and UV-C radiation have been put forward to reduce CI symptoms in banana fruit (Promyou et al., 2008; Pongprasert et al., 2011a). Nevertheless, the main focus of these studies was on the membrane integrity and limited attention was paid to antioxidant system. Considering the speculation and condition mentioned above, the aim of this study is to investigate the effect of H_2S fumigation on banana fruit CI, mainly focusing on the ROS accumulation and antioxidant system. Moreover, to the best of our knowledge, effect of H_2S on proline metabolism during chilling storage has not been evaluated.

2. Materials and methods

2.1. Banana fruit and treatments

Banana fruit (*Musa* spp., AAA group cv. 'Brazil', 70–80% maturity), which were growing under standard commercial conditions, were transported to the laboratory from a local commercial plantation in Hainan Province (geographical coordinates: N 18° 27′ 18″, E 109° 17′ 54″). Fruits were separated into fingers and sorted to eliminate surface defects or physical damage, and selected for uniform maturity and size. Finally, 360 fingers were divided randomly into 6 groups, each of 3 groups comprising three replicates (60 fingers for each replicate). In a preliminary study, banana fruit fumigated with different aqueous sodium hydrosulfide (NaHS) solution concentrations of 0.1, 0.3, 0.5, 0.7 and 1.0 mM before storage at 7 °C for 14 days were investigated to select an optimal H_2S releasing dosage for alleviating banana fruit CI. The results showed that higher concentration of NaHS (1.0 mM) brought some phytotoxic effects and 0.5 mM had better effects to promote chilling injury tolerance in banana fruit, notably with lowest chilling injury index and most brilliant appearance after ripening. Thereafter, the aqueous NaHS solution with 0.5 mM concentration was chosen for the later investigation. For H_2S fumigation, NaHS solution prepared with distilled water (pH 7.1) was placed at the bottom of a sealed 15 L container and banana fruits were exposed to the H_2S gas released for 24 h at 20 °C and 85–90% relative humidity. Water was set for control groups. After fumigation, fruits were placed in untight polyethylene bags and stored at 7 °C for 0, 7 and 14 days after which immersed in 0.1% ethephon for 1 min and stored at 20 °C for 6 days.

2.2. CI index, lightness and firmness

CI index was assessed according to Wang et al. (2013) as the following formula:

$$\text{CI index} = \sum \frac{(\text{CI scale} \times \text{fruit number at that scale})}{(\text{Fruit number in the group} \times 5)}.$$

For fruit color measurement, four different locations around equatorial region on each fruit peel were measured using a Chroma meter (KONICA MINOLTA, CR-400, Japan). Results were presented as L^* (lightness). Correspondingly, four different locations around equatorial region on each banana fruit peel were measured using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalmin, UK) incorporating a 5 mm diameter flat probe. Fruit were compressed 10 mm at a rate of 1.0 mm/s and the maximum force developed during the test was recorded and expressed as N/cm^2.

2.3. Ethylene production, electrolyte leakage, malondialhyde (MDA) content

For ethylene production determination, 5 fruit were incubated in a 2.0 L airtight jar for 1 h at 20 °C. One mL headspace gas was withdrawn and analyzed using flame ionization gas chromatography (SHIMADZU, GC-2014 C PF, Japan) on a 2000 × 3 mm column of aluminum oxide at 85 °C. Four or five replicates were conducted. Electrolyte leakage and MDA content were determined as described previously by Wang et al. (2013).

2.4. Total phenolics and antioxidant capacity assays

Approximately 1.0 g frozen banana peel was homogenized in 8 mL of methanol and extracted for 15 h in the dark and centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was used for measuring total phenolics content, ferric reducing antioxidant potential (FRAP) and 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity analyses. Total phenolics in banana peel were determined according to the Folin–Ciocalteu procedure (Singleton, 1985), results were expressed as mg gallic acid equivalents per gram fresh weight. The FRAP assay was measured according to the method reported by Xu et al. (2007) with slight modifications. 0.1 mL supernatant, which had been diluted with methanol to a suitable concentration, was added to 4.9 mL of the FRAP reagent. The absorbance at 593 nm was recorded after the mixture was stored at 37 °C for 10 min. Results were expressed as μmol trolox equivalents antioxidant capacity (TEAC), μmol/g FW. The DPPH radical scavenging activity was estimated as described by Cao et al. (2011).

2.5. Superoxide production determination

H_2O_2 content was measured by a modification of the method of Ferguson et al. (1983). Frozen banana peel tissues (1.0 g) were homogenized in 5 mL cold acetone and centrifuged at 12,000 × *g* for 10 min. $TiCl_4$ in HCl (10% (v/v) $TiCl_4$ in concentrated HCl, 0.1 mL) and concentrated ammonia water (0.2 mL) were added dropwise to 1.0 mL supernatant. After shaking and reacting for 5 min, the mixture was then centrifuged (12,000 × *g*) at 4 °C for 15 min and the precipitates washed repeatedly with cold acetone until the acetone was colorless. The precipitates were solubilized in 3 mL·2 M H_2SO_4 prior to measurement of absorbance at 415 nm. A standard curve of known concentrations of H_2O_2 was used to calculate the content of H_2O_2 in samples. Results were expressed as μmol/g FW.

To measure O_2^- production rate, the method of Xu et al. (2012) was applied with slight modifications. Frozen banana peel tissues (2.0 g) were homogenized in 8 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA) and centrifuged at 12,000 × *g* for 10 min. The incubation mixture contained 1 mL of the supernatant, 1 mL of 1.0 mM hydroxylammonium chloride and 1 mL of 50 mM potassium phosphate buffer (pH 7.0). After incubation at 25 °C for 1 h, 1 mL of 17 mM sulphanilic acid and 1 mL of 7 mM a-naphthyl amine were added to the incubation mixture. After reaction at 25 °C for 20 min, the absorbance was read at 530 nm. A standard curve with $NaNO_2$ was used to calculate the production rate of O_2^-. Twofold concentration of NO_2^- was regarded as concentration of O_2^- in samples. O_2^- production rate was expressed as nmol/min/g FW.

2.6. Enzyme activity evaluation

2.6.1. Superoxide dismutase (SOD), CAT, APX and glutathione reductase (GR) activity measurement

To get enzyme extracts, frozen banana peel tissues (2.0 g) were homogenized with 8 mL of 50 mM sodium phosphate buffer (PBS, pH 7.0, containing 0.1 mM EDTA) on ice and centrifuged at 12,000 × *g* for 10 min. SOD activity was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium according to Yang et al. (2011). CAT activity was measured according to Pongprasert et al. (2011b) with slight modifications. Briefly,