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Hydrogen gas prolongs the shelf life of kiwifruit by decreasing ethylene biosynthesis

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ABSTRACT

The application of hydrogen-rich water was recently shown to inhibit senescence process of kiwifruit, but it is unclear if hydrogen gas (H_2) exerts a similar function. The present results show that the production of endogenous H_2 in kiwifruit decreases during ripening. Fumigation with H_2 not only increased endogenous H_2 concentrations but delayed the softening and cell wall disassembly in flesh. Ethylene (C_2H_4) production was also inhibited by H_2, which was supported by a decrease in 1-aminocyclopropene-1-carboxylate (ACC) concentration, ACC synthase, and ACC oxidase activities, and the downregulation of the corresponding gene transcripts. Further, ACC-induced C_2H_4 production and ripening were blocked by H_2 and the incidence of natural decay and disease incidence were reduced. Overall, the results suggested that the H_2-induced delay of kiwifruit ripening resulted from an inhibitory effect on C_2H_4 biosynthesis.

1. Introduction

Kiwifruit is classified as a climacteric fruit with two widely known species: *Actinidia deliciosa* and *Actinidia chinensis*. In China, *A. deliciosa* 'Xuxiang' is known for its relatively large fruit size, green flesh, and good flavor (Li et al., 2017a), and has achieved commercially popularity. Despite these attractive fruit qualities, this cultivar is known for its rapid softening, which leads to a short shelf-life and greater susceptibility to fungal infection during ripening.

Ethylene (C_2H_4) is a key regulator of fruit senescence. For most climacteric fruits, C_2H_4 production continues at a slower rate during storage (Mworia et al., 2012). However, in kiwifruit, C_2H_4 production is almost zero at temperatures below 11–14 °C (Antunes et al., 2000); substantial amounts of C_2H_4 could only be detected after long periods of storage (Kim et al., 1999). Kiwifruit are highly sensitive to C_2H_4, and small amounts of C_2H_4 can accelerate fruit softening. As reported by Mcdonald and Harman (1982), 0.005–0.01 μL L^{-1} C_2H_4 could induce the ripening and softening of kiwifruit. The control of C_2H_4 level is therefore crucial in postharvest handling of kiwifruit. Some techniques, including temperature management and controlled atmosphere storage, are often applied to minimize the effects of C_2H_4 on kiwifruit (Hertog et al., 2016; Antunes and Sfakiotakis, 2002). 1-Methylcyclopropene (1-MCP), a potent inhibitor of ethylene perception, delays kiwifruit ripening during shelf life period (Koukounaras and Sfakiotakis, 2007). Several plant hormones, such as acetylsalicylic acid (Yin et al., 2013) and polyamines (Jhalegar et al., 2012), and certain gaseous molecules, including nitric oxide (NO) (Zhu et al., 2010) and ozone (Minas et al., 2014), also delayed the softening of kiwifruit stored at ambient temperature, mainly through the suppression of ethylene biosynthesis. These results indicate that the softening of kiwifruit at ambient temperature is dependent on C_2H_4.

Molecular hydrogen (H_2) has recently been proposed as a multifunctional signalling gas. In animals, this gas exerts beneficial effects on many physiological and pathological processes, including the protective effects of antioxidant activity, anti-inflammation, and antiapoptosis (Hong et al., 2010). Hence, it acts as a therapeutic agent in biomedical fields, clinical, and in experimental models of many diseases (Hong et al., 2010). In plants, older studies reported the evolution of H_2 in several species (Torres et al., 1984) and the seed germination of Winter rye is induced under an atmosphere of H_2 (Renwick et al., 1964). However, the physiological roles of H_2 have only recently been characterized. For example, by using hydrogen-rich water, H_2 was revealed to orchestrate a wide range of processes in plants, such as the regulation of stomatal closure (Jin et al., 2016) and root development (Zhu and Liao, 2016). H_2 was also proposed to be a broad-spectrum anti-stress molecule, because hydrogen-rich water confers protection against salt (Xu et al., 2013), drought (Xie et al., 2014), paraquat (Jin et al., 2013), and heavy metal stresses (Chen et al., 2014). These results indicate that

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the protective function of H_2 is mainly dependent on the increased antioxidant ability exerted through various regulatory mechanisms. Previous work also suggested that hydrogen-rich water attenuated the senescence of kiwifruit through a decrease in oxidative damage (Hu et al., 2014). The possible role of hypoxia in the abovementioned actions conferred by hydrogen-rich water could not easily be excluded (Xie et al., 2014).

To gain a better understanding of the role of H_2 in increasing the shelf life of kiwifruit, fruit were fumigated with H_2 gas. Endogenous H_2, the relationship between H_2 and C_2H_4, and disease incidence during ripening of kiwifruit were examined. The results reveal a new functional mechanism of H_2 when acting as a positive regulator of the ripening process in kiwifruit by decreasing C_2H_4 production.

2. Materials and methods

2.1. Plant materials

Kiwifruit (*Actinidia deliciosa* cv. 'Xuxiang') were obtained from a commercial orchard (33°59′–34°19′N, 107°39′–108°00′E) in Mei County, Baoji City, Shaanxi Province, China. Fruit used for experiment I were picked on October 30, 2015. At this stage, the initial firmness was 40–60 N. The fumigation method of H_2 in experiment I was repeated in experiment II and III in the following season, with except that harvest occurred on October 20, 2016. At this stage, the initial firmness was 50–70 N. The fruit used for experiments were selected by uniform size, shape, and the absence of mechanical injuries and diseases.

2.2. Experimental design

2.2.1. Experiment I: effects of fumigation with different concentrations of H_2

In total, 660 kiwifruit were selected for the experiment and divided into 11 treatment groups with 60 fruit in each group. The fruit were fumigated with different concentrations of H_2 (0, 2.25, 4.5, and 45 μL L^{-1}) in a sealed plastic container (21 L, Lock & Lock). Different H_2 concentrations were obtained from a cylinder that contained 1000 ± 50 μL L^{-1} H_2 in nitrogen (Nanjing Special Gas Factory Co., Ltd. Nanjing, China), and injected into the container by a syringe through an injection port on the container. Fruit were fumigated in an atmosphere containing H_2 for 24 h at ambient temperature (25 ± 1 °C). Control fruit were placed in a similar container for an identical incubation duration without any H_2. During fumigation, the accumulated CO_2 in each chamber was absorbed by 15 g CO_2 absorbent (Calcium hydroxide, purchased from Shanghai Guang shao Metallurgical Technology Co., Ltd. Shanghai, China).

To optimize the treatment conditions, given the above restraints, the effects of two different treatment modes [continuous fumigation for 24 h (with 24 h defined as a one-stage treatment) and continuous fumigation for 12 h, treated again, and continuous fumigation for another 12 h (12 h + 12 h, defined as a two-stage treatment)] on the quality of the fruit were simultaneously compared.

Fumigation with 1.0 μL L^{-1} 1-methylcyclopropene (1-MCP) was used as the positive control, owing to its established beneficial effect in delaying the senescence of kiwifruit (Koukounaras and Sfakiotakis, 2007), and exogenous C_2H_4 fumigation treatment at different concentrations (0.5, 1.0, and 1.5 μL L^{-1}) was used as the negative control. The concentration of required exogenous ethylene was obtained through the injection of an appropriate amount of pure ethylene (99.99%, Nanjing Special Gas Factory Co., Ltd. Nanjing, China) into the experimental container. After the gas within the chamber was circulated using a small fan, the ethylene concentration inside the container was determined by gas chromatography (Li et al., 2017b).

All treated fruit were stored at 25 ± 1 °C. After a shelf life of 9 d, 60 fruit subjected to each treatment (three replicates of 20 fruit each) were sampled, and flesh firmness was determined to choose the most suitable concentration and mode of H_2 gas treatment.

2.2.2. Experiment II: effects of postharvest H_2 fumigation on C_2H_4 biosynthesis and kiwifruit quality

To confirm the roles of H_2 on kiwifruit C_2H_4 biosynthesis, a separate experiment was conducted. As described in experiment I, fruit were exposed to 4.5 μL L^{-1} H_2 (two-stage, based on above result) in an air-tight 21-L chamber for 24 h at 25 ± 1 °C. Fruit sealed in the same containers for 24 h were used as the control. After treatments, the fruit were stored at 25 ± 1 °C to allow ripening to proceed. At 3 d intervals, 36 fruit from each treatment (each replicate contained 12 fruit) were randomly sampled and stored at −70 °C prior to various experimental assays, and fresh samples were used for the determination of firmness, C_2H_4 production, and cellular structure.

2.2.3. Experiment III: treatment of exogenous C_2H_4 (ACC) or H_2, and co-treatment of C_2H_4 + H_2 and ACC + H_2

The combination of C_2H_4 + H_2: Fruit were sealed in air-tight containers for 24 h and exposed to H_2 (4.5 μL L^{-1}, two-stage), C_2H_4 (1.0 μL L^{-1}), or a co-treatment of C_2H_4 (1.0 μL L^{-1}) + H_2 (4.5 μL L^{-1}, two-stage). Control fruit were placed in a similar container for an identical incubation duration in the absence of H_2 or C_2H_4, as described in experiment I.

The combination of ACC + H_2: Fruit were submerged in 5 mmol L^{-1} ACC and distilled water (control) for 5 min and the fruit were air-dried. The combined treatment consisted of fruit that were either treated by ACC or distilled water and fumigated with H_2.

Subsequently, fruit were stored at 25 ± 1 °C and 85–90% relative humidity (Testo 177H1, Germany) for 9 d to simulate the ripening behavior during shelf life. At 3 d intervals, 36 fruit from each treatment (each replicate contained 12 fruit) were randomly sampled and used for the determination of C_2H_4 production and flesh firmness.

2.3. H_2 production

The endogenous H_2 level from fresh pulp tissue was determined by a previously described chromatographic system (Gas chromatography 7890, Agilent Technologies Inc., USA) (Jin et al., 2013), with minor modifications. The gas chromatography was equipped with a thermal conductivity detector (TCD) and a column containing a Molecular Sieve 5 Å stationary phase. Fresh pulp samples were pulverized in liquid nitrogen by using a cryomill (IKA A11, Germany). Approximately 0.5 g of the frozen powder was homogenized with 7 mL distilled water for 2 min and placed in a vial; 5 μL octanol and 0.5 mL 5 mol L^{-1} H_2SO_4 were added. Afterwards, pure N_2 gas (99.99%) was bubbled into the vial by a nitrogen blowing instrument (An Jian Beijing Co., Ltd. Beijing, China) to fully displace the air and the vial was capped and shaken vigorously for 2 min. Subsequently, the vial was heated at 70 °C for 1 h to liberate H_2 from plant tissues and allowed to cool at room temperature before the analysis of a 100-μL sample of the headspace gas.

2.4. Firmness

Flesh firmness was determined on two opposite sides at the equator of each fruit with a TMS-Touch texture analyzer fitted with 10-mm flat probe (Food Technology Corporation, USA). The compression test was performed by using a trigger force of 0.7 N, a test speed of 1 mm s^{-1}, and a penetration depth of 8 mm.

2.5. Transmission electron microscopy (TEM)

The morphological examination of flesh tissues by TEM was conducted as described by Hu et al. (2014).