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Effects of acetylsalicylic acid on kiwifruit ethylene biosynthesis and signaling components

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ABSTRACT

The effects of acetylsalicylic acid (ASA) on kiwifruit (*Actinidia deliciosa* cvs Bruno and Hayward) ethylene biosynthesis and signaling were investigated. Exogenous application of ASA inhibited ethylene production in both whole fruit, and in vitro with flesh discs, and enzymes associated with ethylene biosynthesis (ACS and ACO). The effect of ASA treatment on kiwifruit softening was relatively weak. Combination treatments also had inhibitory effects on fruit ripening, with ASA + C_2H_4 more effective than C_2H_4 + ASA. In order to evaluate the effects of ASA on ethylene signaling, twenty-four ethylene signaling components (five ethylene receptors, two *CTR1* like genes, four EIN3-like genes and thirteen *ERF* genes) were analyzed at the transcriptional level. The results indicated that ASA treatment generally inhibited ethylene-induced modulation of ethylene receptor genes, and had little effect on softening-related ethylene signaling components, which suggested that ASA inhibits fruit ripening mainly by interfering directly with ethylene biosynthesis and perception. In addition, the ethylene response factors *AdERF1*, *AdERF3* and *AdERF12* were characterized as ASA-responsive genes, and their roles in fruit stress response are also discussed.

1. Introduction

Salicylic acid (SA) is associated mainly with plant responses to biotic and abiotic stresses (Loake and Grant, 2007; Peleg and Blumwald, 2011), and aspects of plant growth and development such as seed germination, seedling and cell growth, and senescence (Vlot et al., 2009). In fruit, SA influences fruit yield, aroma, antioxidant capacity, postharvest pathogen resistance, and chilling injury (Xu and Tian, 2008; Vlot et al., 2009; Tieman et al., 2010; Luo et al., 2011; Sayyari et al., 2011). SA can also affect fruit ripening and senescence (Asghari and Aghdam, 2010), and consequently SA, and its derivatives acetylsalicylic acid (ASA) and methyl salicylate (MeSA), have been applied to various fruit species to manipulate the ripening process (Li et al., 2010). For instance, SA has been shown to inhibit banana fruit ripening and softening (Srivastava and Dwivedi, 2000).

Ripening inhibition by SA, or its derivatives, may be due to inhibition of ethylene synthesis, as SA and ASA have both been shown to inhibit ethylene production in pear cells and tissue discs, apple discs and carrot cell cultures, and in strawberry fruit (Leslie and Romani, 1986, 1988; Romani et al., 1989; Roustan et al., 1990; Babalar et al., 2007). In tomato fruit, SA has been shown to inhibit wound-inducible ACC synthase (Li et al., 1992), which is responsible for the increase in ethylene production following wounding. The effect of SA on fruit ripening extends beyond inhibition of ethylene synthesis, however, to include fruit softening and texture change. For instance, ASA applied as a postharvest treatment to loquat fruit, reduced the lignin content of the fruit flesh and delayed the characteristic increase in loquat fruit firmness (Cai et al., 2006).

SA has been associated with ethylene signaling. For example, SA and ethylene coordinate plant responses to pathogens (Devadas et al., 2002), and many ethylene signaling components, such as tomato *Pti4* (Gu et al., 2000), and apple *MdETR1*, *MdERS1* and *MdCTR1* (Li et al., 2006), are transcriptionally regulated by SA treatment. All these data suggest that the effect of SA on fruit ripening might involve regulation of genes associated with ethylene signaling. Although the effects of SA on delaying fruit ripening, softening and lignification have been confirmed in several fruit species, there is still a lack of information about the molecular mechanism of action of SA on ripening fruit and on the modulation of ethylene signaling components.

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Kiwifruit is a typical climacteric fruit, with an ethylene burst during postharvest ripening (Yin et al., 2008). It is very sensitive to ethylene, and extremely low concentrations (0.1 $\mu L\,L^{-1}$) can stimulate fruit ripening and softening (McDonald and Harman, 1982). A range of conventional postharvest treatments has been successfully applied to kiwifruit to delay ripening, including 1-MCP, low temperature, controlled atmospheres, NO, etc. (McDonald and Harman, 1982; Bauchot et al., 1999; Boquete et al., 2004; Koukounaras and Sfakiotakis, 2007; Yin et al., 2008, 2009; Zhu et al., 2010; Mworia et al., 2011). We have found previously that ASA could also delay kiwifruit softening in some treatments, and this was accompanied by reductions in activity of LOX, ACC synthase, ACC oxidase and ethylene production (Zhang et al., 2003). We also observed that ASA could inhibit ethylene production from flesh discs of 'Bruno' kiwifruit (Zhang et al., 2002). Similar effects have also been observed with MeSA vapor treatment of kiwifruit (Aghdam et al., 2010). However, the antagonistic interaction between ASA and exogenous ethylene was not studied, and the effects of ASA treatment on ethylene perception and signaling have not yet been reported.

In the present research, ASA was applied both to whole fruit (cv. Bruno) and fruit flesh discs (cvs. Bruno and Hayward), with the purpose of analyzing its effect on kiwifruit ethylene production and ripening. In order to explore the relationship between ASA treatment and ethylene action, the transcriptional responses of twenty-eight ethylene signaling components (*AdETR*, *AdCTR*, *AdEIL* and *AdERF*) were analyzed in ASA-treated 'Hayward' kiwifruit discs.

2. Materials and methods

2.1. Plant material and treatments

2.1.1. ASA, ethylene and combined treatments on whole 'Bruno' fruit

Kiwifruit (*Actinidia deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Bruno) were harvested from a commercial orchard in Wuyi, Zhejiang, China. Fruit of uniform size were selected, free of mechanical damage and decay, and divided into six batches of about 270 fruit each. The first (C_2H_4) and second (C_2H_4 + ASA) batches were exposed to C_2H_4 (100 $\mu L\,L^{-1}$ for 12 h) in an airtight 360 L chamber at 20 °C. After treatment, the first batch of fruit was stored at 20 °C, while the second was submerged in 1.0 mM ASA (pH 3.5) for 5 min, after which the fruit were air-dried and stored at 20 °C. The third (ASA) and fourth (ASA + C_2H_4) batches were first treated with ASA, as described above, and then either transferred directly to 20 °C or treated with C_2H_4 (100 $\mu L\,L^{-1}$) for 12 h. The fifth and sixth batches of fruit were both controls, with the fifth batch submerged in water (pH 3.5, acidified with HCl), for 5 min, and the sixth sealed in air-tight containers for 12 h. After treatments, all of the fruit were stored at 20 °C to allow ripening (softening) to proceed. At each sampling point, fruit flesh (without skin, core and seeds) were frozen in liquid nitrogen and stored at −80 °C for further use.

2.1.2. ASA treatment of 'Hayward' kiwifruit flesh discs

In order to confirm the roles of ASA on kiwifruit ethylene production, a separate experiment was performed on 'Hayward' kiwifruit flesh discs. The fruit were harvested from The Plant & Food Research Institute orchard at Te Puke, New Zealand, in the 2008 fruit season, as described in our previous report (Yin et al., 2012a). The preparation of flesh discs (no skin, seeds or core; 12 mm diameter, 2 mm thickness) was as described in our previous report (Zhang et al., 2009). Mannitol solution (0.4 M) was used as the medium, supplemented with 1.0 mM ASA (pH 3.5) or acidified with HCl to pH 3.5 (control). The effects of a single ASA treatment on ethylene production were repeated with discs from two different stages of fruit ripening, with firmnesses of approximately 70 N and 50 N.

For each experiment, about 420 discs were prepared and gently mixed, and then separated into 7 groups associated with 7 sampling points (as described in Table 1). Each group consisted of 60 discs, separated into 3 replicates of 20 discs each and were cultured in 100 mL bottles (Duran, filled with 40 mL solution). During the treatment, the bottles were placed on a shaker (23 g) and incubated at 28 °C for 1 h, 6 h and 12 h, separately. After treatment/incubation, the discs were dried with filter paper, and 10 discs were frozen in liquid nitrogen, while the other 10 discs were used for ethylene production measurements.

2.2. Fruit evaluation

2.2.1. Ethylene production

For whole fruit ('Bruno') experiments, six fruit were separated into three replicates of two fruit each, and were sealed in 500 mL rubber-topped flasks for 1 h at 20 °C. One mL of head space gas was removed from each flask, and the ethylene was analyzed with a gas chromatograph model SP 6800 (Lunan Chemical Engineering Instrument, Shandong, China) as described in Yin et al. (2012b).

For the disc experiments, the paper-dried discs (as described above) were sealed in 15 mL tubes with rubber stoppers for 30 min. Then 1 mL of gas was removed from each tube using a syringe, and ethylene production was measured with a gas chromatograph (Philips, UNICAM pro. GC) fitted with 1.5 m × 4 mm glass Alumina column. The injector, detector, and oven temperatures were 130, 130, and 200 °C, respectively.

2.2.2. Fruit firmness

Firmness was measured, using a hand-held penetrometer (Effegi or GY-1), on each of 10 fruit at two points separated by 90° at the equator of each individual fruit, after removal of a 1 mm thick slice of peel.

2.2.3. ACC content, ACC synthase and ACC oxidase

In order to estimate ASA effects on ethylene production, changes in concentrations of 1-aminocyclopropane-1-carboxylic acid (ACC), activities of ACC synthase (ACS) and ACC oxidase (ACO) were measured. ACC was extracted with 6 mL ethanol from fruit flesh (3 g), and the measurements were made according to the method of

Table 1
Effects of 0.5 mM ASA on ethylene production of 'Hayward' kiwifruit flesh discs.

Whole fruit		Disc						
Firmness (*N*)	Ethylene (ng kg^{-1} s^{-1})	Ethylene (ng kg^{-1} s^{-1})						
		0 h	CK-1 h[a]	ASA-1 h	CK-6 h	ASA-6 h	CK-12 h	ASA-12 h
73 ± 0.50[b]	N/A[c]	N/A	5 ± 0.1	N/A	72 ± 5.0	N/A	433 ± 16.7	7 ± 1.3
51 ± 1.11	N/A	N/A	3 ± 0.2	N/A	66 ± 4.6	N/A	373 ± 28.8	1 ± 1.0

[a] CK, control.
[b] n = 10 and 3 for firmness and ethylene respectively.
[c] N/A, non detectable.