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Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage

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Abstract

The effects of nitric oxide (NO) on ethylene biosynthesis and lipoxygenase (LOX) activity in a climacteric peach fruit (*Prunus persica* (L.) Batsch., cv. Feicheng) were studied. It was observed that, in peaches treated with 5 and 10 $\mu l\,l^{-1}$ NO, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity, ethylene production and LOX activity were reduced. This led to the accumulation of ACC and 1-malonyl aminocyclopropane-1-carboxylic acid (MACC) during storage. There was no evidence that ACC synthase activity was affected significantly by any concentration of NO. A plausible mechanism is proposed that NO is bound to ACC oxidase to form an ACC oxidase–NO complex, which is chelated by ACC to produce an ACC–ACC oxidase–NO complex, leading to a decrease in ethylene production. The increase in concentration of ACC in NO-treated peaches may result in the redirection of ethylene to MACC production. This is a secondary effect of NO.

Keywords: ACC; ACC oxidase; Ethylene biosynthesis; Lipoxygenase; Nitric oxide; Peach fruit

1. Introduction

'Feicheng' peach is a traditional speciality cultivar in China, with a large demand from both domestic and foreign markets. However, its rapid softening and browning after harvest lead to losses in the marketing chain. Storage strategies, such as modified atmosphere storage, intermittent warming and delayed storage, have been developed to aid in slowing the ripening process (Wright and Kader, 1997; Zhou et al., 2000, 2002). Some plant growth regulators, such as aminoethoxyvinylglycine and gibberellic acid, have been used to inhibit fruit ripening and softening during storage (Zilkah et al., 1997; Ju et al., 1999). However, the storage life of peaches is limited by storage disorders and loss of quality (Fernández-Trujillo et al., 1998; Zhou et al., 2000).

Ethylene is involved in storage disorders of peach (Dong et al., 2001; Zhou et al., 2001), and fruit softening is partially controlled by ethylene; application of exogenous ethylene to fruit causes faster softening (Abdi et al., 1997). In recent years, 1-methylcyclopropene (1-MCP) has been used as an ethylene action inhibitor to delay ripening and to improve the postharvest quality of apricots, plums (Dong et al., 2002), nectarines (Dong et al., 2001; Liguori et al., 2004) and peaches (Liguori et al., 2004). However, it also caused flesh browning in apricots (Dong et al., 2002) and peaches (Fan et al., 2002) and increased storage flesh disorders in nectarines (Dong et al., 2001).

At the end of the 1990s, nitric oxide (NO) was proven to be an endogenetic and bioactive molecule that takes part in normal physiological processes in animals (Palmer et al., 1987); it was subsequently shown to play a crucial role in the regulation of normal plant physiological processes, including stomatal closure, growth and development (Neill et al., 2002; Guo et al., 2003; Pagnussat et al., 2003). Some previous work has demonstrated that NO could delay ripening and improve the postharvest quality of strawberries (Wills et al., 2000; Zhu and Zhou, 2005), avocados (Leshem and Pinchasov, 2000) and carnations (Bowyer et al., 2003), when applied as short-term fumigation at low concentrations. Although it is suggested that NO might exert a profound influence on fruit by inhibiting ethylene production (Leshem, 2000),

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the mechanism by which NO effects this process is still not clear.

Recently, the effects of combining NO with ACC oxidase in vitro were reported (Tierney et al., 2005), although the effect of NO on ethylene biosynthesis was not explained. In the present work, we fumigated 'Feicheng' peach fruit with different concentrations of NO, and determined the relevant parameters of ethylene biosynthesis for the treated peach fruit during storage. Our objectives were to disclose the possible path through which NO affects ethylene biosynthesis in 'Feicheng' peaches.

2. Materials and methods

2.1. Plant materials

Peach fruit (*Prunus persica* (L.) Batsch, cv. Feicheng) were picked from trees growing in Feicheng, Shandong, in the summer of 2004 at a pre-climacteric, but physiologically mature, stage. They were selected for uniformity of size and ground color, and freedom from defects and mechanical damage. The experiments were carried out at two temperatures (5 and 25 °C). Four treatments of NO (0, 5, 10 and 15 $\mu l\,l^{-1}$) were assessed. There were 20 units of peaches in each treatment at each temperature. One unit comprised 20 peaches. The fruit were placed in sealed 30 l containers, and the containers were put under vacuum and flushed with nitrogen gas to displace all the oxygen. NO was then injected into the containers with nitrogen gas as a carrier to keep the concentrations of NO at 5, 10 and 15 $\mu l\,l^{-1}$. The control peaches were also flushed with nitrogen gas but without NO. After 3 h of exposure to NO, the controls and all of the peaches that were treated with concentrations of NO were divided into two groups for storage in unsealed bags. One group of fruit was placed immediately at 5 °C and the other was held at 25 °C. The following measurements were taken with the newly picked peaches before treatment. These results were expressed as time point 0 day.

2.2. Firmness

Firmness was determined using a Hunter-Spring penetrometer that was fitted with an 8 mm long probe with a diameter of 11 mm. Two measurements were taken from the mesocarp without the skin, on the opposite faces of each fruit; the results were expressed as the mean ± S.E. of the determinations made from 10 replications. The unit of measurement was $N\,cm^{-2}$.

2.3. Soluble solids contents

Soluble solids contents were measured twice in each fruit using a refractometer WYT-4 (Shanghai cany precision instrument Co. Ltd., China). The results were expressed as the mean ± S.E. of the determinations made from 10 replications.

2.4. Measurement of ethylene production

Ethylene production was determined as follows: 25 fruit from each concentration of NO treatment were selected randomly, and lots of 5 fruit were sealed in a 10 l chamber. The chamber was sealed with a rubber septum for 2 h at room temperature prior to ethylene sampling. The five chambers were regarded as five concentration replicates. A 5 ml sample of the headspace gas was withdrawn using a gas-tight syringe from each chamber through the septum stopper, and injected into a gas chromatograph (GC-9A, Shimadzu, Japan) that was equipped with a GDX-502 column and a flame ionization detector (FID). The column temperature was 70 °C and the injection temperature was 120 °C. The carrier gas was N_2 with a rate of 40 ml min^{-1}. The results were expressed as the average ethylene production of peaches treated at each concentration of NO. The rate of ethylene production was expressed as nmol $C_2H_4\,g^{-1}\,FW\,h^{-1}$. Following ethylene measurements, five fruit from each treatment were assessed randomly for the following enzyme activity measurements.

2.5. Measurement of ACC synthase activity

Extracts for ACC synthase activity assays were prepared according to the method of Boller et al. (1979), with slight modifications. Mesocarps (2 g FW) were homogenized in 4 ml of extraction buffer consisting of 100 $mmol\,l^{-1}$ Hepes buffer (pH 8.5), 0.5 $\mu mol\,l^{-1}$ pyridoxal phosphate (PLP) and 10 $mmol\,l^{-1}$ EDTA in the presence of 4 $mmol\,l^{-1}$ dithiothreitol (DTT). After centrifugation of the homogenate at 25,000 × *g* for 20 min, the supernatant was passed through a 0.45 μm membrane filter and eluted with 4 ml of 2 $mmol\,l^{-1}$ Hepes buffer (pH 8.5) containing 0.2 $\mu mol\,l^{-1}$ PLP and 0.1 $mmol\,l^{-1}$ DTT. The eluents were used for enzyme assays. All steps in the enzyme extraction were performed at 4 °C. ACC synthase activity was assayed in a reaction mixture containing 0.5 ml of 60 $\mu mol\,l^{-1}$ *S*-adenosylmethionine and 1 ml of enzyme extract. After incubation of the reaction mixture at 30 °C for 2 h, the amount of ACC formed was determined by the method of Lizada and Yang (1979). The determination was made from five replications using five different fruit.

2.6. Measurements of ACC and MACC contents

Peach mesocarp tissue (5 g FW) was homogenized with 0.5 g insoluble polyvinylpyrrolidone in 10 ml of 80% ethanol at 4 °C and centrifuged at 13,000 × *g* for 25 min. The supernatant was evaporated under vacuum at 50 °C. Residues were dissolved in 5 ml of distilled water and 2.5 ml of chloroform. After being stored at 4 °C overnight, 1 ml of aqueous solution was taken and free ACC contents were measured by the method of Lizada and Yang (1979). A total of 400 μl of aqueous solution was hydrolyzed with 100 μl of 12 $mol\,l^{-1}$ HCl at 100 °C for 3 h. The hydrolyzed sample was neutralized with NaOH and used for quantification of total ACC. These determinations were made from five replications using five