RESEARCH NOTE

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Research Note

Effects of auxin and jasmonates on 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase gene expression during ripening of apple fruit

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ABSTRACT

1-Aminocyclopropane-1-carboxylate (ACC) synthase and oxidase activities, their gene expression, and ethylene production in apple fruit [*Malus sylvestris* (L.) Mill. Var. *domestica* (Borkh.) Mansf.] treated with a synthetic auxin 2,4-dichlorophenoxy-propionic acid (2,4-DP) and *n*-propyl dihydrojasmonate (PDJ), a jasmonic acid derivative, has been investigated to clarify the action of auxin and jasmonates on ethylene production. The fruit was harvested at 103 d after full bloom (preclimacteric). The expression of *MdACS4* messenger RNA (mRNA) at 48 and 96 h after treatment was higher in fruit treated with 2,4-DP than in the untreated control, but those of *MdACS1* and *MdACO1* were not affected by treatment. The ethylene production in 2,4-DP-treated fruit increased at 96 h after treatment. In contrast, expression of mRNAs hybridized with *MdACS1* and *MdACO1* probes in the skin of PDJ-treated fruit were higher than those in the untreated control. In addition, ACC synthase activity and ethylene production also increased after treatment. These results show that the ethylene production rate may differ with the kind of genes which were stimulated by auxin or jasmonates.

1. Introduction

Plant hormone substances have been used in cultural practice of apples to improve fruit quality. For instance, the synthetic auxin 2,4-dichlorophenoxy-propionic acid (2,4-DP) has been used to prevent pre-harvest drop in apples, and *n*-propyl dihydrojasmonate (PDJ), an *n*-propyl ester rather than a methyl ester jasmonic acid (JA) derivative, has been used to promote apple coloration. Generally in the field, 2,4-DP or PDJ has been applied 20 d before harvest (100 d after full bloom: DAFB). Endogenous ethylene and jasmonates change greatly around this time. In a previous study on 'Tsugaru' apples (Kondo et al., 2000), we showed that the endogenous jasmonate concentrations increased at 120 DAFB, which is also the commercial harvest date of this cultivar. Phytohormones have a correlation with each other, that is, 2,4-DP or PDJ application before harvest increases ethylene production in fruit and promotes fruit ripening (Kondo and Hayata, 1995; Kondo et al., 2007). The mechanism of this correlation should be clarified, because phytohormone application may influence fruit storability. 1-Aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase can regulate ethylene production. Auxin influences ACC synthase in the ethylene pathway, but the mRNA levels increased by the application differed among fruit (Ishiki et al., 2000; Coenen et al., 2003). Furthermore, the effect of PDJ on ACC synthase and ACC oxidase genes is unclear in apples.

We investigated the effects of 2,4-DP or PDJ application before harvest on the transcription of ACC synthase and ACC oxidase genes, their activities, ACC and 1-malonylamino cyclopropane-1-carboxylic acid (MACC) concentrations, and ethylene production in apples.

2. Materials and methods

2,4-Dichlorophenoxy-propionic acid (a.i. 10.0%) was purchased from Nissan Chemical Industries, Ltd. (Tokyo). *n*-Propyl dihydrojasmonate was a gift from Nippon Zeon Co. (Tokyo). 1-Aminocyclopropane-1-carboxylic acid was obtained from Sigma–Aldrich Co. (Milwaukee, WI, USA).

Ten randomly selected 8-year-old 'Tsugaru' apple trees [*Malus sylvestris* (L.) Mill. Var *domestica* (Borkh.) Mansf.] growing in an open field at the Prefectural University of Hiroshima, on Malling 9 (M. 9 EMLA) rootstocks, were used in this study. Each tree was trained as

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a central leader, and the trees were planted in a single row from east to west with a spacing of 2.5 m × 4.0 m. The fruit were harvested at 103 DAFB at a preclimacteric stage as shown in Fig. 1. Immediately after harvest, the fruit were randomly divided into three groups. In the first group, untreated control fruit, were dipped for 5 min in a deionized water solution containing 0.1% (v/v) Approach BI® (Kao, Osaka, Japan) only. In the second group, fruit were dipped for 5 min in a deionized water solution containing 0.1% (v/v) Approach BI® and 45 μL L^{-1} 2,4-DP. In the third group, fruit were dipped for 5 min in a deionized water solution containing 0.1% (v/v) Approach BI® and 0.39 mM PDJ. Fruit were air-dried after treatment and placed in the dark at 25 °C. Thirty fruit were sampled at 6, 24, 48, and 96 h after treatment. Skin samples were separated carefully with a knife, frozen immediately in liquid nitrogen, and then stored at −80 °C until analysis. The skin sample should be considered as the epidermal tissues of fruit because a small amount of flesh remained on the skin.

The activities of ACC synthase and ACC oxidase, and the concentrations of ACC, MACC, in the skin samples were analyzed as previously reported (Kondo et al., 2005, 2007). Ethylene production was measured by gas chromatography with flame ionization

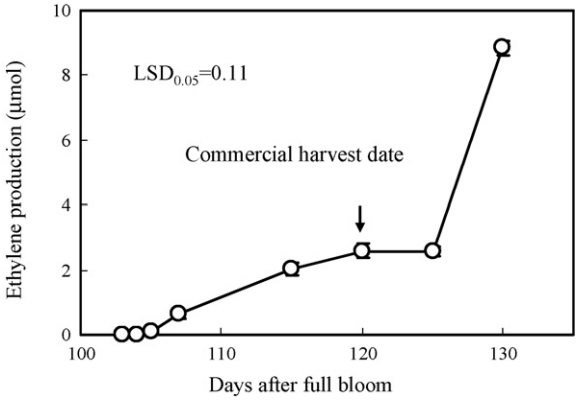

Fig. 1. Ethylene production in 'Tsugaru' apple fruit. Ethylene production in untreated control fruit on each harvest date was measured immediately after the harvest. Data are the means of five fruit.

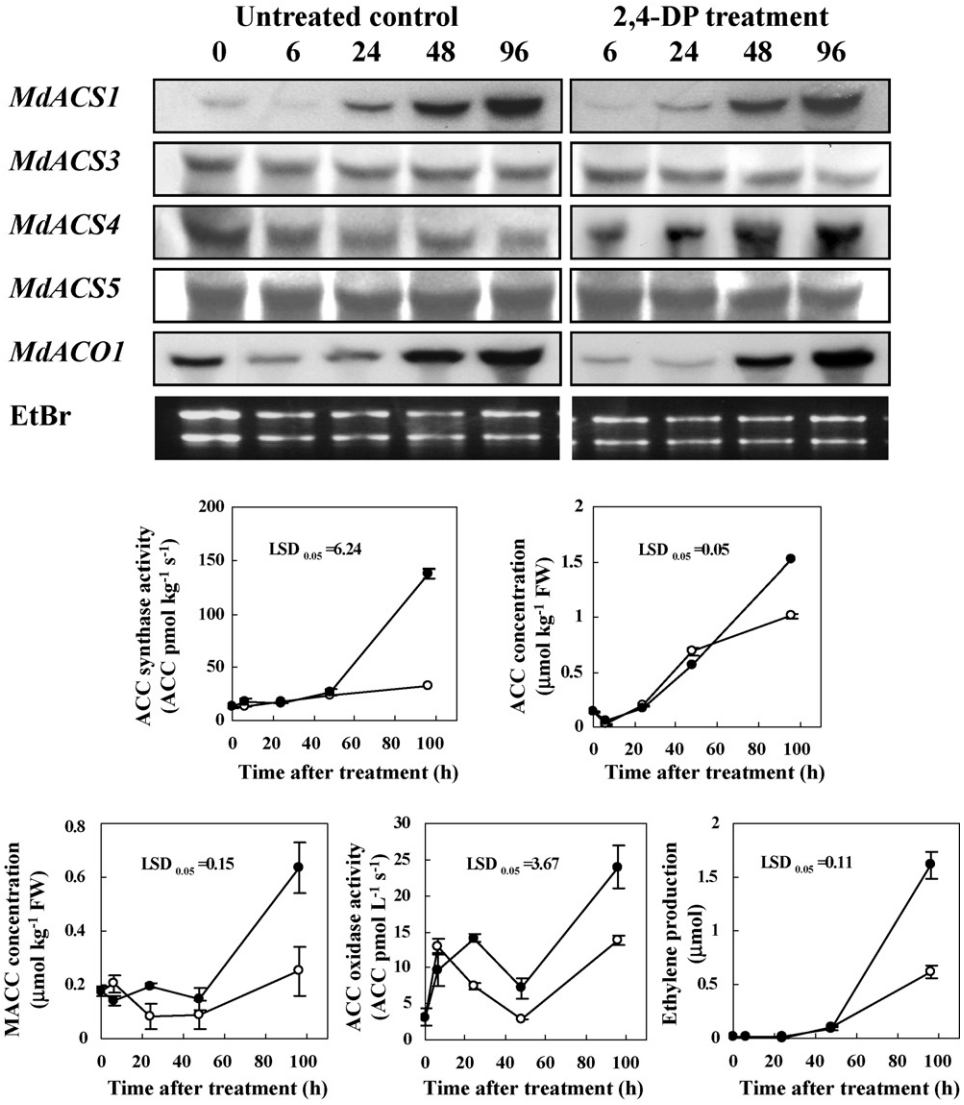

Fig. 2. Northern blots, 1-aminocyclopropane-1-carboxylate (ACC) synthase activity, ACC, and 1-malonylaminocyclopropane-1-carboxylic acid (MACC) concentrations, ACC oxidase activity, and ethylene production in apple fruit skin at preclimacteric stage. The analysis was repeated three times. The bottom panel shows the ethidium bromide (EtBr)-stained gel as a loading gel. (○) Untreated control; (●) 2,4-DP treatment.