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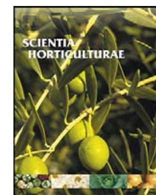

Methyl jasmonate plays a role in fruit ripening of 'Pajaro' strawberry through stimulation of ethylene biosynthesis

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The role of methyl jasmonate (MJ) in strawberry (*Fragaria* × *ananassa* Duch. cv Pajaro) fruit ripening was investigated by monitoring its endogenous concentrations in fruit at various stages of development and the effects of exogenously applied MJ at these stages on ethylene biosynthesis. The concentration of endogenous *trans*-MJ was significantly higher in the white fruit (31.7–162.2 ng g^{-1}) and decreased sharply in half and fully ripe fruit. Higher concentrations of endogenous *trans*-MJ at the white stage of strawberry fruit development followed by a decline during fruit ripening indicate that MJ may play an important role in modulating fruit ripening. Significantly increased ethylene production was measured in the fruit when MJ was applied at white, half ripe and at fully ripe stage. The application of MJ (50 μM) resulted in significantly highest ethylene production and increased activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase as compared to all other treatments. The effect of exogenously applied MJ on ethylene production, ACC synthase and ACC oxidase activities was dependent on concentration of MJ applied and on fruit developmental stage. In conclusion, MJ in strawberry modulates fruit ripening, as its concentration is higher in white fruit and is declined with the progression of ripening and exogenous application of MJ increases ethylene production, activities of ACC oxidase and ACC synthase depending upon the concentration of MJ applied and fruit developmental stage.

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1. Introduction

Jasmonic acid (JA) and its methyl ester (methyl jasmonate), are cyclopentanone compounds and are regarded as naturally occurring plant growth regulators (Sembner and Parthier, 1993; Fan et al., 1998). Jasmonic acid and MJ are present in low concentration in various plant parts including buds, shoots, leaves, flowers, fruits, and seeds (Meyer et al., 1984) and largest amount in fruits. MJ has been reported to modulate chlorophyll degradation and anthocyanin formation (Creelman and Mullet, 1997; Perez et al., 1997), aroma development (Olias et al., 1992), and ethylene production (Lalel et al., 2003; Khan and Singh, 2007; Kondo et al., 2007). In apples [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.], the concentration of endogenous MJ has been reported to be low at the initial stages of fruit development followed by general increase toward harvest (Kondo et al., 2000). Likewise, Lalel et al. (2003) reported that the concentration of *trans*-MJ in the pulp of mango (*Mangifera indica* L.) fruit was higher at harvest and decreased as the ripening progressed. But endogenous MJ in non-climacteric fruits has been reported to be higher at the immature stage and steadily decreasing during fruit development such as strawberry (Gansser et al., 1997), sweet cherries (*Prunus avium* L.) (Kondo et al., 2000) and grape (*Vitis vinifera* L.) berries (Kondo and Fukuda, 2001). Moreover, *in vitro* application of MJ to immature green strawberries has increased respiration, ethylene production, and transitory induction of anthocyanin biosynthesis and degradation of chlorophyll, suggesting a role of MJ in ripening of this fruit (Perez et al., 1997). It is surmised that endogenous MJ may act as inducer of fruit ripening in strawberry. Some sporadic and inconclusive research reports are available on changes in endogenous level of MJ in strawberry at various stage of fruit development (Perez et al., 1997; Gansser et al., 1997).

Ethylene is thought to play an essential role in regulation of ripening of climacteric fruits. But it has only a minor effect on non-climacteric fruit such as strawberry (Given et al., 1988; Abeles and Takeda, 1990). At present, hormonal regulation of strawberry ripening is not fully understood. Auxins produced by achenes are probably the key hormone in strawberry development and ripening (Given et al., 1988). GA_3 has been reported to inhibit strawberry fruit ripening (Martinez et al., 1994). Abscisic acid has been reported to accelerate sucrose uptake and advance colour development in tissue-cultured strawberry fruit and cortex discs (Archbold, 1988; Kano and Asahira, 1981). The role of key ripening hormone ethylene in strawberry fruit ripening remains unclear

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and inconclusive with contradictory results from various investigations (Perez et al., 1997; Abeles and Takeda, 1990; Basiuomy, 1989; Atta-Aly et al., 2000).

The exogenous application of MJ affects ripening parameters including ethylene production in various fruits such as apple (Fan et al., 1998); mango (Lalel et al., 2003); Japanese plum (*Prunus salicina* Lindl.) (Khan and Singh, 2007); pear (*Pyrus communis* L.) (Kondo et al., 2007) and aroma development (Olias et al., 1992; Lalel et al., 2003; Fan et al., 1997), and pigment changes (Lalel et al., 2003; Perez et al., 1993). For immature strawberries, some preliminary research work on the effect of MJ has indicated increased respiration, ethylene production and transitory induction of anthocyanin biosynthesis and chlorophyll degradation (Perez et al., 1997). Recently, Yilmaz et al. (2007) reported that response of 'Tufts' and 'Cruz' strawberries fruit ripening to jasmonic acid is concentration dependant. Postharvest exogenous application of MJ has also been reported to suppress fruit decay caused by *Botrytis cinerea* during storage at 5 °C (Zhang et al., 2006). No research work has been reported on the role of exogenously applied MJ on enzymes involved in ethylene biosynthesis, including ACC synthase, and ACC oxidase, in strawberry during fruit ripening. We hypothesized that externally applied MJ might affect ACC synthase, ACC oxidase and ethylene biosynthesis leading to enhanced ripening. We therefore investigated the dynamics of endogenous MJ concentrations in strawberry fruit at various developmental and ripening stages and the effects of exogenously applied MJ at these stages on ethylene production including activities of ACC synthase and ACC oxidase.

2. Materials and methods

In experiment 1 we investigated the dynamics of endogenous methyl jasmonate in fruit at various developmental stages and in experiment 2 we studied the effects of exogenously applied methyl jasmonate (Sigma–Aldrich, Castle Hill, NWS, Australia) on strawberry fruit discs at various maturity stages in relation to ethylene biosynthesis and activities of ACC synthase and ACC oxidase.

2.1. Experiment 1: Endogenous methyl jasmonate in fruit at various developmental stages

Strawberry fruit (*Fragaria* × *ananassa* Duch. cv Pajaro) fruit at fully ripe, half ripe and white stage were harvested from a commercial farm in Wanneroo (31°42′S, 115°46′E), Western Australia. Fruit were put into punnets and kept at 20 ± 1 °C for 6 d. Each punnet contained 250 ± 10 g fruit and it was considered as an experimental unit and replicated three times. Concentrations of endogenous MJ were determined at 0, 3 and 6 days after harvest.

2.1.1. Estimation of endogenous methyl jasmonate

MJ was analysed using the method described by Fan et al. (1998) and Kondo et al. (2000). Fruit (50 g) were homogenised with a 50-mL saturated NaCl solution, 2.5 mL of 1 M citric acid, and 50 mL of diethyl ether containing 10 mg L^{-1} butylated hydroxytoluene (BHT) as an antioxidant and 4.8 μg of 9,10-dihydro methyl jasmonate as an internal standard. The ether phase was removed after centrifugation for 10 min at 2000 × *g*, and the aqueous layer was extracted with 150 mL diethyl ether containing 10 mg L^{-1} BHT. The extracts resulted from ether phase were dried under N_2. The dried residue was dissolved in 5 mL *n*-hexane and passed through a silica gel column (5 mm i.d. × 140 mm) (250 mg of silica gel 60 Fluka, Steinheim, Germany). The pooled sample was then eluted with 7 mL of *n*-hexane/ether (2:1, v/v), and dried under N_2. Dried samples were redissolved in 50 μL *n*-hexane/ether (2:1, v/v), and 1-μL samples were injected into a GC (Hewlett Packard 5890 series, Walnut Creek, CA) fitted with flame ionisation detector (FID) and DB5MS capillary column (50 m × 0.2 mm i.d., 0.33 μm film thickness; J&W Scientific, Folsom, CA). The injector temperature was 250 °C. The column temperature was maintained at 100 °C for 1 min, increased to 190 °C at the rate of 5 °C/min. The temperature then increased to 200 °C at the rate of 2 °C/min, held for 2 min and increased again to 280 °C at the rate of 15 °C/min. It was then maintained for 5 min. The detector temperature was maintained at 290 °C. Hydrogen was used as the carrier gas. MJ was identified using MJ standard by comparing their retention time (RT). To reconfirm MJ, a GC (Hewlett Packard 5890 series II, Walnut Creek, CA) coupled to a mass detector (MS, Hewlett Packard 5971 series, Walnut Creek, CA) was used. The ultra performance capillary column, Hewlett Packard model 19091B-105 (30 m × 0.2 mm; 0.33 μm film thickness), was coupled directly to the ion source (70 eV) of the MS detector. The inject port temperature of GC–MS was 240 °C. The temperature of column was held at 10 °C for 3 min, increased to 120 °C (at 8 °C/min), then increased to 290 °C at the rate of 10 °C/min and kept for 3 min. MJ was identified by matching its mass spectra with the spectra of MJ standard and WILEY275.L Library. The concentration of MJ was calculated as ng g^{-1} using internal standard.

2.2. Experiment 2: Effect of methyl jasmonate on strawberry discs ethylene biosynthesis and activities of ACC synthase and ACC oxidase

Discs (20 mm diameter, 3 mm thickness) from strawberry fruit were placed into Petri dishes containing 20 mL of 0.4 M mannitol with 0, 10 and 50 μM MJ and incubated for 24 and 48 h at 20 °C. The discs were transferred to MJ-free Petri dishes containing a filter paper moistened with 2 mL of 0.4 M mannitol. The discs from each strawberry were treated as a replicate and three strawberries were used. Ten fruit were randomly selected and used for preparing the discs in each replication. Ethylene production was measured at 0, 1, 2, and 3 days after MJ treatment. After ethylene determination, the discs were used to estimate the activities of ACC oxidase and ACC synthase.

2.2.1. Estimation of activities of ACC synthase and ACC oxidase

The ACC synthase and ACC oxidase activities were determined from fruit tissues according to the method described by Mathooko et al. (1993). ACC synthase activity was expressed as nmol ACC g $protein^{-1}$ h^{-1}. ACC oxidase activity was expressed as nmol C_2H_4 mg^{-1} protein h^{-1}.

2.2.2. Estimation of ethylene

Ethylene production was measured by sealing 5 g fruit in 25-mL Erlenmeyer flasks for 1 h. Ethylene in the headspace was measured using GC (Varian series Star 3400 CX, Walnut Creek, CA), fitted with flame ionisation detector and Porapak-Q column (2 m long, o.d. 3.175 mm, 80/100 mesh). The injector, column and detector temperatures were maintained at 100, 100 and 150 °C, respectively. Nitrogen was used as the carrier gas. Ethylene was calculated and expressed as nmol kg^{-1} h^{-1}.

2.2.3. Estimation of protein

The protein content of the fruit was estimated using the method of Bradford (1976). Bovine serum albumin (BSA) was used as a standard and the concentration of protein in enzyme extract was determined from the standard curves. Protein was calculated and expressed as g kg^{-1} fruit.

2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA), using Genstat release 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Effects of different MJ concentrations, duration of treatment and fruit development stages and the

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