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Regulation of Ethylene Synthesis of Harvested Banana Fruit by 1-Methylcyclopropene

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Summary

Treatment with 1-methylcyclopropene (1-MCP), a potent inhibitor of ethylene action, delayed the decrease in fruit firmness and the time in peaks of respiration and ethylene production rates, 1-aminocyclopropane-1-carboxylic acid (ACC) concentration, and ACC oxidase (ACO) activity of banana fruit, but exogenous ethylene treatment was not able to induce fruit ripening when the fruit was treated with 200 nL/L of 1-MCP. Furthermore, 1-MCP treatment delayed expression of ACC synthase (ACS) and ACO genes, two ripening-related genes in ethylene synthesis. These results demonstrated that ripening process of banana fruit was delayed at a physiological level by 1-MCP, while ethylene synthesis could be regulated at suppressed transcript levels of ACS and ACO genes.

Key words: 1-aminocyclopropane-1-carboxylic acid, banana, ethylene, 1-methylcyclopropene, respiration, synthesis

Introduction

Ripening of climacteric banana fruit is associated with a marked peak in ethylene evolution (*1*). Ripening is initiated by either natural evolution of endogenous ethylene as banana fruit reaches full maturity or by using commercial exogenous ethylene ripening procedures. In this regard, elucidation of the role of ethylene in banana fruit ripening has brought powerful tools, such as transgenic plants with altered ethylene production levels (*2*) and chemical inhibitors of ethylene perception (*3*).

1-Methylcyclopropene (1-MCP) gas blocks ethylene receptors and can prevent ethylene-induced effects in plant tissues for extended periods (*4*). 1-MCP acts by binding, apparently irreversibly, to ethylene receptors (*5*). Thus, ethylene cannot elicit subsequent signal transduction and translation. 1-MCP is a potentially useful tool for studying the role of ethylene in banana fruit ripening (*2*). Golding *et al*. (*1*) and Jiang *et al.* (*6*) reported that 1-MCP is effective in protecting banana from the effects of ethylene.

The current research investigates the effects of 1-MCP on banana fruit softening, respiration and ethylene production in relation to 1-aminocyclopropane-1-carboxylic acid (ACC) concentration, ACC oxidase (ACO) activity, and mRNA levels of ACC synthase (ACS) and ACC oxidase, two ripening-related genes of ethylene synthesis.

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Materials and Methods

Plant materials

Mature green banana (*Musa* sp., AAA group, cv. Zhonggang) grown in Guangdong Province, China, was purchased from a wholesale agency. The fruit fingers were cut out from banana hands, and selected for uniformity and size. Any bruised or diseased fruit was discarded. The fruit, before any treatment, were dipped for 3 min in 250 μL/L of Sportak™ fungicide solution, and then allowed to air-dry. Sportak prevents post harvest diseases of banana fruits.

1-MCP treatment

1-Methylcyclopropene (1-MCP) was released from a commercial powdered formulation (EthylBloc, Rohm and Haas China, Inc.) as described before (*7*).

Three experimental repeats were carried out. Bananas (about 5 kg, 30 fruit units for each repeat, altogether 15 kg) were exposed to 200 nL/L of 1-MCP in a 50-L plastic-film tent for 24 h at 20 °C (80–90 % RH) and then stored openly under the same conditions of temperature and humidity. Control fruit were subjected to the same conditions without exposure to 1-MCP.

Measurements of firmness, respiration and ethylene production rates, ACO activity, ACC concentration

Fruit firmness was measured at the middle cross section of the fruit with a firmness tester (Gaoke Inc., Jiangshu, China). Five fruit fingers were measured at each sampling time.

For assays of respiration and ethylene production rates, the fruit (6 fruit fingers, about 1 kg) were sealed in glass jars (10 L, 3 repeat jars) for 2 h at 20 °C on the sampling day. Afterwards, the fruit was taken out of the jars and stored open at 20 °C (80–90 % RH). Gas samples for analysis of ethylene and CO_2 were withdrawn from the jar through a rubber septum in each lid. Ethylene content in the gas samples was determined by gas chromatography (Shimadzu, GC17A) using an activated alumina column and a flame ionization detector. CO_2 concentration in the gas samples was also determined by a gas chromatography according to Jiang *et al.* (*8*).

ACO activity in the pulp was determined by measuring the conversion of exogenous ACC to ethylene as described previously (*8*). Pulp tissues (2 g of fresh mass) were cut into 0.1-mm discs and placed in a 20-mL vial containing 3 mL of 2 mM ACC. Sealed flasks were then incubated at 25 °C for 1 h, and gas samples were taken thereafter for ethylene analysis by gas chromatography. Samples incubated in the absence of ACC served as controls. The activity of ACO was expressed as ethylene production in μL/(kg·h).

For ACC concentration assay, about 2 g of fresh pulp tissue were homogenized with 5 mL of 95 % ethanol and centrifuged at 10 000 × *g* for 15 min at 4 °C. The supernatant was collected for ACC content analysis. ACC was measured directly in the aqueous extract according to the method by Lizada and Yang (*9*).

The analyses described above were done in triplicate.

Isolation of cDNAs and preparation of the probes

cDNAs of ACC synthase and ACC oxidase were supplied by Professor Akitsugu Inaba (Faculty of Agriculture, Okayama University, Japan) and then amplified by polymerase chain reaction (PCR) using RNA from the banana fruit pulp as the template. Probes were synthesized using a DECA prime II DNA Labeling Kit (TaKaRa, Japan) with [^{32}P] dATP as the label by the method of Liu *et al.* (*10*).

RNA extraction and Northern blot analyses

Total RNA was extracted from banana flesh tissue as described by Liu *et al.* (*10*). Northern blot analyses were carried out according to Jiang *et al.* (*11*). RNA samples (15 μg) were resolved on 1 % agarose/formaldehyde gels, and blotted to nitrocellulose filters. RNA blots were pre-hybridized and hybridized in formamide buffer at 42 °C. Final wash of the RNA blots was done in 0.5 × SSC, 0.1 % sodium dodecyl sulfate (SDS) at 60 °C. Probes were ^{32}P-labeled by the random priming method, and included cDNAs of ACC synthase and ACC oxidase. Equal reactivity and amount of RNA in all samples were verified by hybridization with ^{32}P-labeled actin.

Statistical analysis

All statistical analyses were performed with SPSS10.0. Data were analyzed by Duncan's multiple tests. Mean separations were performed using the least significant difference method.

Results and Discussion

Effects of 1-MCP treatment on fruit firmness

Firmness in control banana fruit decreased as holding time progressed at 20 °C, but there was not significant difference ($p>0.05$) in the firmness between control fruit and 1-MCP-treated fruit in less than 4 days of storage. After 4 days of storage, the firmness of the control fruit decreased rapidly (Fig. 1A). The firmness of control fruit decreased to 0.2 kg/cm^2, while MCP-treated fruit had a firmness of 4.2 kg/cm^2 after 14 days of storage, which indicated that 1-MCP treatment inhibited fruit softening. Furthermore, a rapid decrease in firmness of 1-MCP-treated fruit was observed after 21 days of storage, and the firmness decreased to 0.3 kg/cm^2 when the 1-MCP-treated fruit were stored for 28 days. Similar results were observed by Jiang *et al.* (*12*), who reported that banana ripening was inhibited effectively by the addition of 1-MCP into sealed polyethylene bags.

Effects of 1-MCP treatment on respiration and ethylene production rates

Respiration rate of banana fruit increased, then reached a peak after 12 days of storage, and finally decreased rapidly (Fig. 1B). 1-MCP treatment significantly delayed the peak in ethylene production. There was a lower respiration rate of 1-MCP-treated fruit compared to that of control fruit in less than 6 days of storage. However, the respiration rate of 1-MCP-treated fruit increased after 12 days of storage and reached a peak after 20 days of storage (Fig. 1B).

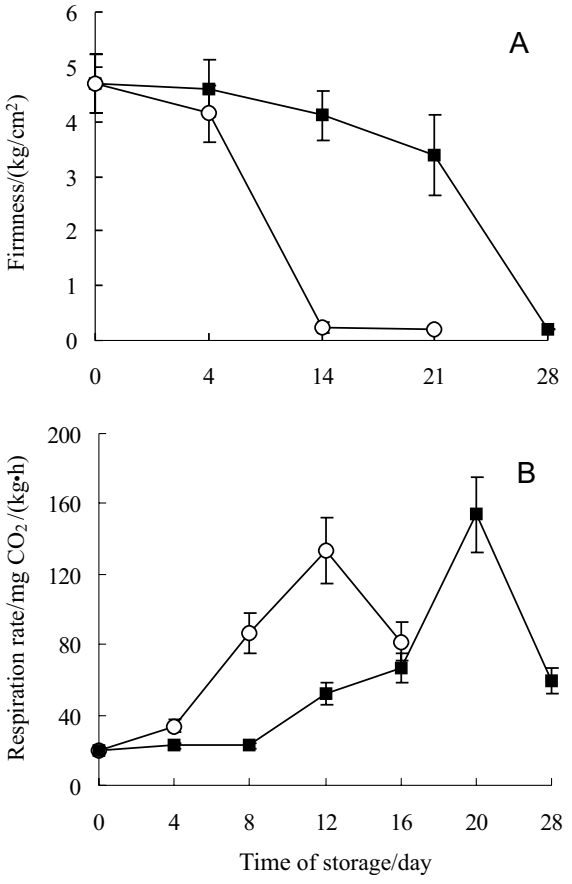

Fig. 1. Effects of 1-MCP on firmness (A) and respiration rate (B) of banana fruit. Banana fruit were exposed to 200 nL/L of 1-MCP for 24 h, then constantly stored in air (■). Control fruit (○) was subjected to the same conditions without exposure to 1-MCP. The data is mean value of 6 replicates ± S.E. (as vertical bars) for firmness or mean of 3 replicates ± S.E. for the respiration rate

Ethylene production rate increased during storage, and then reached a peak after 12 days of storage. 1-MCP treatment significantly delayed the peak of ethylene production. The 1-MCP-treated fruit exhibited an ethylene production peak when stored for 20 days (Fig. 2A). In addition, exposure of 1-MCP-treated fruit, stored for 4, 8 or 12 days, to 20 μL/L of ethylene had no significant effects on the time in the ethylene production peak (data not shown), which confirmed further that response of the 1-MCP-treated fruit to ethylene depended on the synthesis of new ethylene sites needed to mediate banana fruit softening (*6*).

Effects of 1-MCP treatment on ACO activity

ACO activity of control fruit increased slowly during storage and then reached a peak after 12 days of storage (Fig. 2B). 1-MCP treatment delayed the peak in the ACC activity, which was associated with the delayed peak in the ethylene production of 1-MCP-treated fruit. The delay in the ACO activity peak by 1-MCP treatment may be attributed to blockage of ethylene reception, which prevents downstream ethylene-induced effects (*5*,*13*).

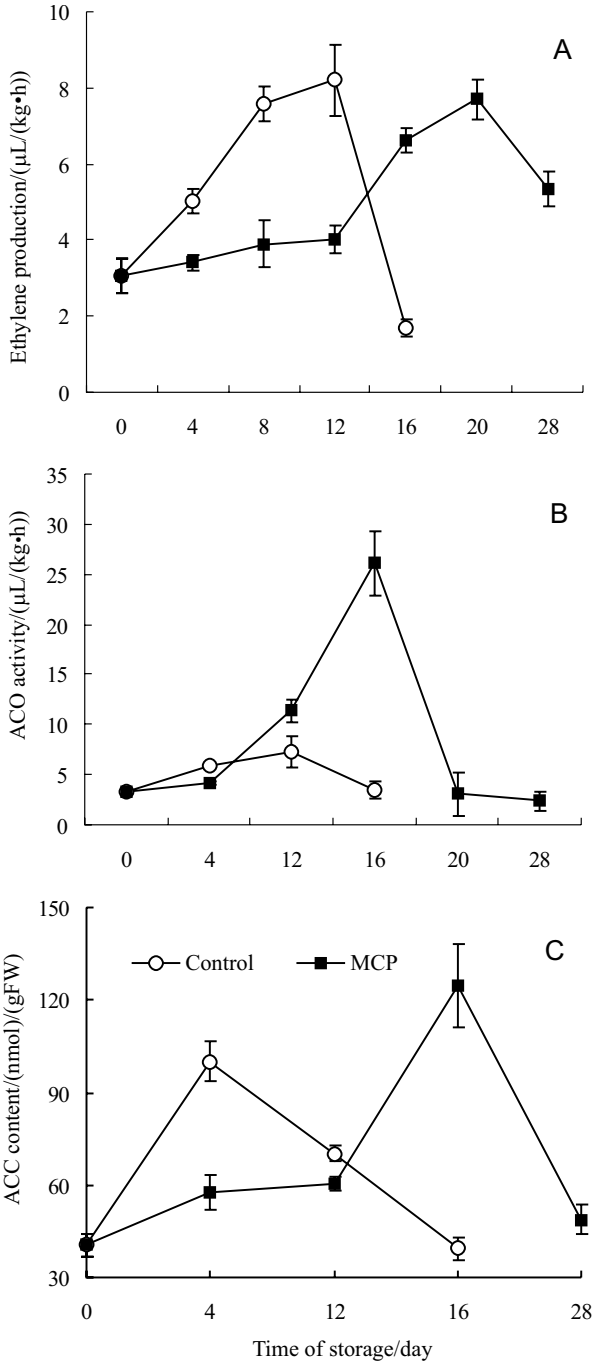

Fig. 2. Effects of 1-MCP on ethylene production (A), ACO activity (B) and ACC content (C) in the banana fruit. Banana fruit were exposed to 200 nL/L of 1-MCP for 24 h, then constantly stored in air (■). Control fruit (○) was subjected to the same conditions without exposure to 1-MCP. The data is mean value of 3 replicates ± S.E. (as vertical bars)

Effects of 1-MCP treatment on ACC concentration in banana pulp

As shown in Fig. 2C, ACC concentration in control fruit increased rapidly during storage and then reached a peak after 4 days of storage, whereas increase in the ACC concentration was delayed significantly by 1-MCP treatment. The 1-MCP-treated fruit reached a maximum

value of ACC concentration after 16 days of storage. Thus, reduced ethylene production of banana fruit by 1-MCP treatment was attributed to blockage of ACC synthesis. However, 1-MCP treatment could not completely inhibit ACC accumulation in banana fruit (Fig. 2C), which is in agreement with the result of Pathak *et al.* (*14*). These results further elucidate why the 1-MCP--treated banana fruit can also ripen, showing changes such as softening (Fig. 1A).

Effects of 1-MCP treatment on expression of ACS and ACO genes

Control fruit showed a maximum expression of ACS and ACO genes after 4 days of storage (Fig. 3). The increases in ACO and ACO mRNAs with fruit ripening were delayed by 1-MCP treatment. The expression of ACS and ACO genes during fruit ripening is believed to be stimulated by ethylene (*13*,*14*). Various inhibitors of ethylene action have been used to repress ethylene-related gene expression (*3*,*5*). Here, it is shown that ACS and ACO transcript levels were suppressed by 1-MCP treatment (Fig. 3).

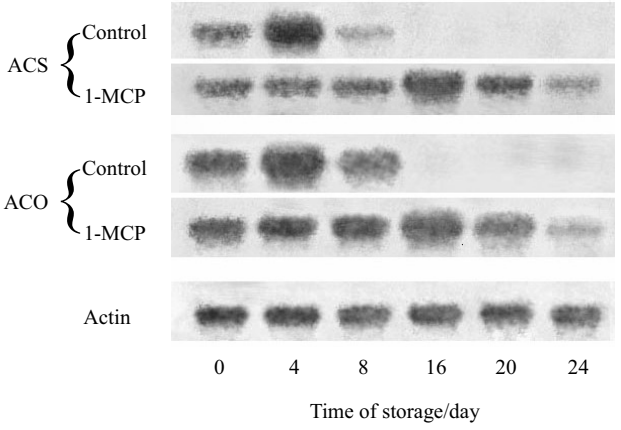

Fig. 3. Effects of 1-MCP on expression of ACS and ACO genes of banana fruit. Banana fruit were exposed to 200 nL/L of 1-MCP for 24 h, then constantly stored in air. Control fruit was subjected to the same conditions without exposure to 1-MCP. Each lane contains 15 μg of mRNA for ACS and 2 μg of mRNA for ACO. Actin was used as an internal control to normalize the amount of mRNA loaded

Conclusion

Banana is a climacteric fruit and its ripening depends on the perception of ethylene (*1*,*15*). In this study, non-1-MCP-treated fruit reached peaks in ACC concentration and expression of ACS and ACO genes after 4 days of storage, which preceded the time in peaks of respiration and ethylene production rates (Figs. 1B, 2 and 3). Thus, enhanced ability of ACC synthesis is the characteristic that triggers fruit ripening, whereas increased conversion of ACC to ethylene, and respiration and ethylene production rates, and decreased firmness can be ripening-related physiological changes. The study corroborates the theory that expression of ACO and ACO genes of 1-MCP-treated fruit, based upon Northern blot analyses of ACO and ACO mRNAs, enhanced over their holding time. In conclusion, physiological changes associated with banana fruit ripening were delayed by inhibiting ethylene perception, while ethylene synthesis of banana fruit by 1-MCP can be regulated at suppressed transcript levels of ACS and ACO genes.

Acknowledgments

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Regulacija sinteze etena s 1-metilciklopropenom u bananama

Sažetak

Obrada s 1-metilciklopropenom (1-MCP), snažnim inhibitorom djelovanja etena, usporava snizivanje čvrstoće banane i vrijeme dostizanja maksimalne respiracije, brzine proizvodnje etena, zatim koncentraciju 1-aminociklopropan-1-karboksilne kiseline (ACC) i aktivnosti ACC oksidaze (ACO). Međutim, egzogena obrada etenom nije mogla inducirati sazrijevanje ploda ako su banane bile obrađene s 200 nL/L 1-MCP. Nadalje, obrada s 1-MCP usporila je ekspresiju ACC sintaze (ACS) te ACO gena, jer oba gena sudjeluju u sintezi etena tijekom sazrijevanja. Ovi rezultati pokazuju da je proces sazrijevanja banana usporen na fiziološkoj razini, dok se sinteza etena može regulirati prigušivanjem razine transkripcije ACS i ACO gena s 1-MCP.