Ethylene Biosynthesis in Mume (*Prunus mume* Sieb. et Zucc.) Fruits

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Abstract : It was ascertained that ethylene from mume (*Prunus mume* Sieb. et Zucc.) fruits during the ripening was possibly produced according to the pathway in which 1 — aminocyclopropane — 1 — carboxylic acid (ACC) is an intermediate. A prominent occurrence on ethylene production in mume fruits at the pre-climacteric stage began with an increase of ACC synthase activity, causing ACC to accumulate. Ethylene level, then, attained to the maximum peak following a rapid decrease of ACC synthase activity. Both activities of ACC synthase and ethylene forming enzyme (EFE), catalyzing the conversion of ACC to ethylene, were disappeared by cycloheximide, but the latter was not affected by aminoethoxyvinylglycine in the presense of sufficient ACC at all. These results indicated the presense of the pathway, S — adenosylmethionine → ACC → ethylene, in ripening mume fruits. It was also proposed that the short period having a sharp peak of ACC synthase activity just before the climacteric rise might be the most significant in the ripening process of mume fruits.

Introduction

Mume originated from China grows within the limits of Eastern Asia. Japanese apricot (mume) is a kind of sour fruits and especially available to preservative foods such as pickles, jams and liqueur. Mume is one of the climacteric fruits and matures evolving a large amount of ethylene.[1] The fruits after harvest rapidly deteriorate in the quality at the market. Since ADAMS and YANG[2] demonstrated the pathway of ethylene biosynthesis in apple tissue : methionine → SAM → ACC → ethylene, the existence of this pathway has been proved in various plants including wound tissues.[3-9] It would be important from aspect of the establishment of the quality control of fruits and vegetables to recognize the mechanism of ethylene biosynthesis in individual species. In the present study we examined the biosynthetic pathway in mume fruits during the ripening.

Materials and Methods

Plant materials and chemicals Mume (*Prunus mume* Sieb. et Zucc. cv. Nanko) fruits were obtained from the Kochi Fruit Tree Experiment Station at the normal harvest time or the green-mature stage.

1 — Aminocyclopropane — 1 — carboxylic acid (ACC), S — adenosylmethionine (SAM), pyridoxal phosphate and aminoethoxyvinylglycine (AVG) were from Sigma Chemical Co., U.S.A. Cycloheximide (CHI) was from Tokyo Kasei Chemical Co., Tokyo and 4 — (2 — hydroxyethyl) — 1 — piperazinepropanesulfonic acid (Hepps) from Wako Pure Chemical Industries, Ltd, Tokyo. All other chemicals used were of analytical reagent grade.

Extraction and Assay for ACC Mume tissue, about 1 g fresh weight, was homogenized in 8 mℓ of

80% ethanol by means of an Ilado laboratory disperser in an ice bath. The homogenate combined with additional 5 mℓ of 80% ethanol for washing the shaft was filtrated through a Toyo filter paper (No. 5 B), and the filtrate was centrifuged for 10 min at 10,000 x *g* at 5℃. The supernatant was evaporated under reduced pressure (20 mmHg) at 45℃. The extract was brought to a volume of 2 mℓ with water, and 2 mℓ chloroform were added. The mixture was shaken vigorously and centrifuged for 10 min at 4,000 x *g*. The water phase was used for the assay of ACC by the method of LIZADA and YANG.[10]

Extraction and Assay for ACC synthase activity Extraction of ACC synthase from mume tissue was performed by a slight modification of the method of SITRIT et al.[9] Mume tissue, about 2.2 g fresh weight, was homogenized in 20mℓ of 50 mM potassium phosphate buffer (pH 7.2) containing 5 % $(NH_4)_2SO_4$, 4 mM dithiothreitol and 5 μM pyridoxal phosphate in an ice bath. The following procedures were carried out below 5 ℃. The homogenate was filtrated through a Toyo filter paper (No. 5 B), then the filtrate was centrifuged for 10 min at 10,000 x *g*. The supernatant was saturated to 90% with $(NH_4)_2SO_4$ and allowed to stand for 1 hr at 2 ℃. After centrifugation the precipitate was dissolved in 4 mℓ of 10 mM potassium phosphate buffer (pH 7) containing 0.1 mM dithiothreitol and 2 μM pyridoxal phosphate. The solution was dialyzed overnight against the same buffer. The dialyzed solution was used for assay of ACC synthase activity. ACC synthase activity was assayed in a reaction mixture containing 0.5mℓ of enzyme solution, 40 μmol Hepps buffer (pH 8.2) and 200 nmol SAM in a total volume of 0.6mℓ. After incubation for 1.5 hr at 30℃, the ACC formed was assayed by the method of LIZADA and YANG.[10]

Assay for EFE activity EFE activity was determined by measuring the conversion of applied ACC to ethylene.[11] Eight discs, 10mm in diameter and 3 mm thick (about 2.0 g), were incubated for 1.5 hr at 30℃ with contant shaking in the dark in a sealed 50mℓ-Erlenmeyer flask containing 6 mℓ of 10 mM potassium phosphate buffer (pH 6.0) with or without 5 mM ACC. A half milliliter of head space gas was sampled with 1 −mℓ gas tight syringe and analyzed for ethylene.

Treatment with inhibitors Eight discs, weighing about 2.2 g, submerged in a solution of 1 mM CHI or AVG, or in water were vacuum-infiltrated for 10 min. After blotting on a filter paper, the discs were put in a 50mℓ-Erlenmeyer flask and incubated at 30℃ in the dark as follows; 1) The discs treated with AVG were incubated in a solution of 1 mM ACC or in water. EFE activity was assayed after 4 hr. 2) The discs treated with CHI were incubated for 10 hr, then, ethylene production, ACC level, ACC synthase and EFE activities were determined.

Fruit ripening after harvest Intact fruits were allowed to ripen naturally at 20℃ in the dark. Fifteen fruits were placed in a about 3 −liters desiccator and these same fruits were assayed daily for ethylene evolution. The desiccator was enclosed only for 2 hr a day when ethylene assay was carried out. This ethylene production rate was used as the criterion in this experiment. Ten other fruits were daily taken up and each of them were placed separately into a 150−mℓ glass bottle and assayed for ethylene. Five fruits of them, having the closer level of ethylene evolution to that of the criterion at that day, were taken up for assay of ethylene production, ACC level, ACC synthase and EFE activities.

Ethylene determination Ethylene was measured by a Shimadzu GC−7A gas chromatograph with FID equipped with an activated alumina column (1 m × 3 mm i. d.) at 70℃. A half milliliter of gas always taken from the head space of containers by a 1 −mℓ gas tight syringe and analyzed.

Results

Ethylene production, ACC level and ACC synthase activity Mume fruit is one of the typical fruits developing the climacteric rise, therefore, the detached fruits should trace the normal ripening process accompanied with evolution of ethylene. Individual fruits, however, vary minutely in the ripening degree, even if they were harvested on the same time. Each fruit used for assay should be in a similar degree of ripening, otherwise it will be difficult to reveal rigidly periodical relations between ethylene production and ACC accumulation and ACC synthase action. In order to overcome this difficulty a sample of 15 fruits was preserved for a criterion of the ripening extent monitored by ethylene level throughout this experiment, being referred to individual fruits taken up at that day.

Fig. 1 shows the changes of ethylene production, ACC level and ACC synthase activity of intact fruits from the onset of the climacteric rise to the post-climacteric stage. Mume fruits produced a large amount of ethylene during the ripening. Ethylene production amounted to the 380 nl/g/hr peak, which occurred after 8 -day storage. ACC level enhanced steeply after 6 days in accordance with an increase of ACC synthase activity. ACC level reached a maximum after 7 days, then decreased gradually. On the other hand, the peak of ACC synthase activity preceded a day that of ACC level. Fig. 2 shows the occurrence of EFE in intact fruits. EFE activity was low at the earlier stage, but as the ripening proceeded it increased reaching a maximum at the climacteric peak. The increase of EFE activity by application of ACC indicates that ethylene is derived from ACC.

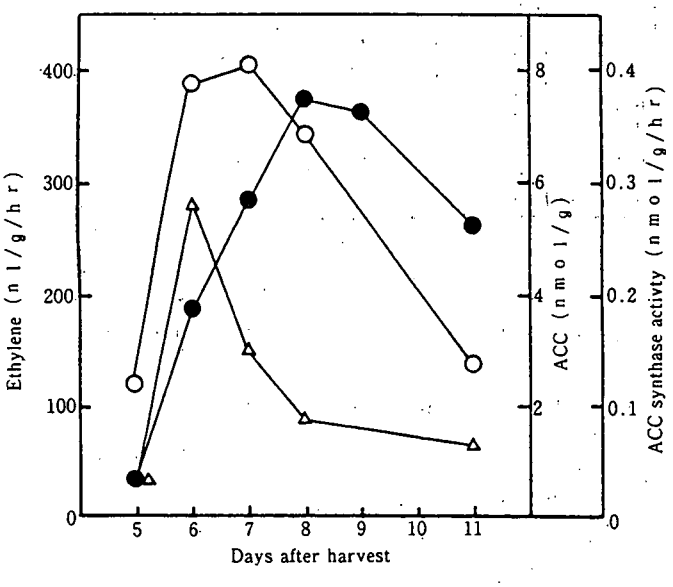

Fig. 1. Changes in ethylene production rate, ACC level and ACC synthase activity of mume fruits after harvest.
●, ethylene production rate ; ○, ACC level ; △, ACC synthase activity.

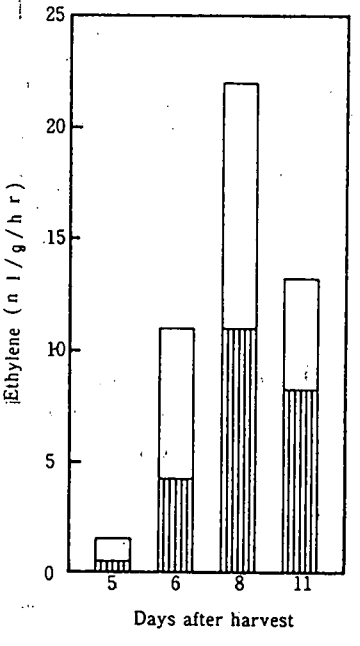

Fig. 2. Changes in EFE activity of mume fruits during ripening.
□, +ACC ;
▥, −ACC.

Effect of CHI on ethylene biosynthesis system CHI is known as an inhibitor of protein synthesis.[12] Fig. 3 shows the result of 10−hr incubation of the discs treated with or without CHI. Since the discs were sampled from the fruit near the climacteric peak, all the three controls untreated with CHI gave high level or activity. When the discs were treated with CHI, all of ethylene production, ACC level and ACC synthase activity decreased, especially the last entirely disappeared. As given in Table 1, EFE activity remained in the CHI−free discs, while the activity decreased in the discs treated with CHI, even though sufficient ACC was available. It is suggested that the occurrence of endogenous ethylene would involve the protein synthesis.

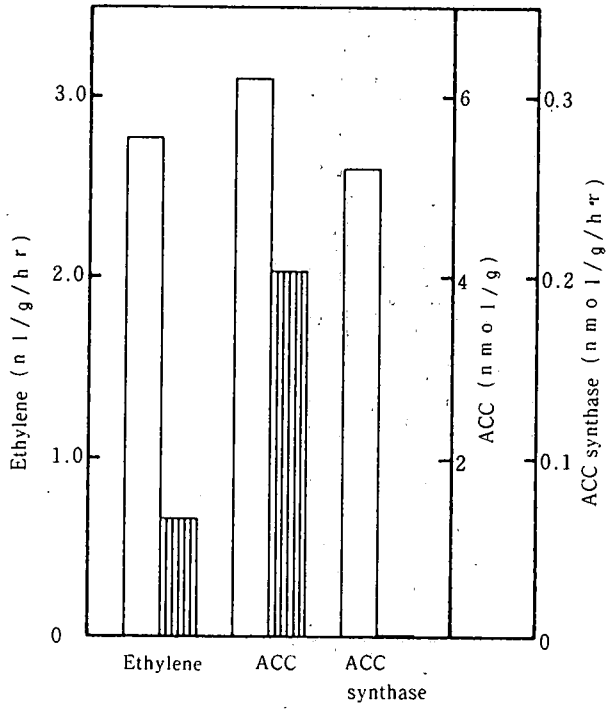

Fig. 3. Effect of cycloheximide on ethylene production, ACC level and ACC synthase activity in discs of mume fruits.
□, cycloheximide−free disc ; ▥, cycloheximide−treated disc.

Table 1. Effect of cycloheximide on EFE* activity

Treatment	Ethylene** (nl/hr/g fresh weight)	
	−ACC	+ACC
H_2O	5.08	21.5
Cycloheximide	2.00	3.31

* Ethylene forming enzyme.
** Cycloheximide−free or −pretreated discs were incubated for 10 hr, then EFE activity was determined by application of 6 mℓ of ACC or 10 mM potassium phosphate buffer (pH 6.0).

Effect of AVG on EFE activity AVG inhibits the conversion of SAM to ACC by blocking ACC synthase.[3,13] As shown in Fig. 4, ethylene production (EFE activity) in AVG-treated discs almost paralleled that of AVG-free discs as long as ACC was supplied. It was suppressed in AVG-treated discs without ACC. These data likewise indicate that the ethylene synthesis in mume fruits proceeds through the pathway of ACC synthesis.

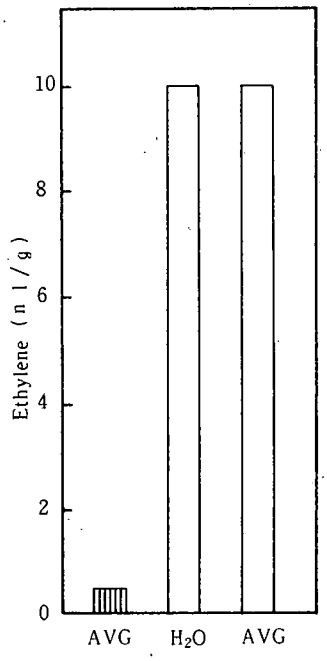

Fig. 4. Effect of exogenous ACC on ethylene production in aminoethoxyvinylglycine-pretreated discs of mume fruits.
□, +ACC; ▨, −ACC.

Discussion

It was ascertained in the present study that ethylene production of mume fruits was also caused according to the pathway as described by ADAMS and YANG.[2] The conversion of SAM to ACC, which is catalyzed by ACC synthase, is reported to be the major step limiting ethylene production in various fruits.[4,9,13-15] ACC synthase activity in mume fruits only existed just before the climacteric peak and rapidly diminished thereafter (Fig. 1). EFF that converts ACC to ethylene retained sufficient activity to produce ethylene, even after the climacteric peak. The level of ACC being newly formed during the post-climacteric stage would be very low because of the slight activity of ACC synthase. Most of the ethylene production after the climacteric peak would result from the destruction of ACC which have been accumulated up to then. These data as for mume fruits also supports the view that the induction of ACC synthase is a key step for ethylene production.

Ethylene production is much more stimulated by wound tissues, not only in the plant having the climacteric ability, but also lacking such as citrus fruits.[4,16,17] ACC synthase activity, ACC level and ethylene production enhanced greatly in mume fruits near the climacteric peak (Fig. 1 and 2). Similar results were obtained in the excised or wound tissues untreated with CHI just before the climacteric peak (Fig. 3 and Table 1). It is, therefore, suggested that ethylene biosynthesis in wound tissue would proceed as well as that in intact fruits.

In ACC synthase assay the final concentration of SAM was 333 μM. This enzyme is reported to be deactivated by SAM as a substrate depending on the time-course of the enzymatic reaction.[3,18,19] In our preliminary experiment using apple tissues the activity for 1 −hr incubation was about one fifth lower than that for 0.5−hr incubation. The declining rate of ACC synthase activity at the concentration of SAM in the range of 50 μM to 400 μM under 1.5−hr incubation was linear. The present data could be, therefore, compared with each other, though the estimates for the activity were considerably low.

It has been known that CHI gives an almost complete effect of inhibition on ethylene production, ACC level and the synthesis of ACC in immature fruits at the climacteric stage such as tomato[4] and

avocado fruits.[9] The decrease of ACC level and ethylene production would likewise result from the disappearance of ACC synthase by CHI (Fig. 3). It is possibly due to a large amount of ACC accumulated already that the drop of ACC level was not as large as those of ACC synthase activity and ethylene production. It is supposed that ACC accumulated near the climacteric peak would contribute to the source of ethylene evolution at the post-climacteric stage when ACC is little formed because of a slight activity of ACC synthase.

AVG inhibits specifically ACC synthase and CHI blocks either ACC synthase or EFE. The conversion of SAM to ACC was surely suppressed by the treatment with AVG in the absence of ACC (Fig. 4). The conversion of ACC to ethylene, however, proceeded as long as ACC was available even if the discs were treated with AVG (Table 1). Similar results were reported in kiwifruit,[8] citrus fruits[7] and their leaves.[20] These date also support that ethylene in mume fruits is evolved by the pathway indicated by ADAMS and YANG.[2]

Among the fruits at the same age, detached fruits generally ripen earlier than attached fruits to the plant. The author,[21] e.g. previously observed a similar phenomenon in the case of tomato fruit. The ripening of mume fruits is also hastened by harvest.[1] It is assumed that the acceleration of the ripening by detaching may be based on the disappearance of some inhibitory materials which are translocated from leaves and/or stems into the fruits. Ethylene is surely a trigger of the fruit ripening, but some factor regulating the biosynthetic system preceding to ethylene formation may be rather a more substantial trigger. As for the ripening mechanism of the fruit might be further investigated from aspect of a synergistic effect of ACC synthase and some plant hormones.

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