Relationship of Polyamine Content to ACC Content and Ethylene Evolution in Japanese Apricot Fruit

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Summary
The polyamine and ACC contents in mesocarp of Japanese apricot (Prunus mume Sieb. et Zucc.) were determined during fruit development. Polyamine and ACC levels peaked in the early fruit development stage, followed by 'June drop'. Then, polyamine and ACC levels decreased rapidly and changed little until fruit was harvested. The content of spermidine (Spd) in the mesocarp sharply declined during the post-harvest period, but the levels of spermine (Spn) and putrescine (Put), did not change. ACC and ethylene levels peaked 3–4 days after harvest. Exogenous polyamines, applied to fruit 20 days before normal harvest, delayed fruit drop and ethylene evolution after harvest as compared to the control.

Introduction
Polyamines are widespread in many plant species; they are important in normal growth and development, such as cell division, embryogenesis, root formation, flowering, pollen tube growth, and senescence (Evans and Malmberg, 1989). However, very little is known about their mode of action (Bagni and Pistocchi, 1992). In higher plants, some evidence indicates that polyamines are physiologically important in fruit development and senescence (Evans and Malmberg, 1989). Senescence usually occurs in plants when ethylene is released in relatively large quantities. Ethylene arises from S-adenosyl-methionine (SAM) through the intermediate substance 1-aminocyclopropane-1-carboxylic acid (ACC). SAM is not only the precursor for ethylene but also a precursor of polyamine (Tiburcio et al., 1993).

Physiological effects of ethylene in plants are known to be antagonized by treatment with polyamines. Because of the potential metabolic connection between polyamines and ethylene through the propylamine group of: SAM, a great deal of attention has been given to the involvement of polyamines in growth and senescence (Evans and Malmberg, 1989).

In climacteric fruits such as peach, it has been reported that ACC content and ethylene evolution followed similar patterns during Stage I and Stage II of fruit development. Peak ACC content and ethylene production occurred during late Stage I, with ethylene production increasing again during ripening (Miller et al., 1988).

Moreover, growth rate, ACC content, and ethylene evolution are correlated. However, few scientific papers investigating ethylene and polyamines metabolism in Japanese apricot fruit have been published. In this study, the possible relationship of endogenous polyamines to ACC content and ethylene evolution during fruit growth and post-harvest life of the Japanese apricot was investigated.

Materials and Methods
The study was carried out in 1993 on three trees of Japanese apricot, 'Shirokaga' planted at the experimental farm of Chiba University. During fruit development, fruits were collected weekly from 32 days after anthesis (March 30) until harvest, and kept at −60°C for analysis of polyamine and ACC contents. Fruit growth is expressed as fresh weight, whereas fruit drop rate was calculated from fruit set percentage in relation to the changes of endogenous polyamine and ACC contents. At 20 days before normal harvest date (June 30, 1993), the exogenous polyamines were applied to the fruit.
fruits were painted with 3 ml/fruit containing 10 mmol ACC, or one of 3 polyamines; 0.1 mmol putrescine (Put), 0.1 mmol spermidine (Spd), 1.0 mmol spermine (Spn). Our previous research on fruit ripening showed that those concentrations effectively delayed fruit drop (Unpublished data). Ethylene evolution and fruit drop rate were measured 10 days after application at 2 and 5 day intervals, respectively. At harvest, 45 fruits were collected and kept at room temperature. Five fruits per day were transferred to −60 °C for analysis of polyamine and ACC contents.

**Measurement of ethylene evolution**

Three fruits were sealed in each of three 1.2-liter chambers, and incubated at room temperature for 1 hr. To determine ethylene content, three 1-ml gas samples from the chamber headspace were injected into a gas chromatograph (GC) fitted with a flame ionization detector. The column (1 m × 3 mm i.d.) was packed with 80/100 mesh of active alumina; column, detector, and injector temperatures were 80 °C, 140 °C, and 140 °C, respectively. Flow rate of N₂ as a carrier gas was 30 ml·min⁻¹.

**Measurement of ACC content**

The frozen mesocarp was homogenized in 80% MeOH (12.5 ml·g⁻¹), extracted for 30 min at 70 °C, then filtered. Total ACC content was estimated by the method of Lizada and Yang (1979); the concentration of HgCl₂ reagent was modified to 1 μmol (Suzuki, 1988).

**Results and Discussion**

Fruit growth and fruit drop rate of Japanese apricot are shown in Fig. 1. During early development, fruits contained a high concentration of ACC, which peaked at 59.86 μmol·g⁻¹FW 39 days after anthesis and sharply declined to a low level until the ripening stage. After the peak of ACC, rate of fruit abscission 'June drop' greatly increased. The decrease in ACC content and rate of fruit drop followed a similar pattern. While cumulative fruit weight increased, ACC content rapidly decreased to 4.65 μmol·g⁻¹FW on May 25. A slight increase in ACC content preceded normal harvest date. These results are similar to those reported for peach fruit development (Miller et al., 1988).

The changes in polyamine content during fruit growth and development are shown in Fig. 2. Spd peak of 822.52 nmol·g⁻¹FW occurred concurrent-
ly with that of ACC, April 6. From April 6 to May 11, the rate of fruit growth increased rapidly, and polyamine contents, especially Spd, significantly decreased; levels of the three polyamines remained constant until harvest. Polyamines have been shown to be associated with cell division during early development, usually increasing when rapid cell division occurs (Faust and Wang, 1992). Polyamines are also related to fruit growth rate. In young tomato fruit, polyamines are metabolized during cell division to other compounds (Egea-Cortines and Mizrahi, 1993). These by-products, which are necessary for fruit development, include γ-aminobutyric acid, glutamic acid, putative sugars, β-alanine, and organic acids (Rastogi and Davies, 1989; Terano and Suzuki, 1978). Spraying polyamines in apple trees at the time of fertilization increased fruit growth (Bagni and Pisticci, 1992). Polyamines may act as a source of nitrogen to stimulate growth (Smith, 1985).

The polyamine content of Japanese apricot occurred after fruit was harvested, the level of Spd sharply decreasing from 81.11 to 35.57 nmol·g⁻¹FW over 2 days, and maintained at that level until fruit deteriorated (Fig. 3). In contrast, Spn and Put contents did not change much; whereas, the changes in ACC content and ethylene evolution during post-harvest rapidly increased, and reached a maximum 3 days after harvest (Fig. 4). Ethylene evolution peak trailed that of ACC due to the conversion of ACC, an intermediate precursor to ethylene. SAM is also an intermediate of ethylene biosynthesis pathway in plant tissues, ethylene being formed from methionine via SAM and ACC (Yang and Hoffman, 1984).

Research in avocado fruit also showed that ACC is related to fruit ripening, with the relative concentration of polyamines decreasing rapidly while ethylene production was increasing (Winer and Apelbaum, 1986). During normal harvest, Japanese apricot fruits did not produce detectable levels of ethylene; they become detectable 4 days later. A similar result occurred on ‘Nanko’ Japanese apricot in that the rate of ethylene evolution in intact fruits remained low until they matured; in detached fruits, ethylene evolution peaked after harvest (Inaba and Nakamura, 1981). Our results show an inverse relationship between the contents of Spd and ACC, suggesting that high Spd levels may suppress ethylene production in Japanese apricot fruit.

Exogenous polyamines applied 20 days before harvest delayed ripening. Polyamine treatments delayed fruit abscission (Fig. 5), and decreased post-harvest ethylene evolution (Fig. 6). In contrast, ACC treatment resulted in earlier fruit drop; the rate of ethylene evolution was significant at harvest. Application of exogenous ACC caused an increase in endogenous ACC content, and as a result, fruits reached the ripening stage more quickly; indicating that the ripening process of Japanese apricot was controlled by the high endogenous polyamines content until fruits were picked. In polyamines biosynthesis, SAM serves as the
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source of the aminopropyl groups of Spd and Spn by becoming decarboxylated, converting the aminobutyrate group to an aminopropyl group. This aminopropyl moiety is then transferred to Put, forming the other polyamines. In both of these biosynthetic paths, methionine plays an important role in sustaining the continued production of ethylene and polyamines. The possible competing effects between ethylene and polyamine biosynthesis depended on SAM, the precursor, because inhibition of the synthesis of one may promote the synthesis of the other (Miyazaki and Yang, 1987).

Figure 4. Changes in cumulative weight loss, ACC content and \( \text{C}_2\text{H}_4 \) evolution of Japanese apricot fruit during post-harvest storage at room temperature.

Fruit weight decreased by 16.25% over the first 4 days after harvest (Fig. 4). In apple, ethylene

Figure 5. Effect of exogenous polyamines and ACC on cumulative fruit drop percentage of Japanese apricot.

Figure 6. Effect of exogenous polyamines and ACC on \( \text{C}_2\text{H}_4 \) evolution of Japanese apricot fruit during post-harvest storage at room temperature.
synthesis may be inhibited by a product transported from the parent tree. When fruits are harvested, the inhibitor source disappears and the rate of ethylene production rises (Moore, 1989). In the Japanese apricot fruit, a concurrent weight loss and reduction of polyamine content occur, after which the ripening process may be induced by an increase in ACC content and ethylene evolution.

**Literature Cited**


ウメ果実における ACC 含量、エチレン発生量とポリアミン含量との関係について

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摘 要

ウメ果実の発育に伴うポリアミンおよび ACC 含量の変化を測定した結果、両者ともに、果実の発育初期にピークを示したが、その後ジューン・ドロップ終了期にかけて減少した。しかし、それ以後は果実が収穫されるまでポリアミンおよび ACC 含量ともにほとんど変化しなかった。

収穫後、果実中のポリアミン、特にスペルミジン(Spd)含量は急激に減少したが、スペルミン(Spa)、プトレシン(Put)含量の変化はみられなかった。ただし、ACC 含量とエチレン発生量は収穫3～4日後にピークを示した。

収穫20日前の樹上果実に対するポリアミン処理は、無処理果実に比較して果実の落下や収穫後のエチレン発生量を抑制した。