Comparison of 1-Aminocyclopropane-1-carboxylic Acid, Abscisic Acid and Anthocyanin Content of Some Apple Cultivars during Fruit Growth and Maturation

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Summary

The changes in 1-aminocyclopropane-1-carboxylic acid (ACC), abscisic acid (ABA), internal ethylene concentration (IEC), anthocyanin and sugar content of the apple cultivars, Tsugaru, Senshu and Fuji, which have different harvest times, were investigated. The relation between these changes and maturation are discussed.

1. The sucrose content became greater than that of glucose at 95 days after full bloom (DAFB) in 'Tsugaru', 107 DAFB in 'Senshu' and 124 DAFB in 'Fuji'. The increase in IEC also initiated at about the same times.

2. The increase in ACC content in the pulp and peel coincided with that of IEC in 'Tsugaru'. However, this relationship was not evident in the late-harvest cultivar.

3. The ABA content differed among the cultivars. In the early-harvest cultivar, the changes in ABA content in the pulp showed a similar pattern to the increases in ACC and IEC. A concomitant rise in ABA and anthocyanin content was also noted in the peel. Thus, this suggested that ABA, as well as ethylene, might be related to maturation and/or senescence, and might play a role in the coloring of apple in the early-harvest cultivar.

Introduction

The physiological changes that occur in apple fruit during development are considered to differ among various cultivars. Although there have been numerous reports on changes in sugar content during fruit development, there have been only a few studies on this subject on the more recent cultivars. Furthermore, while it is well known that internal ethylene evolution increases during maturation (3, 23), there have been only a few reports on the changes of 1-aminocyclopropane-1-carboxylic acid (ACC) on fruit development during attachment to the tree. It has been suggested that both abscisic acid (ABA) and ethylene increase with maturation or ripening in peach (21), pear (8, 20) and Japanese apricot (11), whereas ABA does not necessarily have an influence on maturation in plum (13) and sour cherry (5). Moreover, it has been shown that ABA decreases, despite the increased ethylene evolution, during ripening of kiwi fruit (20). Thus, the relationship between ABA and maturation has not been clearly established.

While the harvest time of apple fruit differs considerably among cultivars, it is estimated that the interaction of ACC, ethylene and ABA during fruit development varies from the early-season to late-season cultivars.

In the present report, the changes in ACC, ABA, internal ethylene concentration (IEC), and the anthocyanin and sugar contents of the apple cultivars Tsugaru (an early-season cultivar), Senshu (a mid-season cultivar) and Fuji (a late-season cultivar), which have different harvest times, were investigated. The relations between these changes and maturation are discussed.

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Materials and Methods

Fifteen- to 25-year-old trees of the apple cultivars Tsugaru, Senshu and Fuji, grafted onto a domestic clonal rootstock, were selected for this study. The fruits were collected for analysis at intervals of 5 to 24 days from 43 to 45 days after full bloom (DAFB) to the harvest time of each cultivar. After the juice was extracted and filtered, the sugar content was determined quantitatively by HPLC (Hitachi 655; column: GL-C610). The IEC of the fruit was determined from the extracted gas, under the surface of saturated ammonium sulfate for reducing the solubility of ethylene under a partial vacuum (2). The ACC content was estimated according to the method of Lizada and Yang (15). The above measurements were performed by gas-chromatography (Hitachi 163; column: Porapak Q). The ABA content was determined as previously reported (22), including extraction by HPLC and identification by GC-MS. The anthocyanin content was determined according to the method of Harborne (9).

Results

1. Sugar content

Fig. 1 shows the changes in sugar content. Glucose content was approximately constant during fruit growth and maturation. Although the glucose content was more than that of sucrose at 43 to 45 DAFB in each cultivar, this relationship reversed from 95 DAFB in 'Tsugaru', 107 DAFB in 'Senshu' and 124 DAFB in 'Fuji'. The rate of increase of fructose decreased from 109 DAFB in 'Tsugaru', 107 DAFB in 'Senshu' and 109 DAFB in 'Fuji'.

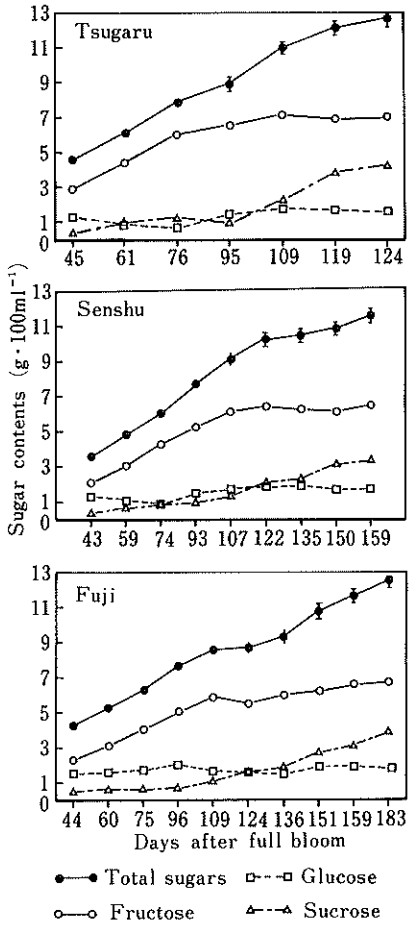

Fig. 1. Sugar contents of 'Tsugaru', 'Senshu' and 'Fuji' apples during fruit growth and maturation. Vertical bars indicate S.E.

2. ACC content and IEC

The ACC contents in the peel and pulp differed greatly among the cultivars (Fig. 2). The ACC content in the pulp of 'Tsugaru' increased from 109 DAFB, and amounted to 1.194 μmols·g^{-1}dw at 119 DAFB, while that in the peel increased from 95 DAFB and amounted to 3.854 μmols·g^{-1}dw at 124 DAFB (harvest time). In addition, the IEC changed in a similar manner to the ACC content. Although the changes in the ACC content of the peel of 'Senshu' did not show a clear trend, that in pulp increased from 135 DAFB and amounted to 0.235 μmols·g^{-1}dw at 159 DAFB (harvest time). This increase of the ACC content in the pulp generally corresponded with that of the IEC. The ACC content in the pulp of 'Fuji' increased slightly from 151 DAFB, and there was only a small amount, between 0.016 and 0.063 μmols·g^{-1}dw, during fruit growth and maturation in the peel. Thus, the relationship between the increases in ACC content and IEC was not evident in this late-harvest cultivar.

3. ABA content

The ABA contents in the pulp and peel are shown in Fig. 3. There was a dramatic increase in the ABA content in the pulp of 'Tsugaru' after 95

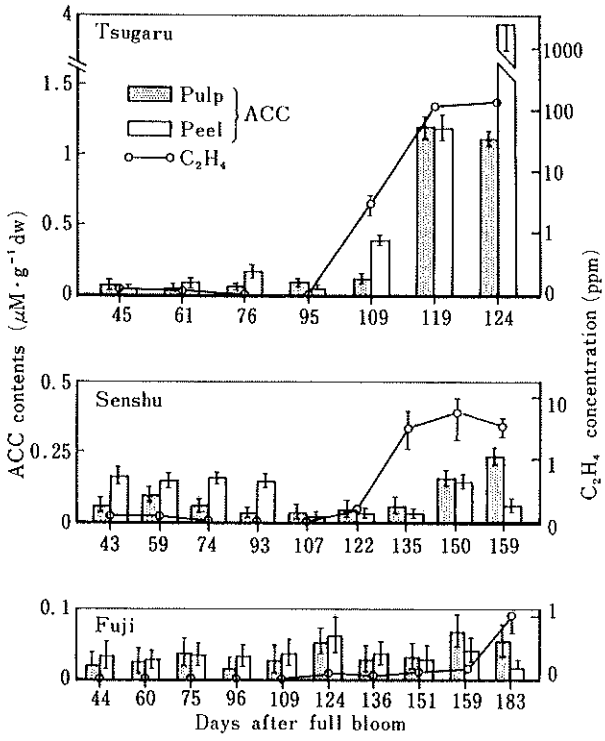

Fig. 2. Changes in ACC contents in the pulp and peel, and internal ethylene concentration of 'Tsugaru', 'Senshu' and 'Fuji' apples during fruit growth and maturation. Vertical bars indicate S.E.

DAFB, which amounted to 429 ng·g^{-1}dw at 124 DAFB (harvest time), while that in the peel increased from 76 DAFB and amounted to 532 ng·g^{-1}dw at harvest time. The ABA content in the pulp of 'Senshu' increased from 74 DAFB, while that in the peel increased from 107 DAFB. A slight increase in the ABA content of the pulp of 'Fuji' was observed at 124 DAFB, but there were fluctuations in the levels until harvest time. Although the ABA content in the peel totaled 1.79 ng·g^{-1}dw at harvest time (183 DAFB), the amount was very little during fruit growth and maturation.

4. Anthocyanin content

The changes in anthocyanin content in the peel are shown in Fig. 4. Anthocyanin contents increased from 95 DAFB in 'Tsugaru', 135 DAFB in 'Senshu', and 151 DAFB in 'Fuji'.

Discussion

Kato et al. (12) indicated that non-reducing sugars increased during the maturation period, while the reducing sugar level was almost constant in 'Golden Delicious', 'Starking Delicious' and 'Fuji' apples. Similar results were obtained for the three early to late-season cultivars in the present experiment (Fig. 1). Moreover, the time at which the sucrose content became more than that of glucose, generally coincided with the time at which the IEC increased rapidly, that is, from 95 DAFB in 'Tsugaru', 107 DAFB in 'Senshu' and 124 DAFB in 'Fuji' (Figs. 1 and 2). From these results, it was considered that this time marked the change from the growth to the maturation of the fruit.

The internal ethylene level of apple fruit, which is a climacteric rise type, increases with maturation (3, 14). In this study, this rate of increase was

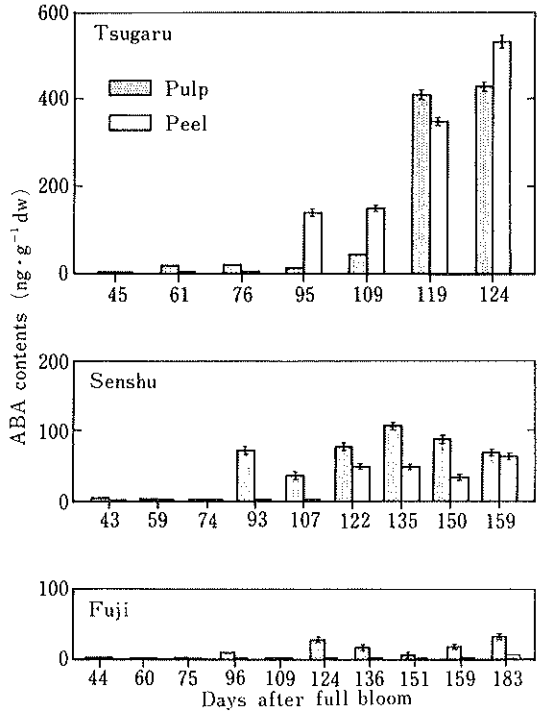

Fig. 3. Changes in ABA contents in the pulp and peel of 'Tsugaru', 'Senshu' and 'Fuji' apples during fruit growth and maturation. Vertical bars indicate S.E.

higher in the early-season cultivar (Fig. 2). The increase in ACC content also showed a similar tendency. In particular, the increase in ACC of the pulp and peel in 'Tsugaru', and of the pulp in 'Senshu', were significantly correlated with the increase in IEC (Fig. 2). These results indicate that the increase in ACC accumulation is accompanied by the onset of ethylene production, as reported in avocado (10), peach (1) and apple fruits (14). However, in the late-season cultivar 'Fuji', the ACC content hardly varied until harvest, and the IEC increased slightly only at harvest time (Fig. 2). Miller et al. (17) and Brecht and Kader (4) also reported a similar observation in peach. Sitrit et al. (19) have shown that although the activity of ethylene-forming enzyme (EFE) is low in preclimacteric avocado fruit, it may stimulate ethylene production, whereas the ACC concentration does not increase. Hence, it is necessary to investigate the relationship between EFE activity and ethylene evolution in the late-season cultivar.

In this study, the relative ABA content in the pulp during maturation was the highest in 'Tsugaru', followed by 'Senshu' and then 'Fuji' (Fig. 3). The change in ABA content in the pulp of 'Tsugaru' apparently showed a similar pattern to the ACC content and IEC (Figs. 2 and 3). In addition, the ABA content increased greatly around harvest time. Although the ABA content in late-harvest cultivars, such as 'Senshu' and 'Fuji', increased towards maturation, the content fluctuated until harvest time (Fig. 3). Kitamura et al. (13) indicated that although the ABA content increased with maturation in plum fruit, the relation with maturation was not evident because the content was at a high level before the climacteric rise. In addition, Tsay and Mizuno (20) have shown that the 'Kosui' pear, which contains a high level of

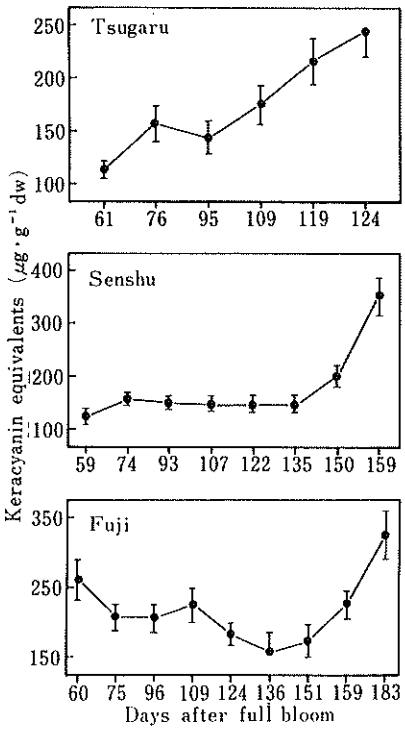

Fig. 4. Anthocyanin contents in the peel of 'Tsugaru', 'Senshu' and 'Fuji' apples during fruit growth and maturation. Vertical bars indicate S.E.

ABA, aged earlier than the 'Nijisseiki' pear which has a low ABA content. Moreover, Tsuchida et al. (21) indicated that the ABA content in peach fruit increased 35-fold after five days in storage, compared to that at harvest time. According to these reports, and the results of this study which compared early to late-season cultivars, it is considered that the differences in ABA content among cultivars may be related to the storability of each cultivar. That is to say, in the five developmental stages proposed by Watada et al. (24), the period from maturation to senescence is shorter in the early-season cultivars which have the highest ABA content, as also observed in this present report (Fig. 3). Therefore, the increase in ABA may be related to senescence rather than to maturation. This is supported by the report that ABA contents increased rapidly with aging, without ethylene evolution, in young fruits of pear and kiwi fruit which were picked at a young stage of growth and stored at 20°C (20).

Murphey and Dilley (18) indicated that the application of ethylene-releasing compounds promoted anthocyanin biosynthesis in 'McIntosh' apple. Faragar and Brohier (7) also reported a similar observation in 'Jonathan' apple. Therefore, our study, showing that anthocyanin accumulation in the peel coincided with increasing IEC in the fruit (Figs. 2 and 4), supports the previous reports. Furthermore, the concomitant increase in ABA content and anthocyanin accumulation was also noted in the peel of 'Tsugaru' (Figs. 3 and 4). Hence, this result suggests that ABA, as well as ethylene, may be one of the factors which are related to anthocyanin accumulation. Matsushima et al. (6) have shown that ABA treatment promoted anthocyanin accumulation in the grape. However, the ABA content in the late-season cultivar was low, as was the IEC. Faragar (6) demonstrated that temperature was an important factor regulating anthocyanin accumulation, and that the optimum temperature for anthocyanin accumulation was 16° to 24°C in ripe apple. Further studies should be conducted on other factors which may regulate anthocyanin accumulation, such as temperature and enzymes (7), as well as phytohormone substances. Investigations into whether ABA directly affects anthocyanin accumulation are now underway.

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リンゴ数品種の果実の発育および成熟中の1-アミノシクロプロパン-1-カルボン酸，アブシジン酸，ならびにアントシアニン含量の比較

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摘　要

収穫期の異なるリンゴ'つがる'，'千秋'，'ふじ'の3品種を供試して，発育中の果実内糖含量，アントシアニン含量および内生エチレン濃度の変化，また果皮と果肉の部位別に1-アミノシクロプロパン-1-カルボン酸(ACC)，アブシジン酸(ABA)含量の変化を調査し成熟との関わりを検討した．

1．'つがる'では満開後95日以降，'千秋'では満開後107日以降，'ふじ'では満開後124日以降，果実中のショ糖含量がブドウ糖含量を上回り，またこの時期はそれぞれの品種の果実の内生エチレン濃度の増加時期とほぼ一致した．

2．果皮と果肉中のACC含量の増加割合は，早生品種'つがる'では内生エチレン濃度の上昇と一致したが，収穫期の遅い品種ほどACC含量とエチレン濃度との関係は明らかでなかった．

3．果肉中のABA含量の変化は，収穫期の早い品種ほど，ACC含量およびエチレン濃度の増加と類似のパターンを示し，その含量も多かった．さらに'つがる'では果皮中のABA含量とアントシアニン含量が同様な推移を示した．これらの結果から，ABAは成熟(老化)に関係する要因の一つであろうと推察され，また，'つがる'では着色への関連が示唆された．

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