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Distribution of ABA and IAA within a Developing Valencia Orange Fruit and Its Parts

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Summary

Various parts of developing Valencia orange fruits were analyzed for abscisic acid (ABA) and indole-3-acetic acid (IAA) by gas chromatography-electron capture detector and -mass spectrometry-selective ion monitoring with ^{3}H-ABA and [$^{13}C_6$]IAA as internal standards. The flesh and seed weight increased rapidly, while the albedo and flavedo weight increased gradually. The seed had a large peak of ABA concentrations (21 nmol $\cdot g^{-1}$ fresh weight) 150 days after full bloom (DAB), while the flesh had a small peak (5 nmol $\cdot g^{-1}$ fresh weight). IAA concentration in the seed decreased till 150 DAB but remained higher than other parts. In the central vasuclar axis, the IAA concentration reached a peak on 119 DAB. The level remained higher than those of the flesh, albedo and flavedo. The albedo and falvedo had an equally low level of IAA. We speculate from the analyses of the different fruit parts that 1) the ABA in the seed and IAA in the central vascular axis play a role in the accumulation of assimilates; and 2) the difference in ABA concentrations between the pulp and the peel indicates that no cross transfer occurs between these two tissues.

Introduction

A fruit grows relatively more rapidly than a vegetative tissue in woody species. Mobilization of assimilates to the growing fruit often proceeds at a rate faster than that of the leaves' capacity to supply photosynthates (Naylor, 1984). Thus, physiological status in the fruit may be drastically altered. It has been considered that the fruit growth was controlled by phytohormones. Additionally, it is assumed that endogenous hormones (e.g. auxin and gibberellins) produced in the seed diffuse into the surrounding structures giving rise to the fruit and that these structures are themselves dependent on such a hormonal supply for their own growth (Nitsch, 1950; Schwabe and Mills, 1981).

Abscisic acid (ABA) which was originally discovered as an abscission-promoting hormone has been reported to be growth inhibitory substance (Ackerson, 1984; Sakurai et al., 1987; Zeevaart and Creelman, 1988). However, a positive relationship between ABA level and the growth rate has also been reported in seeds of beans (Hsu, 1979), peas (Browning, 1980), and soybeans (Hein et al., 1984). Thus, Brenner (1989) claimed that ABA played a role in the accumulation of assimilates by strengthening sink activity.

In growing tomato fruits, it was reported that ABA level (Kojima et al., 1993b) and indole-3-acetic acid (IAA) level (Kojima et al., 1994) changed in the various parts, independent of each other. Whereas the physiological changes within the Valencia orange have been reported (Waynick, 1927; Bain, 1958), the distribution of ABA and IAA within the growing citrus fruit has not been investigated to our knowledge.

The present work describes the distribution of the naturally occurring ABA and IAA in various parts of Valencia orange and its possible relationship to their growth.

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Materials and Methods

Plant material

Twenty fruits per sample were harvested at 92, 119, 150 and 188 days after full bloom (DAB)

from five 30-year-old Valencia orange trees (*Citrus sinensis* Osbeck). The trees were grafted on trifoliate orange (*Poncirus trifoliata* Raf.) and were growing at the Kuchinotsu Branch. After harvest, the fruit was immediately separated into a flesh, flavedo and albedo. From the separated flesh tissue composed of a central axis region and numerous segments, only central part which includes axial bundles was excised from the central axis region. From the segment, all seeds were collected. All separation procedures were performed in cold box (0°C). The separated tissues were weighed instantly, immersed in 80% ethanol (− 20°C) and stored at −75°C until analyzed.

Purification and fractionation of plant hormones

Hormone purification was performed according to Kojima et al. (1993a). Briefly, fruit tissue adding soluble polyvinylpyrrolidone (PVP, K-30) was homogenized in 80% ethanol. After the filtrates were evaporated to the aqueous phase, the pH was adjusted to 2.5 with H_3PO_4. The acidified phase was filtered through membrane filters (0.22 μm pore size) and the aqueous filtrate partitioned against petroleum ether and diethyl ether. After separation, the organic layer was evaporated to dryness.

Dried extracts were dissolved with 25% CH_3CN, and fractionated with an HPLC system equipped with a UV and a fluorescence detector. The HPLC column was Inertsil ODS-2 (150 × 6.0 mm, GL Sciences Inc. Tokyo). A column temperature was maintained at 40°C. The sample was eluted with a mixture of 25% and 50% CH_3CN solution (20 mM acetic acid) at a flow rate of 1.6 m*l*/min. The starting solvent 25% CH_3CN was eluted isocratically until 12 min of retention time, increased linearly to 50% till 16 min, then held at 50% until 46 min, decrease linearly to 25% until 48 min and held at 25% until 55 min. The effluents corresponding to the retention time of ABA and IAA were collected separately, and were methylated with diazomethane.

ABA analysis

Determination of ABA content was performed according to Kojima et al. (1993a). Briefly, the methylated sample was injected into a GC system equipped with a ^{63}Ni electron capture detector and a fused silica glass capillary column. A portion of methylated sample was injected into the HPLC system and the methylated ABA fraction was collected. The radioactivity of the collected fraction was measured in a scintillation counter. Data were corrected according to recovery rates.

IAA analysis

Determination of IAA content was similar to the methods of Cohen et al. (1986) using [$^{13}C_6$]IAA as an internal standard. The methylated sample was injected into gas chromatography equipped with mass spectrometry (QP-5000, Shimadzu Inc., Kyoto) using the split-less technique. The injection port was maintained at 250°C. The column used was a fused silica capillary column (CBP 1, 25 m × 0.22 mm i.d., 0.25 μm film thickness, Shimadzu Inc.). The oven was programmed from 2 min at 100° to 280°C at 30°C · min^{-1} and then held at 280°C for 15 min.

Results

Figure 1 shows changes in fresh weight of the Valencia orange and its parts from 92 to 188 DAB. The flesh and seed weight increased much more rapidly than the flavedo and albedo weight. The increase in flesh weight mainly contributed to that in whole fruit weight. The axis weight was almost constant because only central tissue which includes axial bundles was sampled from the central axis region.

Figure 2 shows changes in ABA concentration of fruit parts and a whole fruit. The whole fruit value is calculated using the seed, axis, flesh, albedo and flavedo values. The ABA concentration in the seed peaked at 21 nmol · g^{-1} fresh weight at 150 DAB, whereas that of the flesh reached 5 nmol · g^{-1} fresh weight on the same data. The ABA concentration in the axis, albedo, and flavedo were low and gradually increased so that the ABA in seed and flesh at 150 DAB accounted for most of the increase in the whole fruit.

Figure 3 shows changes in IAA concentration in the fruit and its parts. The whole fruit value is calculated using the seed, axis, flesh, albedo and flavedo values. The seed maintained a higher level than any other part through the experiment period. IAA concentration in the axis peaked 119 DAB and kept stayed higher than in the flesh, albedo and flavedo. The IAA concentration in the flesh decreased on 119 and 188 DAB (see insert

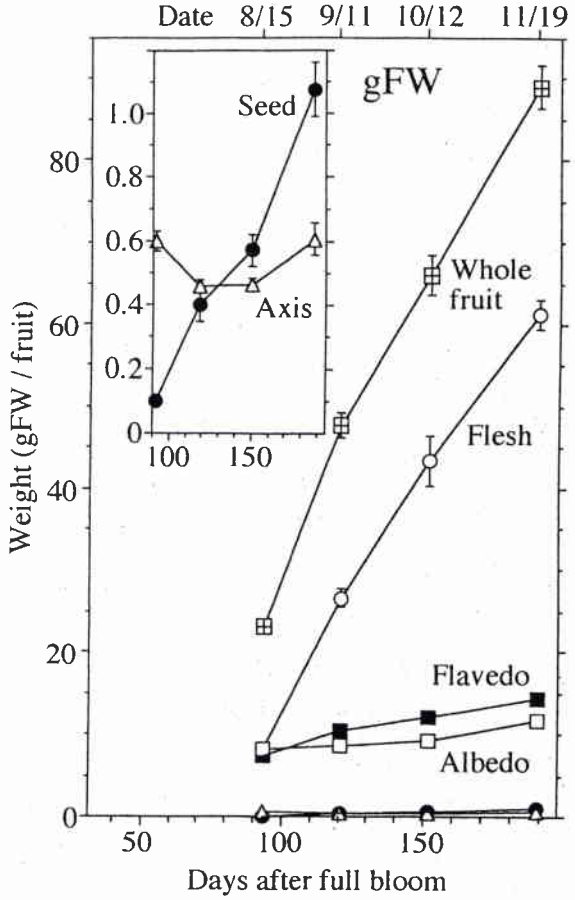

Fig. 1. Changes in fresh weight of Valencia orange fruit and its parts from 92 to 188 days after full bloom. Means and their SE of parts (n=5) and of a whole fruit (n=20) are shown. Where vertical bars are not shown, the limits are within the dimensions of the symbols. The upper horizontal axis shows sampling date.

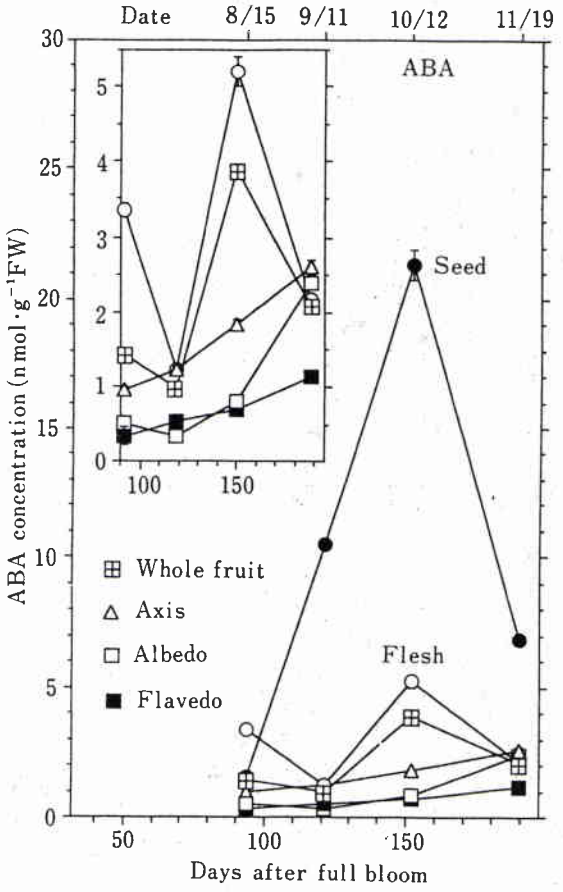

Fig. 2. Changes in ABA concentrations of the developing Valencia orange fruit and its parts. Means of three determinations and their SE are shown (n=3). Where vertical bars are not shown, the limits are within the dimensions of the symbols. The whole fruit value is calculated using the seed, axis, flesh, albedo and flavedo values.

Fig. 3), whereas the nearly equal values in the albedo and flavedo were the lowest of the tissues analyzed.

Discussion

Bain (1958) divided fruit development of Valencia orange into three stage: Stage I is a period of cell divison. Stage II is a period of rapid enlargement of cells. Stage III is regarded as the maturation period. Waynick (1927) reported that maximum growth of Valencia orange fruit occurred about between September and December within stage II. In this study, the period, in which the fruit grows in the highest rate, was established.

The seed of Valencia orange had a large peak of ABA concentration 150 DAB. Ackerson (1984) suggested that the role of ABA in developing seeds was suppression of precocious germination. However, ABA in developing seeds of Valencia orange in stage II seemed to have different roles, because ABA concentration of the seed peaked and declined conspicuously at 188 DAB. Brenner (1989) claimed that ABA functions as a stimulator of unloading of assimilates from the phloem into the sink and as a promoter of sink activity. If the theory is applied, ABA may promote sink activity in the seed of Valencia orange during stage II.

A peel of a citrus fruit has been divided into two regions (Schneider, 1968). A flavedo (exocarp) is composed of the cuticle-covered epidermis and compactly arranged parenchyma cells adjacent to it. An albedo (mesocarp) consists of the spongy

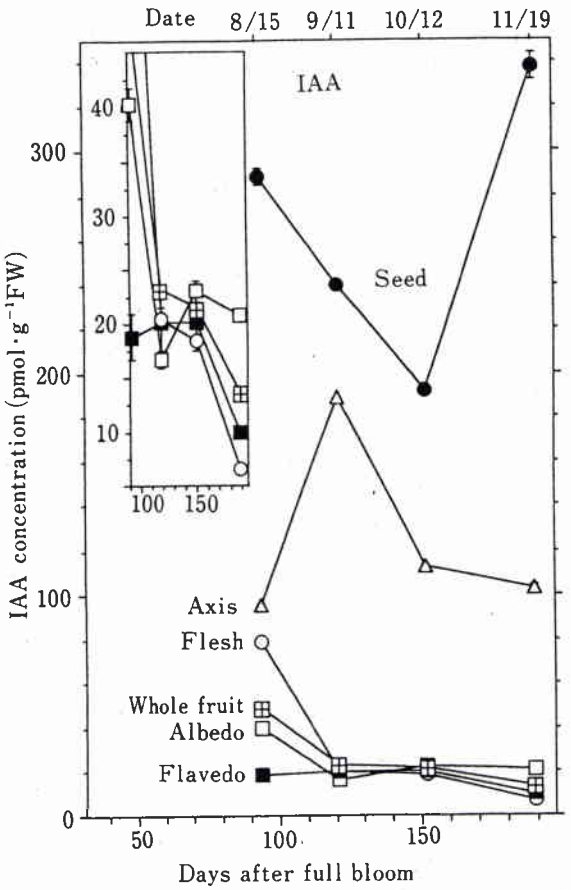

Fig. 3. Changes in IAA concentration of the developing Valencia orange fruit and its parts. Means of three determinations and their SE are shown (n= 3). Where vertical bars are not shown, the limits are within the dimensions of the symbols. The whole fruit value is calculated using the seed, axis, flesh, albedo and flavedo values.

mesophyll cell. In strawberry, it has been assumed that auxin produced within a seed diffuse into the receptacle tissue, thus promoting growth (Nitsch, 1950). In this study, a concentration gradient of IAA exists between the seed and the flesh, suggesting that the hormone also promotes fruit growth as in strawberry. However, in ABA concentration the peel (flavedo and albedo) of Valencia orange had an independent trend of the pulp (seed and flesh). The seed and flesh had the peak of ABA concentration, but the flavedo and albedo had the gradual increasing tendency. Thus, it does not seem that there is the direct transfer of ABA between the pulp and peel.

The central axis region of a citrus fruit, which includes axial vascular bundles with its phloem connected to the developing seed, had a high concentration of IAA. In the tomato fruit, the axis region had a low concentration of IAA (Kojima et al., 1994), but a high concentration of ABA (Kojima et al., 1993b) during the cell enlargement stage. There is a possibility that the reason for the high concentration of IAA in the central axis region is that it contains the axial vascular bundles which feed the developing seed.

Measurement of plant hormones using whole fruits of tomato may hide the changes of hormonal levels in its component tissues (Kojima et al., 1993b). In Valencia orange fruit, ABA levels differed substantially between the seed and other tissues and IAA levels likewise differed substantially among the seed, axis and other tissues. Thus, for a better understanding of the phytohormone role in specific tissues, measurement of its contents of the component tissues is desirable.

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肥大期のバレンシアオレンジ果実内のABAとIAAの分布

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摘　要

本実験ではバレンシアオレンジ果実の生長速度が高い秋期の果実を部分（果芯，種子，果肉，アルベド，フラベド）に分けてアブシジン酸（ABA）とインドール-3-酢酸（IAA）を分析した．ABAとIAAは，内部標準として^{3}H-ABAと〔$^{13}C_6$〕IAAを使用し，ガスクロマトグラフィー電子捕獲型検出器と質量分析器（選択的イオンモニタリング）で測定した．果肉と種子の重さは急激に増加したが，アルベドとフラベドの重さはゆるやかに増加した．150 DAB（開花盛期後の日数）に，種子のABA濃度は大きなピーク（21 nmol・g^{-1}生重量）を示したが，果肉は小さなピーク（5 nmol・g^{-1}生重量）であった．種子中のIAA濃度は150 DABまで減少したが，他の分析した部分よりも高い濃度であった．果芯部のIAA濃度は119 DABにピークを示し，果肉・アルベド・フラベドよりも高い濃度を保った．アルベド，フラベドは同程度の低いIAA濃度であった．得られた部位別の植物ホルモン量より，種子中のABAと同化物集積性や果芯部のIAAと維管束との関係，果肉と果皮間のABAの非移動性，部分別の植物ホルモン分析の必要性が示唆された．