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Changes in Indole-3-acetic Acid and Abscisic Acid Levels during Tomato (*Lycopersicon esculentum* Mill.) Fruit Development and Ripening

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Abstract. Changes in the levels of indole-3-acetic acid (IAA) and abscisic acid (ABA) in tomato (*Lycopersicon esculentum* Mill.) fruit pericarp tissue during development through ripening were measured by GC-SIM-MS using d_3-ABA and $^{13}C_6$-IAA internal standards. In the two cultivars of field-grown tomatoes analyzed, the highest IAA levels (8–24 ng/g fw) were found at the earliest stage of development (7 days after anthesis) followed by a rapid decline in levels of the hormone. ABA levels of 40–60 ng/g fw were found at the earliest stages of development followed by a decline in levels until ripening occurred when elevated ABA levels (125 ng/g fw) were measured.

One of obstacles in examining the role of hormones in developmental processes such as fruit growth and ripening has been the inability to determine the various hormone levels with confidence. Quantitative analysis done with gas chromatography-selected ion monitoring-mass spectrometry using heavy isotope internal standards comprises a technique for hormone analysis that has been used for the validation of other analytical procedures (Cohen et al. 1987). To provide accurate information on changes in levels of two hormones, indole-3-acetic acid and *cis*-abscisic acid in tomato pericarp tissue segments from anthesis through fruit ripening, GC-SIM-MS analysis of the levels of these hormones in the same portion of tissue was done on a series of fruit samples from two genotypes differing in growth pattern. Changes in levels of these two hormones were related to changes in ethylene, the hormone most identified as a regulator of ripening (Tucker and Grierson 1987).

Materials and Methods

Tomato plants (*Lycopersicon esculentum* Mill. cvs. Pik-Red and Ailsa Craig) were field grown at Beltsville, Maryland in 1991 and 1992, respectively. Flowers were tagged, and the developing tomato fruit were harvested at various intervals from 7 days after anthesis to full ripeness. Levels of ethylene and carbon dioxide were obtained from intact comparable fruit by headspace analysis immediately after harvest by use of a Hewlett-Packard GC M5890 modified with a flame-ionization detector for CO_2 after methanization and a photoionization detector for ethylene. Single samples of Pik-Red pericarp tissue (except for two samples at 14 and 44 days) and duplicate samples of Ailsa Craig pericarp tissue were analyzed from anthesis to full ripeness. The fruit were dissected, and 5-g samples of pericarp tissues were frozen in liquid N_2 and stored at −80°C. The samples were ground in cold 80% acetone using a Polytron. Internal standards were added during this first stage of sample preparation, 1100 ng of d_3-*cis*-ABA (Kubik et al. 1992) and 700 ng of $^{13}C_6$-IAA (Cambridge Isotopes). The resulting extract was filtered and acetone was removed by rotary evaporation. The residue was redissolved in a minimum amount of methanol and filtered. After several washings with dichloromethane, a final volume of approximately 30 ml was obtained. The acidic hormones were partially purified by use of an Extra-Sep-NH_2 solid-phase minicolumn (2.5 g/6 ml). After prewashing the column with 50–60 ml dichloromethane, the sample was passed through under a vacuum at a maximum rate of 5 ml/min, followed by successive washings with 12 ml dichloromethane, 12 ml ethyl acetate, and 12 ml methanol. The acidic hormones were eluted from the column with 30 ml of 2% acetic acid in methanol and concentrated. Further purification was done by HPLC using a Whatman Partisil 5 ODS-3 column (4.6 × 250 mm). The initial solvent system was acetonitrile/1% acetic acid (23:77) for 18 min at 1 ml/min. This was followed by a linear gradient to 100% acetonitrile in the succeeding 17 min. Zones of elution corresponding to those of hormonal standards, IAA and ABA, were collected, evaporated, and treated with diazomethane. The methyl ester samples of ABA and IAA were each purified further by HPLC using the same chromatographic procedure with acetonitrile/1% acetic

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acid (10:90) for 30 min. The quantities of ABA and IAA were then determined by GC-MS-SIM by analysis of their methyl esters on a HP5992 unit equipped with a 15-m, 0.32-mm DP-1701-fused silica capillary column (J & W Scientific). Ratios of the heavy isotope ABA ion (193) compared to native ABA ion (190) and another pair for IAA (136/130) were used to obtain weights of the native hormones present in the sample.

Results

The small-fruited Ailsa Craig tomato developed more rapidly and reached full ripeness slightly sooner than did the Pik-Red fruit. However, growth of Ailsa Craig fruit was significant after the breaker stage, while Pik-Red fruit reached 95% of their final weight a week before the breaker stage (Fig. 1).

The levels of IAA (8.2 ng/g fw) in the Ailsa Craig tomato pericarp were highest at 7 days after anthesis, the earliest stage of development that was measured (Fig. 2). These levels of hormone then decreased during the fruit expansion stage and were lowest at the breaker stage (33 days after anthesis) with a small increase in levels during ripening. The levels of IAA (23.5 ng/g fw) in the Pik-Red pericarp were also highest at 7 days after anthesis, and the levels declined rapidly to a minimum at 27 days after anthesis, the immature green stage. There was an increase in hormone levels during ripening with apparently a subsequent decrease at full ripeness.

The levels of ABA (61 ng/g fw) in Ailsa Craig pericarp were relatively high at 7 days after anthesis and then declined to a minimum at 33 days after anthesis (Fig. 3). A large increase in ABA levels to 121 ng/g fw occurred at the pink stage (40 days) followed by a significant decline with ripening. The highest level of ABA (67 ng/g fw) in the Pik-Red pericarp was found at 14 days after anthesis and then ABA levels declined until 34 days (mature green stage) with a short-term increase to 44 ng/g fw at 40 days (breaker stage), followed by a slow decline in ABA levels during subsequent ripening.

These data should be examined in conjunction with the levels of ethylene measured from tomato fruit at the same stages of development. There was a relatively high level of ethylene observed with Ailsa Craig fruit at 7 days after anthesis (Fig. 4) followed by the expected decline until the hormone levels rose at 33 days (the breaker stage), with a maximum level then observed at 40 days after anthesis. The initial high ethylene levels may have been partially a response after wounding, although high levels of ethylene have been reported in the early stages of developing fruit (McGlasson et al. 1978). The Pik-Red fruit did not produce similar levels of ethylene at 7 days after anthesis. Low levels of ethylene were found until the levels rose rapidly from 40 days to a maximum at 51 days after anthesis

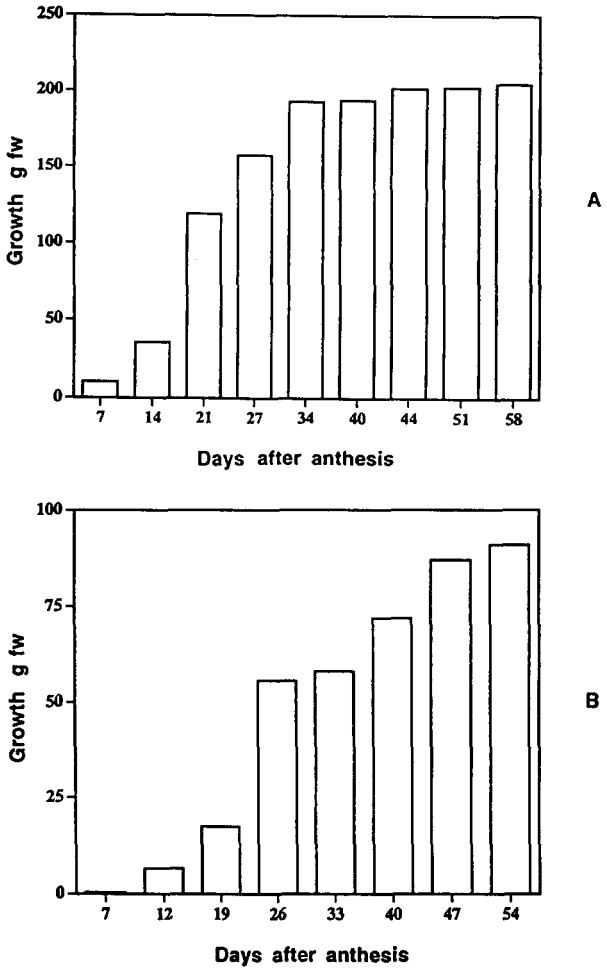

Fig. 1. Tomato fruit growth during development and ripening-Average fresh weight of fruit. (A) Pik-Red with stages of development: days 7–27, immature green; day 34, mature green; day 40, breaker; day 44, pink; day 51, red; day 58, ripe red. (B) Ailsa Craig with stages: days 7–19, immature green; day 26, mature green; day 33, breaker; day 40, pink; day 47, red; day 54, ripe red.

(red stage). Changes in carbon dioxide levels conformed to usual changes for tomato fruit and will not be discussed further.

Discussion

The changes in IAA and ABA levels in tomato pericarp tissue during development and ripening that were measured differ from the combined trends presented by McGlasson (1978) where the levels of both hormones were greatest at the breaker stage of development. Specifically in our determinations, the initial high levels of IAA measured at 7 days after anthesis during the period of cell division were

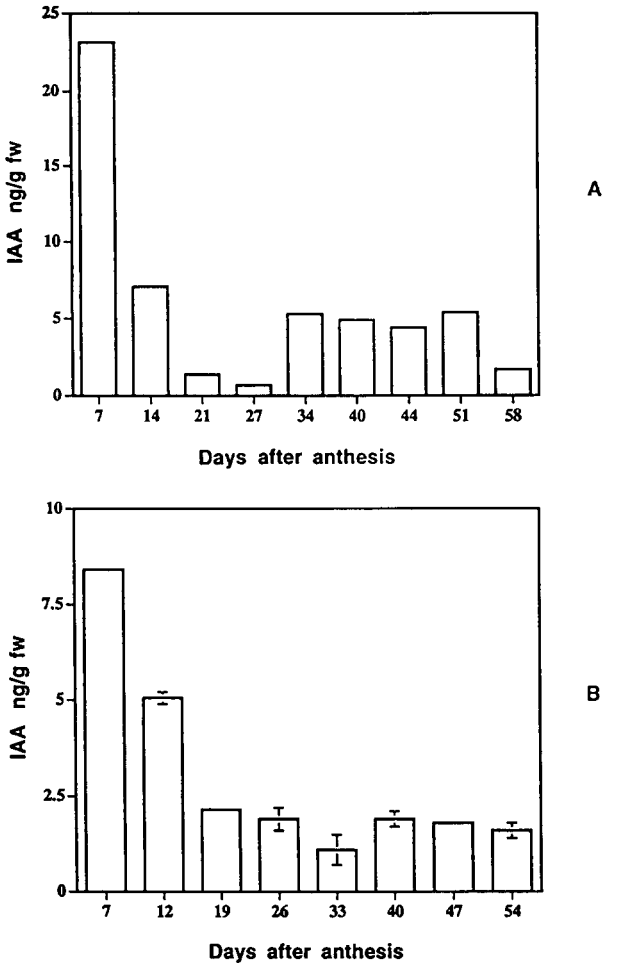

Fig. 2. IAA levels in tomato fruit pericarp during development and ripening: **(A)** Pik-Red, single sample analysis except for duplicate sample analyses at days 14 and 44 where average is shown; **(B)** Ailsa Craig, duplicate sample analysis. Bars: SE (n = 2).

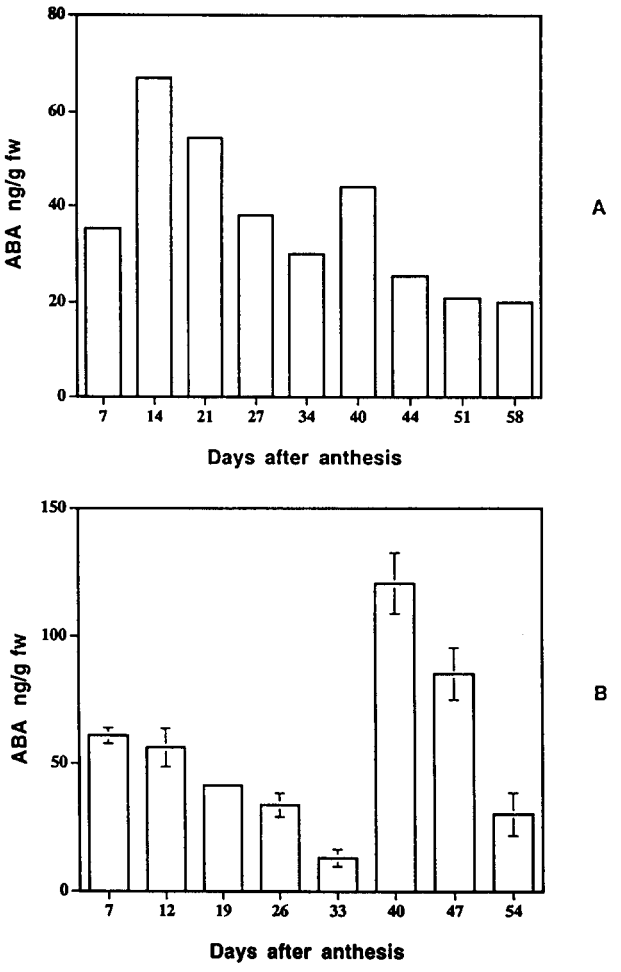

Fig. 3. ABA levels in the same tomato fruit pericarp samples as corresponding IAA levels: **(A)** Pik-Red; **(B)** Ailsa Craig. Both groups of analyses completed as described in Fig. 2.

followed by a decline in hormone levels during the cell-enlargement phase. There was a trend indicating a small increase in IAA levels during ripening. These results are similar to those obtained with the oat coleoptile straight-growth bioassay of whole field-grown tomato fruit where high auxin activity was found at 10 days and another smaller peak of activity at 30 days after anthesis (Mapelli et al. 1978). Later determinations of IAA levels in tomato fruit using the fluorescence of 2-methylindole-α-pyrone method during ontogny of greenhouse-grown tomato fruit indicated the presence of very low levels of IAA (0.1–1.8 ng/g fw). Maximum IAA levels in pericarp tissue were found at 9 days after anthesis and no IAA was detected from 20 to 30 days after anthesis when the study was concluded (Lacheene and El-Beltagy 1986). We found a similar pattern of change in hormone level during tomato fruit development but the magnitude of IAA levels determined by GC-SIM-MS were larger. The storage of harvested tissue at −20°C by Lacheene may have led to major losses of IAA since we have found in earlier experiments that tissue storage at −80°C was necessary to prevent IAA degradation. IAA levels in greenhouse-grown tomato fruit pericarp tissue determined by radioimmunoassay were reported to increase slightly for 30 days after pollination (Bohner and Bangerth 1988). More recently, changes in the levels of IAA during tomato seed development in the greenhouse were determined using an ELISA assay following HPLC purification where levels in the placenta and mesocarp, as well as seeds, were investigated (Hocher et al. 1992). The IAA levels found in the mesocarp tissue were comparable to the hormone levels we have found in the pericarp tissue at the various stages of development.

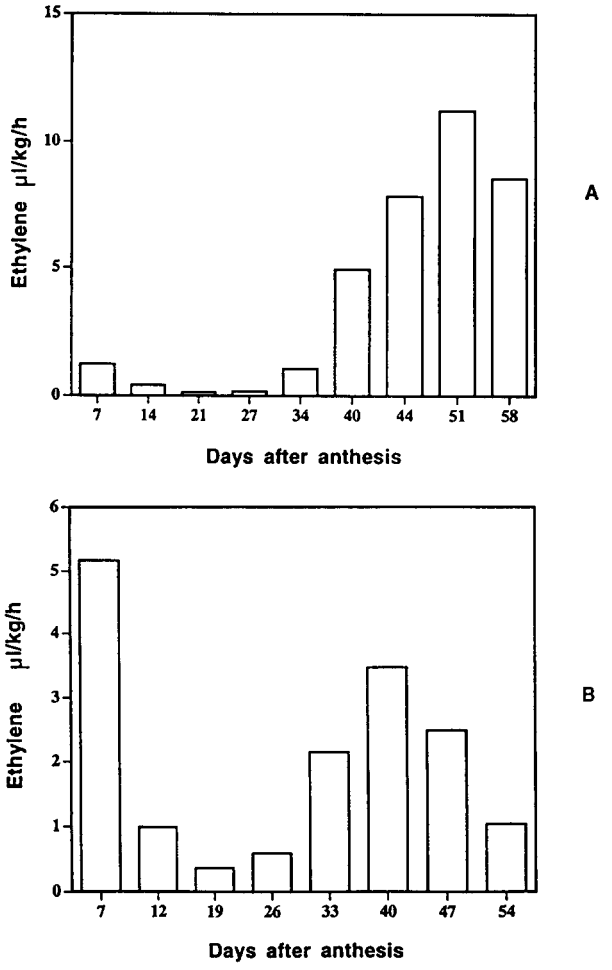

Fig. 4. Ethylene levels produced by tomato fruit measured at times of collection for IAA and ABA analyses: **(A)** Pik-Red; **(B)** Ailsa Craig.

As with IAA, the levels of ABA found in tomato pericarp tissue within the first 2 weeks after anthesis (cell division phase) were higher than levels found during the cell expansion phase prior to the onset of ripening when higher ABA levels were measured. High ABA levels (180 ng/g fw) during the first week after anthesis also had been found in tomato fruit pericarp tissue by glc analysis (Lacheene and El-Beltagy 1986) but not at 10 days after pollination in other studies (McGlasson et al. 1978, Bohner and Bangerth 1988). The earliest ABA levels measured in tomato mesocarp tissue by ELISA after HPLC purification (Hocher et al. 1991) were comparable to those found in tomato pericarp tissue.

Although our measurements were made of field-grown fruit subjected to variable growth conditions, the pattern of changes in levels of the two hormones, IAA and ABA, are similar in the two genotypes of tomato fruit from early stages of development through ripening. The larger-fruited Pik-Red cultivar tomatoes contained higher initial levels of IAA in the pericarp than did the Ailsa Craig, and these higher levels were present throughout fruit development and ripening. The higher levels of IAA found in the larger-fruited cultivar are in agreement with the previous suggestion of high levels of auxin being associated with larger fruit size (Mapelli et al. 1978). The ABA levels in Pik Red pericarp were lower than that in the Ailsa Craig pericarp and remained so throughout fruit development and ripening. This may be related to a slower rate of occurrence of senescence that was observed for the Pik-Red fruit.

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