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Changes in Indole-3-Acetic Acid, Indole-3-Acetic Acid Oxidase, and Peroxidase Isoenzymes in the Seeds of Developing Peach Fruits

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Abstract. Changes in indole-3-acetic acid (IAA) content of peach (*Prunus persica* L. Batsch cv. Merry) seeds were followed during fruit development. The highest concentration of IAA, 2.7 μg/g fresh weight, was found at the beginning of Stage III of fruit development, approximately 50–60 days after anthesis. The IAA-decarboxylating capacity of crude extracts of seeds was also greatest at 55–60 days after anthesis. Four soluble peroxidase isoenzymes were found on anionic electrophoresis. There were no marked changes in two isoenzymes (R_f 0.23 and 0.51), which were present in all three stages of fruit growth. There was a marked increase in a band at R_f 0.59 between Stages II and III, and a decrease in a band at R_f 0.68 from Stages II to III. Neither band (R_f 0.59 and 0.68) was present at Stage I.

The role of hormones in specific plant developmental processes has yet to be definitively established (Hanson and Trewavas 1982) and in particular their role in fruit growth and development. In peach, however, it is known that fruit growth is dependent on seed development (Tukey 1936), and that at certain stages of fruit development exogenously applied auxin can markedly affect fruit growth, as demonstrated by naphthaleneacetic-acid-induced abscission (Leuty and Bukovac 1968). Indole-3-acetic acid (IAA) may be an important factor in this critical relationship since the seed is an important source of this hormone (Lombard and Mitchell 1962, Powell and Pratt 1966). The steady-state concentration of IAA in plant tissue is believed to be the result of a dynamic balance between synthesis, degradation, and chemical conjugation (Bandurski 1984).

The involvement of IAA oxidase activity in the metabolic degradation of IAA in many plant tissues, including peach seeds (Ritzert et al. 1972), has been widely reported. However, two aspects should be noted: (1) that this activity has been associated in most cases with peroxidase activity; and (2) that there are many reports of the existence of multiple peroxidase isoenzymes which vary in their distribution in cells and tissues (van Huystee 1987).

We present herein data on changes in levels of IAA, IAA oxidase activity, and the soluble peroxidase isoenzymes in seeds of peach fruits during the course of seed and ovary wall development in a preliminary attempt to elucidate the relationship between seed hormone levels and fruit growth.

Materials and Methods

Plant Material

Fruits were sampled from a commercial cultivar ('Merry') of peach (*Prunus persica* L. Batsch), having a low chilling requirement, at periodic intervals during fruit development. The fruits were immediately transferred to the laboratory, frozen, and stored at −20°C prior to analysis. Fruit growth Stages I, II, and III represent 0 to 35, 35 to 45, and 45 to 80 days after anthesis, respectively.

Extraction and Measurement of Free and Esterified IAA

The extraction of IAA from the seeds followed the method previously described by Sánchez-Roldán et al. (1988). [1-^{14}C]IAA was used as an internal standard to monitor the loss of IAA during the extraction procedure. The final ether phase was evaporated to dryness under reduced pressure and stored at −20°C.

To determine esterified IAA, an aliquot of the methanolic extract was evaporated to dryness under a stream of nitrogen and hydrolyzed for 1 h in 1 N NaOH at 22–25°C. The mixture was then neutralized with 6 N HCl, and the procedure cited above for free IAA was followed.

The final dry residue was dissolved in 0.5 ml methanol and derivatized by the addition of 0.1 ml acetic anhydride and 0.05 ml trifluoroacetic acid. The reaction was run for 6 min in an ice bath before being terminated by adding 1 ml of 1 N NaOH. IAA was quantified by following the kinetics of the decomposition of the indole-α-pyrone derivative at 25°C by measuring the emission fluorescence intensity at 485 nm, as previously described by Sánchez-Roldán et al. (1988).

Extraction and Assay of IAA Oxidase

Crude extracts were prepared by homogenizing seeds, from the same seed population used for IAA measurements, in 0.05 M acetate buffer at pH 4.0 (buffer:fresh tissue ratio, 1:2) using an Ultra Turrax apparatus. The extracts were used to measure IAA oxidase activity after filtering through Whatman

Table 1. Concentration of free and esterified IAA in seeds of peach fruits during the three stages of fruit development.[a]

Stage of development	Days after anthesis	Free (ng/g fresh weight)	Esterified (ng/g fresh weight)
I	9	81 ± 8	150 ± 24
	20	45 ± 16	967 ± 244
II	27	323 ± 25	845 ± 431
	43	199 ± 17	905 ± 39
III	51	1185 ± 228	1125 ± 251
	58	2739 ± 143	444 ± 13

[a] Values are a mean ± SD for three determinations from a single extraction.

No. 1 paper. Enzyme activity was evaluated using [1-^{14}C]IAA (59 mCi/mmol, Amersham, Arlington Heights, IL, USA), as previously described (Valpuesta and Bukovac 1984), in the presence of 50 mM $MnCl_2$ and 50 mM 2,4-dichlorophenol. The $^{14}CO_2$ released during the 1-h incubation reacted with hyamine hydroxide and radioactivity was quantified by liquid scintillation counting.

Electrophoresis

Electrophoresis was performed following the method described by Davis (1964), using 7.5% polyacrylamide for the separating gel and 4% for the stacking gel.

Peroxidase Activity

Gels were stained to visualize and measure peroxidase activity by incubating in the presence of 6 mM *o*-dianisidine and 8.8 mM H_2O_2 in 20 mM phosphate buffer, pH 6.0, at room temperature for 20–30 min.

Protein Determination

The method of Lowry et al. (1951) was used, with bovine serum albumin as standard.

Results and Discussion

There were marked changes in free IAA levels in the seeds during the three developmental stages of fruit growth (Table 1). Levels in the range of 45–80 ng/g fresh wt^{-1} were found in Stages I and 200–300 ng/g fresh wt^{-1} in Stage II. However, there was a sharp increase during Stage III (Table 1). The marked increase in the concentration of free IAA during seed development is even

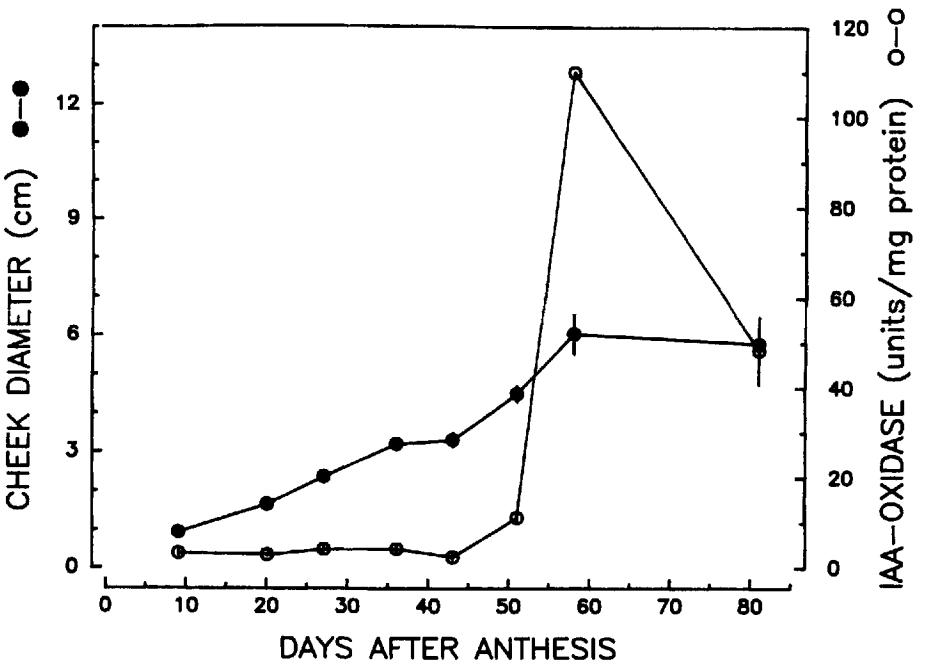

Fig. 1. IAA oxidase activity of crude extracts of peach seeds during the growth and development of the fruit. Fruit growth Stages I, II, and III are equivalent to 0–35, 35–45, and 45–80 days after anthesis, respectively. One unit of enzyme activity corresponds to 10 pmol of IAA decarboxylated per min at 30°C. Vertical bars through means represent SD for fruit growth and SE for enzyme activity. Where no bars are shown they are smaller than the data points.

more dramatic if expressed on a per-fruit basis because of the rapid increase in seed weight.

The kinetic procedure (Sánchez-Roldán et al. 1988) permitted the measurement of IAA in seed tissue very early in fruit development. Previous studies were limited to detection by bioassays (Lombard and Mitchell 1962, Powell and Pratt 1966) or quantified the hormone content on a whole-fruit basis (Miller et al. 1987). The low values found in the seeds of young fruits were unexpected since this actively growing tissue is viewed as an important source of auxin. IAA was found at higher levels during Stages II and III as compared to Stage I in sour cherry (Hopping and Bukovac 1975). Low seed levels of auxin in early stages of fruit growth may be related to high turnover of this hormone in seed tissues and/or it is quickly transported to the surrounding ovary wall.

A recent study on the 'Redhaven' peach, a high chilling cultivar, also documents an increase in seed IAA during the transition from Stage II to III (Miller et al. 1987), with a decrease during Stage III. There was a parallel increase in the mesocarp during the transition from Stage II to III; however, unlike the seed, IAA in the mesocarp continued to increase markedly during Stage III.

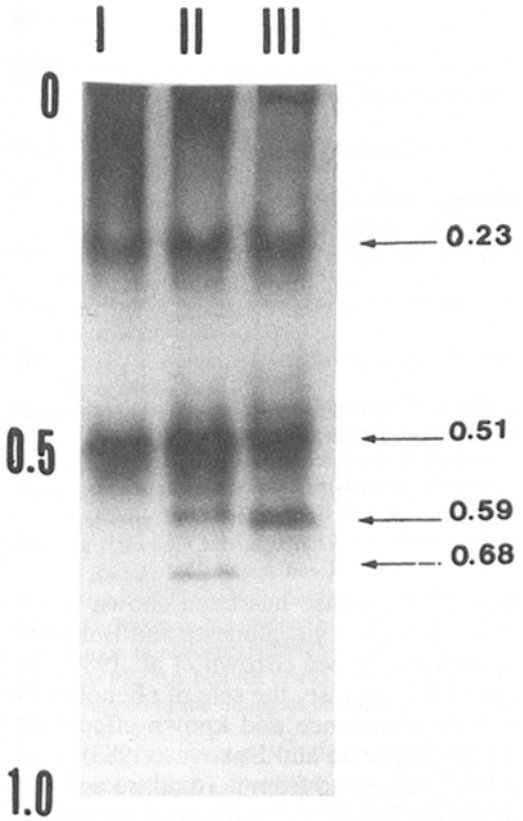

Fig. 2. Profile of the anionic electrophoresis of the soluble extracts from peach seeds from fruits at the three stages of development, stained for peroxidase activity. I, II, and III correspond to the samples taken (15, 35, and 65 days after anthesis, respectively) during the three stages of fruit development. Concentrated crude extract equivalent to 1 g of fresh tissue was loaded on each gel.

This continued increase during Stage III may be related to the rapid cell expansion occurring in the mesocarp during this period.

The esterified IAA concentration in the seed (Table 1) indicates, firstly, that esterified IAA is a significant component of the IAA pool; and, secondly, that some of the increase in IAA during Stage III may have been derived from the esterified fraction. The storage function of this conjugate has been well documented (Cohen and Bandurski 1982). High levels of another possible conjugate, a peptide-bound fraction, has also been detected in peach seed tissue (data not presented). However, the conditions needed for hydrolysis to release IAA from the conjugate (Bandurski and Schulze 1977) gave highly variable results.

IAA oxidase activity profile of seeds (crude extracts) during development of the fruit is illustrated in Fig. 1. The presence of polyvinylpyrrolidone in the extraction medium minimized interference by phenolics (Valpuesta and Bukovac 1984). Relatively low levels of IAA-oxidase activity were observed during Stages I and II of fruit growth (Fig. 1). A marked increase occurred at early Stage III (Fig. 1), which coincided with the increase in IAA (Table 1). Since both enzyme and substrate increased at the same time, the two most

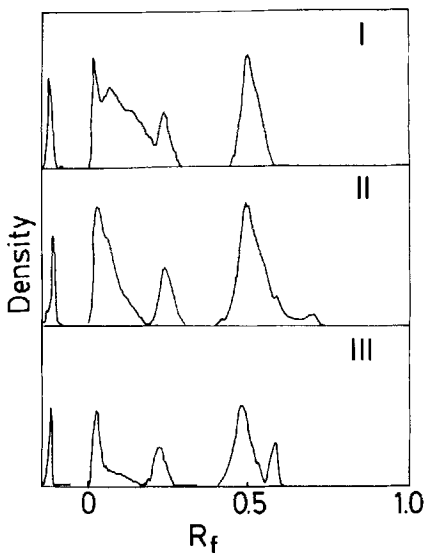

Fig. 3. Scanning-densitometer traces (made from photographs) of electrophoretic gels stained with *o*-dianisidine for peroxidase activity of seeds excised from peach fruits during Stages I, II, and III of fruit development.

likely reside in different compartments. IAA oxidase has been shown to be localized in different seed components of sour cherry (Valpuesta and Bukovac 1983), and even in different cell compartments in pea (Brown et al. 1986). In view of the interaction between IAA and IAA-oxidase, the role of phenolics in vivo must be recognized because of their abundance and known effects on IAA-oxidase activity (Ritzert et al., 1972; Valpuesta and Bukovac, 1983).

Since IAA oxidase activity has not been separated from peroxidase activity (van Huystee and Chibbar 1987), peroxidase isoenzyme profiles of the seeds, at the three stages of fruit growth, were developed (Fig. 2). Four bands (R_f 0.23, 0.51, 0.59, and 0.68) were found with peroxidase activity. Two main bands, R_f 0.23 and 0.51, were present at similar levels at all three stages, while the band at R_f 0.59 increased with fruit development (Stages II and III). A weak band at R_f 0.68 was present during Stage II, but not detected during Stages I and III (Figs. 2 and 3). Two main functions have been assigned to these isoenzymes, they serve either in the degradation of IAA or in the lignification processes (van Huystee 1987). To correlate the specific isoenzymatic changes reported here with either of these functions will require further research on enzyme purification, which is currently underway.

Our data demonstrate significant changes in IAA concentration and IAA degrading activity in seeds of peach during fruit development. These changes are greater in the Stage II–III transition. These data are useful in providing a basis for further study on the relationship of seed-produced auxin and function of IAA oxidase in the role of the seed in fruit growth and development.

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