J Plant Growth Regul (1990) 9:171–174

Journal of
Plant
Growth
Regulation

Indole-3-Acetic Acid Content and Glutamine Synthetase Activity in the Pericarp, and Peroxidase Activity and Isoenzymes in the Meso- and Exocarp of Growing Peach Fruits

Cristina Sánchez-Roldán,[1] Antonio Heredia,[1] Victoriano Valpuesta,[1] and Martin J. Bukovac[2]

[1]Departamento de Bioquímica y Biología Molecular, Universidad de Málaga, 29071 Málaga, Spain; and [2]Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, USA

Received October 13, 1989; accepted January 2, 1990

Abstract. The indole-3-acetic acid (IAA) content in peach pericarp (*Prunus persica* L. Batsch cv. Merry) was highest at early stage I of development (~200 ng/g fresh wt), decreased to the lowest level during stage II, and rose again at stage III to 60–70 ng/g fresh wt. High activity of glutamine synthetase was found in the pericarp during stage I. The soluble peroxidase activity was highest in the meso- and exocarp at stage II, and isoenzymatic changes in this fraction corresponded to the transition from cationic isoenzymes, predominant at stage I, to anionic isoenzymes at stage III. The ionically bound peroxidase activity in these tissues was highest at stage I. The three developmental stages showed marked differences in auxin content and enzyme activities; for peroxidases these changes reflect a developmental expression pattern for the isoenzymes.

Fruit growth and development appear to be well-regulated, genetically determined events where specific changes in enzyme activities have been reported (Brady 1987). The rapid early growth of fruit is probably stimulated by plant growth regulators, especially the auxin, indole-3-acetic acid (IAA). The expression pattern of glutamine synthetase (which provides nitrogenous precursors for many metabolites, structural components, and growth regulators) and peroxidase [which is involved in cell wall formation (Cassab and Varner 1988) and possibly in auxin catabolism (Grambow 1986)] might be expected to change during fruit development.

Two significant features of peach fruit growth are as follows: (1) the presence of three well-defined developmental stages, and (2) the dependence of early stages of fruit growth on seed development (Tukey 1936). Thus, IAA content and peroxidase activity have been previously determined in the seeds of fruits at the three stages of development (Valpuesta et al. 1989). Seed IAA content has been found to reach its highest level during stage III, and the peroxidase isoenzyme pattern has been found to change markedly during development.

We present herein data on IAA and glutamine synthetase of the pericarp, and peroxidase activities of the meso- and exocarp during fruit development. IAA content during development of whole fruits has been reported by Miller et al. (1987). However, these previous findings now are augmented by the study of glutamine synthetase activity during the entire growing period, as a marker for the growing processes (Miflin and Lea 1980), and soluble, ionically and covalently bound peroxidase activities. These probably represent a high percentage of total peroxidase activity in the peach pericarp at early developmental stages.

Materials and Methods

Plant Material

Peach fruits (*Prunus persica* L. Batsch cv. Merry) with a low-chilling requirement were sampled periodically during development and stored as previously described (Valpuesta et al. 1989).

Glutamine Synthetase Extraction and Assay

Pericarp tissue was homogenized (Ultra Turrax homogenizer) in 50 mM Tris-HCl buffer (fresh tissue weight to buffer volume, 1:2) containing 2 mM EDTA and 20 mM 2-mercaptoethanol, pH 8.0. After filtration through two layers of muslin, 1 mM phenyl me-

thyl sulfonyl fluoride (PMSF) was added and then centrifuged at 25,000 *g* for 20 min. The same extraction procedure was repeated with the pellet, and the two supernatants were pooled before assaying for glutamine synthetase activity.

Glutamine synthetase activity was measured by the transferase assay described by Shapiro and Stadtman (1970): units of enzyme activity corresponded to μmole of product, Γ-glutamyl hydroxamate, formed per minute under described experimental conditions.

Extraction and Measurement of Free IAA

IAA was extracted from the pericarp as previously described using 1-^{14}C-IAA as an internal standard and assayed by a spectrofluorimetric kinetic method (Sánchez-Roldán et al. 1988).

Peroxidase Extraction and Assay

The meso- and exocarp were combined and ground in 50 mM sodium phosphate buffer, pH 6.0 (fresh tissue to buffer ratio, 1:2.5, wt/vol), using an Ultra Turrax homogenizer at full speed. After filtration through two layers of muslin and centrifugation at 25,000 *g* for 20 min at 4°C, the supernatant was recovered and the residue reextracted with the same buffer (1:2 ratio). After centrifugation, the two supernatants were pooled and termed the crude soluble extract. The pellet and the filtration residues were resuspended in phosphate buffer containing 1 M KCl (1:2 ratio, wt/vol) and stirred overnight at 0–4°C. Following filtration and cold centrifugation at 25,000 *g* for 20 min, the supernatant contained the ionically bound fraction. The pellet was resuspended in 0.1 M sodium phosphate buffer at pH 5.25 (1:2 ratio, wt/vol) containing cellulase (0.25%) and pectinase (0.15%) and incubated with continuous stirring at room temperature for 4 h. The supernatant obtained after filtration and centrifugation (25,000 *g*, 20 min) contained the covalently bound fraction.

All extracts were exhaustively dialyzed against 25 mM sodium phosphate buffer, pH 6.0, for about 24 h.

Peroxidase activity was defined as the increase in absorbance at 450 nm of enzyme extracts incubated with 0.26 mM *o*-dianisidine and 8.8 mM hydrogen peroxide in 20 mM phosphate buffer, pH 6.0, at 25°C.

Electrophoresis

Anionic polyacrylamide gel electrophoresis (PAGE) was performed as described by Davis (1964) using 7.5% polyacrylamide for the separating and 4% for the stacking gel.

Cationic PAGE electrophoresis was performed using the procedure described by Reisfield et al. (1962).

Results and Discussion

The activity of glutamine synthetase, the enzyme catalyzing the first step in amino acid biosynthesis from ammonia (Miflin and Lea 1980), was extremely high in young peach tissue (Fig. 1). The enzyme's activity decreased sharply during early

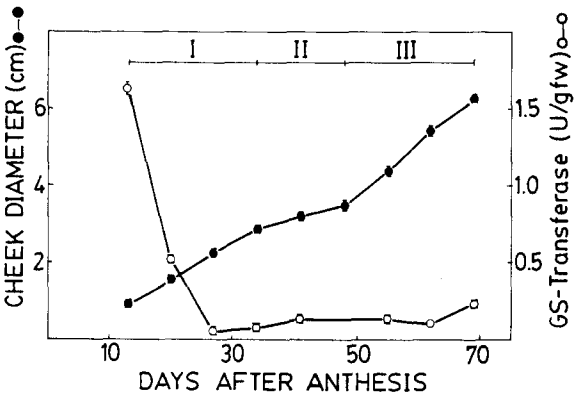

Fig. 1. Glutamine synthetase activity, measured by the transferase assay, in the pericarp of growing peach fruits. Fruit growth was determined by measurement of cheek diameter. Vertical bars represent SD for fruit growth and SE for enzyme activity based on duplicate samples and three determinations for each. Where no bars are shown they are smaller than the symbols.

fruit development, reaching its lowest level at early stage II, and remaining low for the remainder of fruit growth. The high activity of glutamine synthetase during stage I, when the cells of the fruitlet are dividing rapidly, undoubtedly reflects the key role of this enzyme in growth processes; its product, glutamine, is the main source of nitrogen in the synthesis of nucleotides and amino acids and their products, including IAA.

IAA has been shown to alter gene expression in plant tissues (Hagen 1987). Whether the high IAA content of young peaches is responsible for the induction of glutamine synthetase remains to be determined.

Transition between the different developmental stages is also reflected in the IAA content of peach pericarp tissue (Fig. 2). From 200 ng/g fresh wt at early stage I, IAA decreased to the lowest level during stage II, and rose again to 60–70 ng/g fresh wt at the end of stage III. These values are in the same range as those previously reported for "Redhaven" peach fruits at stage III (Miller et al. 1987). Peaks found during stages I and III may be related to the involvement of IAA in cell division and enlargement (Davies 1987), predominant processes occurring during these two developmental stages, respectively. IAA content of the seeds of these fruits was low at stage I but increased at stage III (Valpuesta et al. 1989). Since seeds are often considered the source of hormone for fruit growth (Davies 1987), IAA may be rapidly exported from the seed to the surrounding tissue during stage I, thus explaining the low IAA content of the seed at this time (Valpuesta et al. 1989) and the requirement

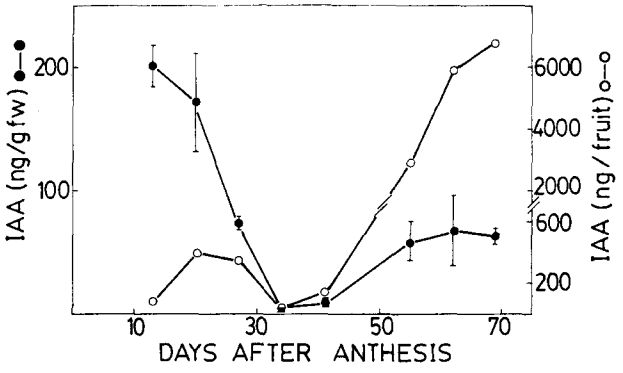

Fig. 2. Levels of free IAA in the pericarp tissue of peach fruits during fruit development. Values are mean ± SE (represented by vertical bars) for three determinations from two extracts.

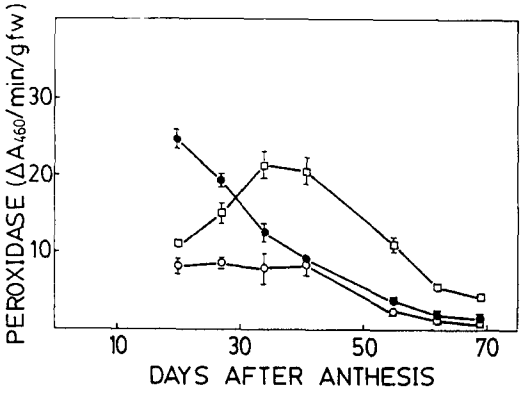

Fig. 3. Peroxidase activity in the soluble (–□–), ionically (–●–), and covalently-bound (–○–) extracts obtained from meso- plus exocarp peach tissues during fruit growth. Vertical bars represent mean ± SE values for three determinations from three extracts. Where no bars are shown they are smaller than the symbols.

of a viable seed for fruit growth at this stage of development (Tukey 1936).

Peroxidase activities in peach meso- and exocarp were measured in the soluble and cell wall bound extracts (Fig. 3). To follow peroxidase activity, only the meso- and exocarp tissues were sampled, since lignification would occur in the endocarp during fruit development; this event could affect the isoperoxidase profile in the whole pericarp. Sampling therefore was initiated 20 days after full bloom when these tissues could be distinguished visually. The most significant changes occurred in the ionically bound peroxidase fraction, which was predominant at stage I and decreased continuously during fruit growth. Cell wall bound peroxidases have been implicated in processes, such as lignin synthesis (Fukuda and Komamine 1982) and extensin crosslinking (Cooper and Varner 1984). Since these processes are required for cell wall formation, which is coupled to cell division (Meyer and Herth 1982), high peroxidase activities would be anticipated during growth stage I.

Peroxidase activity in the soluble fraction increased from stage I to II and then decreased during stage III (Fig. 3). These changes are better understood when compared with the isoenzymatic profiles in Figs. 4 and 5. It should be stressed that accurate quantitative inferences cannot be made from these profiles because *o*-dianisidine was used as the substrate and different isoenzymes vary in their affinities for phenolic substrates (Siegel and Siegel 1986). However, it is clear that the two cationic isoenzymes present at stages I and II disappear during stage III, whereas an anionic isoenzyme becomes dominant during stage III. Previous studies have suggested that cationic isoperoxidases may be responsible for IAA catabolism and anionic isoenzymes for cell wall lignification (Gaspar et al.

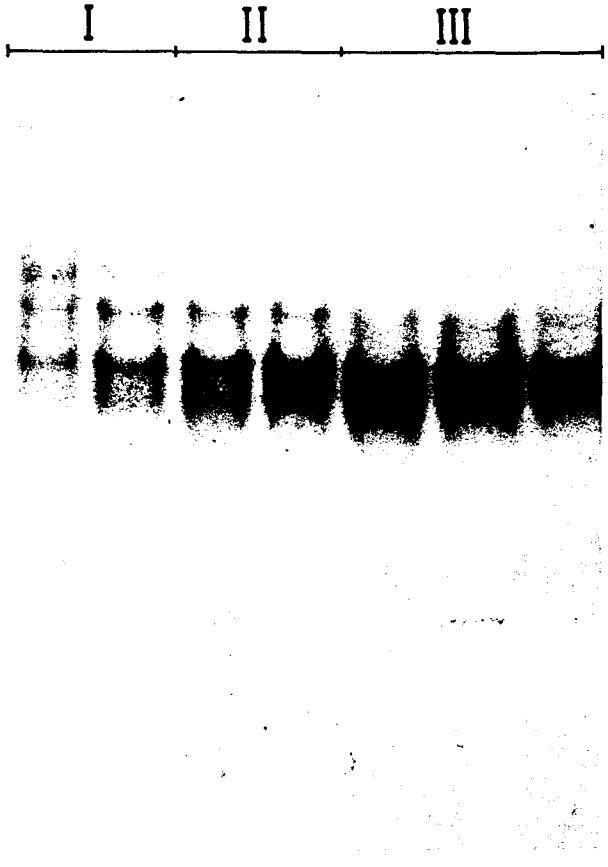

Fig. 4. Peroxidase isoenzyme profiles of soluble extracts from meso- plus exocarp tissues, obtained by anionic electrophoresis and staining for peroxidase activity. Samples were harvested 20 and 27 days after anthesis for stage I; 34 and 41 days for stage II; and 55, 62, and 69 days for stage III.

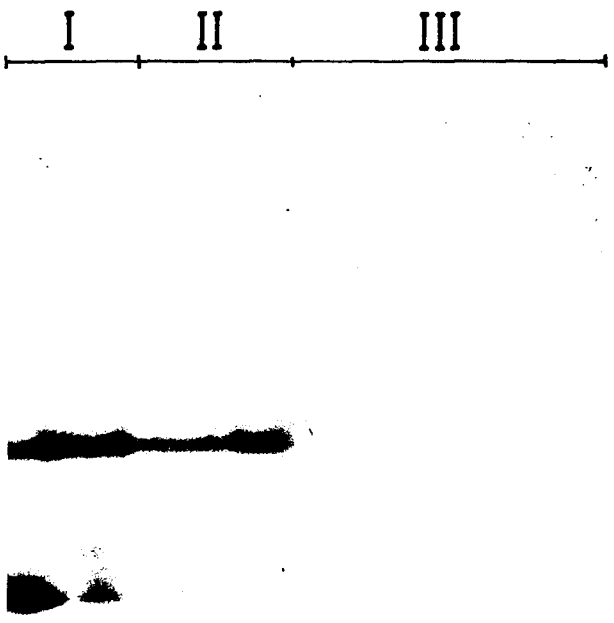

Fig. 5. Peroxidase isoenzyme profiles obtained by cationic electrophoresis of soluble extracts from meso- plus exocarp tissues and staining for peroxidase activity. Sampling dates as in Fig. 4.

1985). More research is needed to link the isoenzymatic changes reported here to these functions. It is noteworthy, however, that specific isoenzyme changes were detected in the meso- and exocarp of peach fruits during development, and that they generally agree with the two-step model previously proposed for peroxidase changes during plant growth and development (Gaspar et al. 1985); an initial increase in the cationic isoperoxidases which is followed by an increase in anionic isoenzymes. Changes in peroxidase isoenzymes in the endocarp tissue of peach fruits are presently under study.

Acknowledgments. This work was supported by Grant no. 8510-001 from the US–Spanish Joint Committee for Scientific and Technological Cooperation and by Grant no. PB86- 0167-CO3-03 from Dirección General de Investigación Científica y Técnica. The authors are indebted to A. R. Treitero for technical help.

References

Brady JC (1987) Fruit ripening. Annu Rev Plant Physiol 38:155–178

Cassab GJ, Varner JE (1988) Cell wall proteins. Annu Rev Plant Physiol 39:321–353

Cooper JB, Varner JE (1984) Cross-linking of soluble extensin in isolated cell walls. Plant Physiol 76:414–417

Davies PJ (1987) The plant hormones: Their nature, occurrence, and functions. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Martinus Nijhoff, Dordrecht, pp 1–11

Davis BJ (1964) Disc electrophoresis. II. Method and application to human serum proteins. Ann NY Acad Sci 121:404–427

Fukuda H, Komamine A (1982) Lignin synthesis and its related enzymes as markers of tracheary-element differentiation in single cells isolated from the mesophyll of *Zinnia elegans*. Planta 155:423–430

Gaspar T, Penel C, Castillo FJ, Greppin H (1985) A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiol Plant 64:418–423

Grambow HJ (1986) Pathway and mechanism of the peroxidase-catalyzed degradation of indole-3-acetic acid. In: Greppin H, Penel C, Gaspar T (eds) Molecular and physiological aspects of plant peroxidases. University of Geneve Press, Geneve, pp 31–41

Hagen G (1987) The control of gene expression by auxin. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Martinus Nijhoff, Dordrecht, pp 159–163

Meyer Y, Herth W (1982) Interactions of cell-wall formation and cell division in higher plant cells. In: Brown RM Jr (ed) Cellulose and other natural polymer systems. Plenum Press, New York, pp 149–165

Miflin BJ, Lea PJ (1980) Ammonia assimilation. In: Stumpf PK, Conn EE (eds) The biochemistry of plants, Vol. 5. Academic Press, New York, pp 169–202

Miller AN, Walsh CS, Cohen JD (1987) Measurement of indole-3-acetic acid in peach fruits (*Prunus persica* L. Batsch cv Redhaven) during development. Plant Physiol 84:491–494

Reisfield RA, Lewis VJ, Williams DE (1962) Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature 195:281–283

Sánchez-Roldán C, Quesada MA, Bukovac MJ, Valpuesta V, Heredia A (1988) Improved procedure for determining free and conjugated indole-3-acetic acid by a spectrofluorimetric kinetic method. Anal Lett 21:1535–1543

Shapiro BM, Stadtman ER (1970) Glutamine synthetase (*Escherichia coli*). Meth Enzymol 17:910–922

Siegel BZ, Siegel SM (1986) Differential substrate specificity among peroxidases: A functional view of phyletic relations. In: Greppin H, Penel C, Gaspar T (eds) Molecular and physiological aspects of plant peroxidases. University of Geneve Press, Geneve, pp 131–142

Tukey HB (1936) Development of cherry and peach fruits as affected by destruction of the embryo. Bot Gaz 98:1–24

Valpuesta V, Quesada MA, Sánchez-Roldán C, Tigier HA, Heredia A, Bukovac MJ (1989) Changes in indole-3-acetic acid, indole-3-acetic acid oxidase, and peroxidase isoenzymes in the seeds of developing peach fruits. J Plant Growth Regul 8:255–261