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INTRACELLULAR LOCALIZATION OF POLY-PHENOLOXIDASE IN AVOCADO FRUIT

AHP ENGELBRECHT
DEPARTMENT OF BOTANY, RAND AFRIKAANS UNIVERSITY

OPSOMMING

Die intrasellulêre lokalisasie van PPO in die endokarp van avokadovrugte is ondersoek. PPO-positiewe reaksieprodukte is aanwesig in die tilakoiede en prolamellare liggaampies van die etioplaste van normale vrugte, maar is afwesig in die etioplaste van die pulpvlekvrugte.

SUMMARY

The intracellular localization of PPO in the endocarp of avocado fruit was studied. PPO positive reaction products are present in the thylakoids and prolamellar bodies of the etioplasts of normal fruit, but are absent in the etioplasts of pulp spot affected fruit.

INTRODUCTION

The browning potential of avocado fruit is directly related to the polyphenoloxidase (PPO) activity and the phenol content of the fruit (Kahn, 1975; Golan, Kahn & Sadovski, 1977). The PPO activity is localized in the plastid thylakoids (Martyn, Samuelson, Freeman, 1979). The present study was initiated to determine the intracellular locality of PPO in avocado fruit and the possible role of PPO in the development of pulp spot.

MATERIALS AND METHODS

A modification of the techniques developed by Czaninski and Catesson (1974) was used.

Endocarp tissue of mature normal and pulp spot affected fruit was used. The tissue was fixed in a buffered (0,05 M sodium cacodylate, pH 7,2) solution of 4% glutaraldehyde for 90 min. After rinsing in buffer the sample was separated into three groups. One group was boiled in distilled water for 10 min. The second group was incubated in 0,02 M sodium diethylcarbamate (DIECA) an inhibitor of PPO, at 25 C for 60 min. The third group had no additional treatment.

The samples were then preincubated overnight at 4 C in a DOPA solution (50 mg D-beta-3,4-dihydroxiphenylalanine or L-beta-3,4-dihydroxyphenylalanine in 10 mf of 0,067 M phosphate buffer, pH 7,0). This was followed by incubation in the same solution for

60 min at 37 C. The samples were rinsed five times in 0,5 M sucrose and postfixed in 2% OsO_4 in 0,067 M phosphate buffer, pH 7,0. After rinsing in buffer and dehydration in an ethanol series, the samples were embedded in a low-viscosity resin. Unstained sections or sections stained with 5% $KMnO_4$, were examined.

RESULTS

The normal tissue preincubated in DOPA directly after fixation showed electron-dense deposits in the thylakoids and prolamellar bodies of the etioplasts (Fig. 1, 2 and 3). The deposits were formed by the positive reaction of PPO with DOPA. No deposits were present in the mitochondria, dictyosomes and endoplasmic reticulum of this tissue (Fig. 4).

Normal tissue treated by boiling or incubation with DIECA before DOPA incubation, showed no positive PPO reaction in the etioplasts. The enzyme was apparently inactivated by the heat and inhibited by DIECA.

No PPO positive reaction products were found in the pulp spot affected tissue.

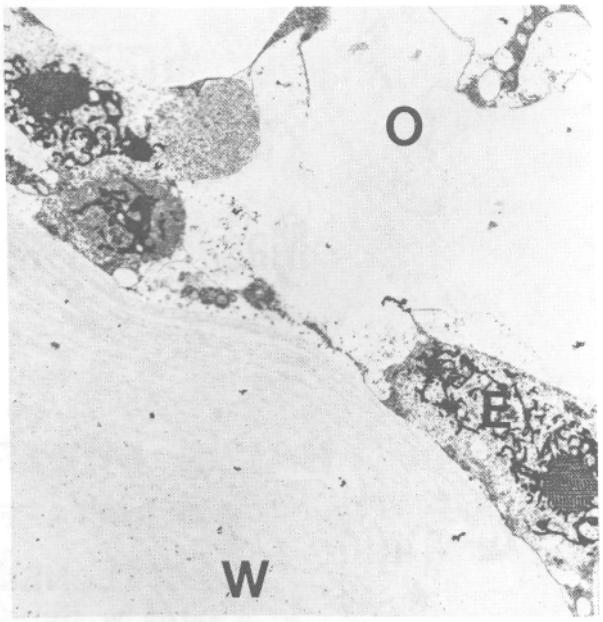

FIG. 1: Endocarp parenchyma cell from normal tissue. Electron-dense deposits are present in the etioplasts (E). (O, oil; W, cell wall)

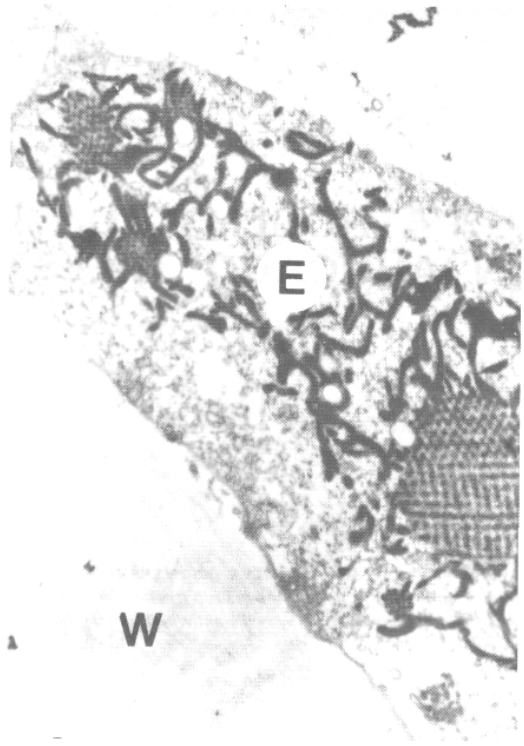

FIG. 2: Etioplast (E) from normal endocarp tissue. (W, cell wall)

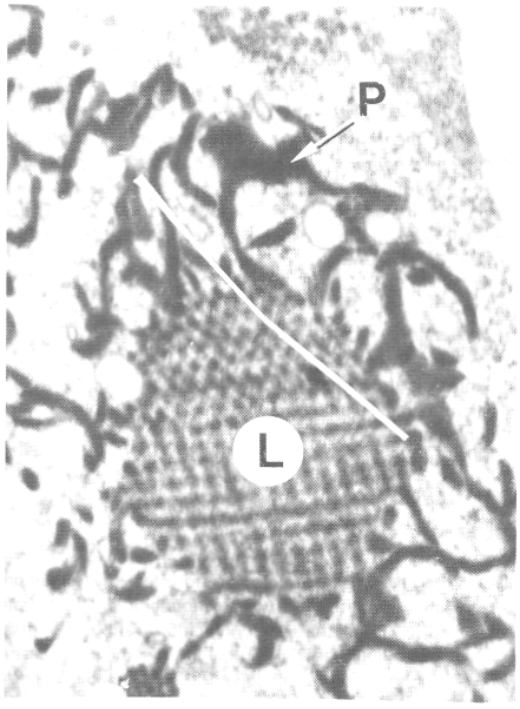

FIG. 3: Electron-dense deposits (P) in the thylakoid space and prolamellar body (L) of an etioplast from normal endocarp tissue

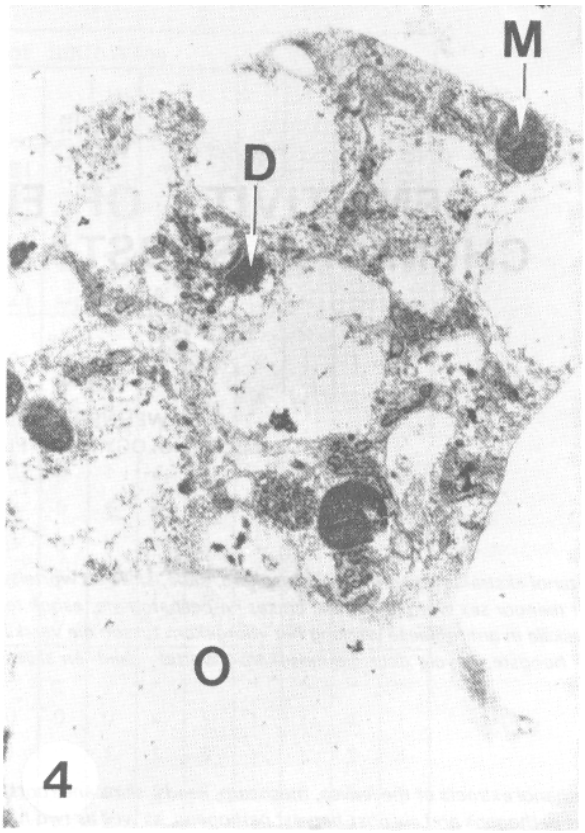

FIG. 4: Endocarp parenchyma cell from normal tissue. (D, dictyosome; O oil; M, mitochondrion)

DISCUSSION

The presence of PPO activity in the normal tissue and its absence in the pulp spot affected tissue, indicates a possible involvement of PPO in the development of pulp spot. Precisely what this involvement was, could not be determined in this study.

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