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Localisation of polyphenol oxidase activity in avocado fruit

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SYNOPSIS
Polyphenol oxidase positive reaction products are present in the thylakoids of plastids from normal mesocarp tissue, and absent in the thylakoids of plastids from brown abnormal mesocarp tissue.

INTRODUCTION

The browning potential of avocado fruit is directly related to the polyphenol oxidase (PPO) activity and the phenol content of the fruit (Kahn, 1975; Golan et al, 1977). In higher plants the PPO is present intracellular or in the cell walls(Coombs et al, 1974). Intracellular it is present in the plastids (Czaninski & Catesson, 1974; Mayer & Harel, 1979; Shomer et al, 1979), microbodies (Sharon & Kahn, 1979), peroxisomes and microsomes (Ruis, 1972; Flurkey & Jen, 1978). In plastids the PPO activity is limited to the thylakoids (Olah & Mueller, 1981).

The present study was initiated to determine the locality of PPO in avocado fruit and the possible role of PPO in the development of postharvest browning of the mesocarp.

MATERIALS AND METHODS

Mesocarp samples were taken from soft post-climacteric avocado (*Persea americana* Mill cv Fuerte) fruit with post-harvest browning symptoms. Samples were taken from the green, yellow and brown areas of the mesocarp and fixed in a 4 per cent glutaraldehyde solution in 0,05 mol dm^{-3} sodium cacodylate buffer (pH 7,2) for 90 min. After fixing and rinsing in buffer, each sample was separated into three groups. One group of each sample was boiled in distilled water for 10 min. The second group of each sample was incubated in a 0,002 mol dm^{-3} solution of sodium diethylcarbamate (DIECA) in distilled water at 25°C for 60 min. DIECA is an inhibitor of PPO. The third group of each sample had no additional treatment. The samples were then incubated overnight at 4°C in a DOPA solution (50 mg D-beta-3,4-dihydroxiphenylalanine [Czaninski & Catesson, 1977] or L-beta -3,4-dihydroxiphenylalanine) in 10 mt of 0,067 mol dm^{-3} phosphate buffer (pH 7,0). This was followed by incubation in the same solution for 60 min at 37°C. After incubation, the samples were rinsed five times in 0,5 mol $dm^{-}3$ sucrose and post-fixed in two per cent phosphate-buffered osmium tetroxide for 90 min, rinsed in buffer and dehydrated through a graded ethanol series, followed by 1,2-epoxypropane and embedded in a low viscosity resin. Unstained sections or sections stained with 5 per

cent potassium permanganate were examined.

RESULTS

The normal tissue incubated in DOPA directly after glutaraldehyde fixation shows electron-dense deposits in the prolamellar bodies of the etioplasts (Figure 1) and the. thylakoids of the chloroplasts (Figures 2 & 3). The deposits are formed by the positive reaction of PPO with DOPA. No similar deposits are present in the rest of the cell organelles from the yellow area (Figure 4) and green area (Figure 5) of the normal part of the mesocarp.

Normal tissue treated by boiling (Figure 6) or pre-incubation with DIECA before DOPA incubation, shows no positive PPO reaction products in the plastids.

No PPO positive reaction products are found in the plastids of the brown abnormal mesocarp tissue (Figures 7 & 8) and the thylakoid membranes appear structurally normal.

DISCUSSION

The presence of PPO activity in the plastids of normal mesocarp tissue, and its absence in the plastids of the abnormal brown mesocarp tissue, indicate a possible involvement of PPO in the development of mesocarp browning in avocado fruit. The movement of the PPO through the thylakoid membranes causes no visible structural changes in the membranes. This movement is probably triggered by the presence of the substrate in the surrounding cytoplasm.

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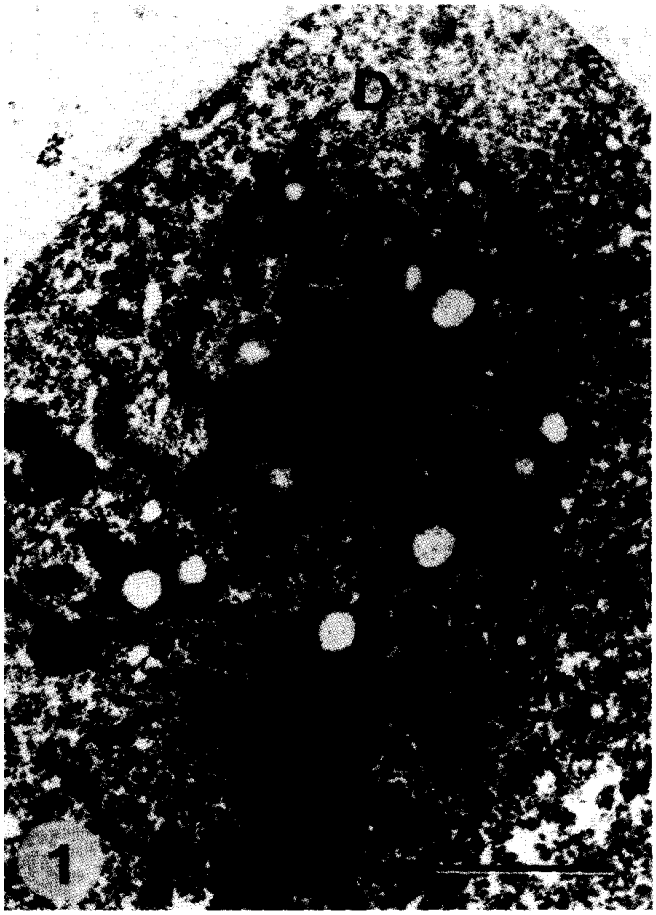

Fig 1 Yellow tissue + DOPA. Etioplast with PPO positive reaction product deposits (D) in the prolamellar body. (Marker = 0,5 µm).

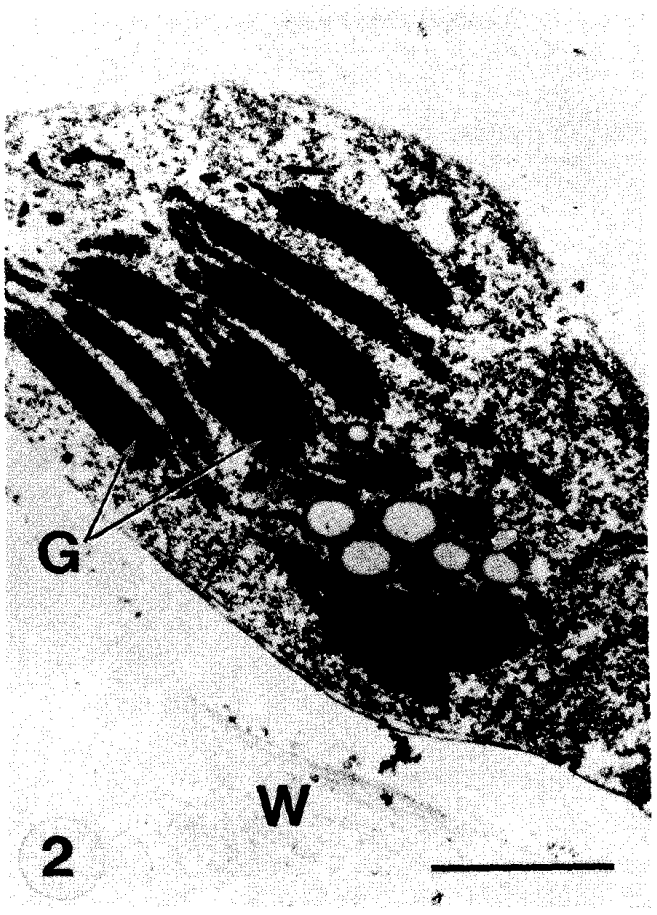

Fig 2 Green tissue + DOPA. Chloroplast with PPO positive reaction product deposits in the thylakoids of the grana (G). (W, cell wall; marker = 1,0 µm).

Fig 3 Green tissue + DOPA. Higher magnification of the chloroplast in Figure 2. (D, PPO positive reaction product deposits; marker = 0,5 μm).

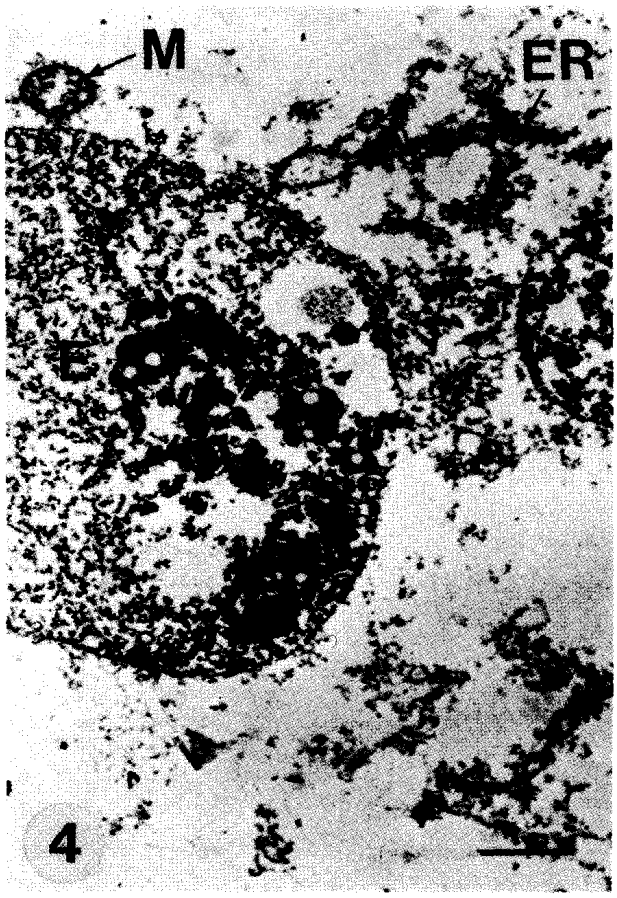

Fig 4 Yellow tissue + DOPA. Part of a mesocarp cell. Etioplast (E) with PPO positive reaction product deposits. No deposits present in the mitochondria (M) and endoplasmic reticulum (ER). (Marker = 1,0 μm).

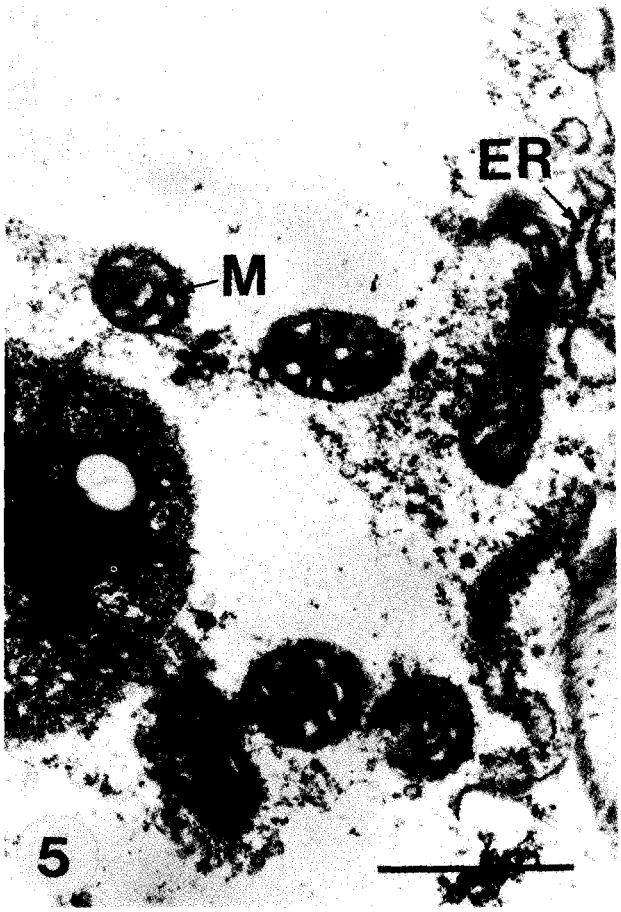

Fig 5 Green tissue + DOPA. No PPO positive reaction product deposits present in the mitochondria (M) and endoplasmic reticulum (ER). (Marker = 1,0 µm).

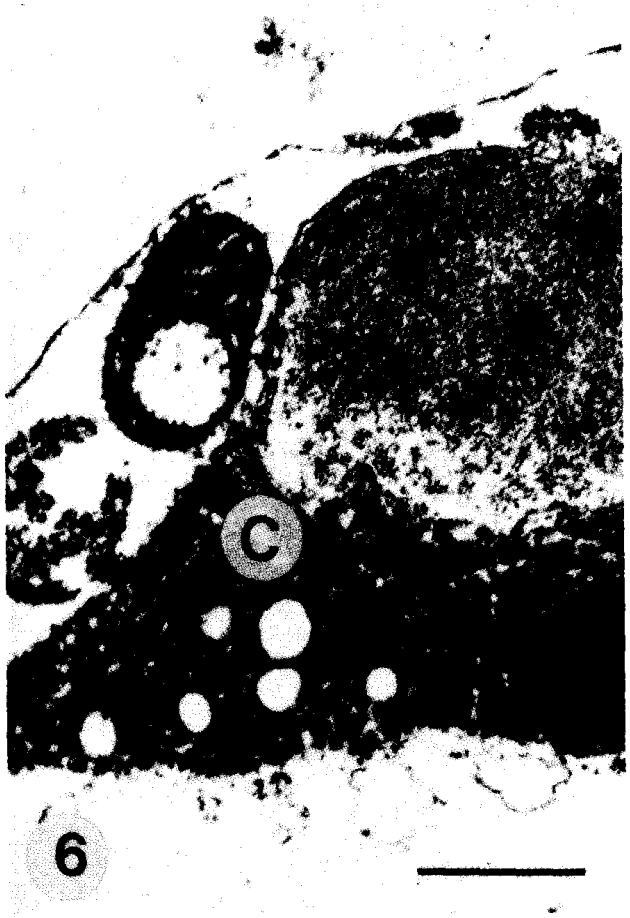

Fig 6 Green tissue + boiling. No PPO positive reaction product deposits present in the chloroplast (C). (Marker = 0,5 µm).

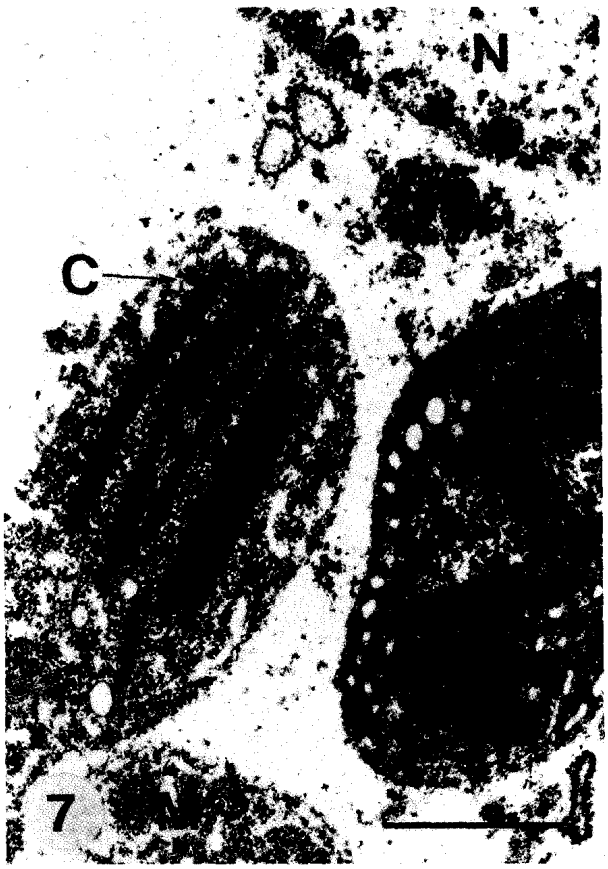

Fig 7 Brown tissue + DOPA. Chloroplasts (C) without PPO positive reaction product deposits. (N, nucleus; marker = 1,0 μm).

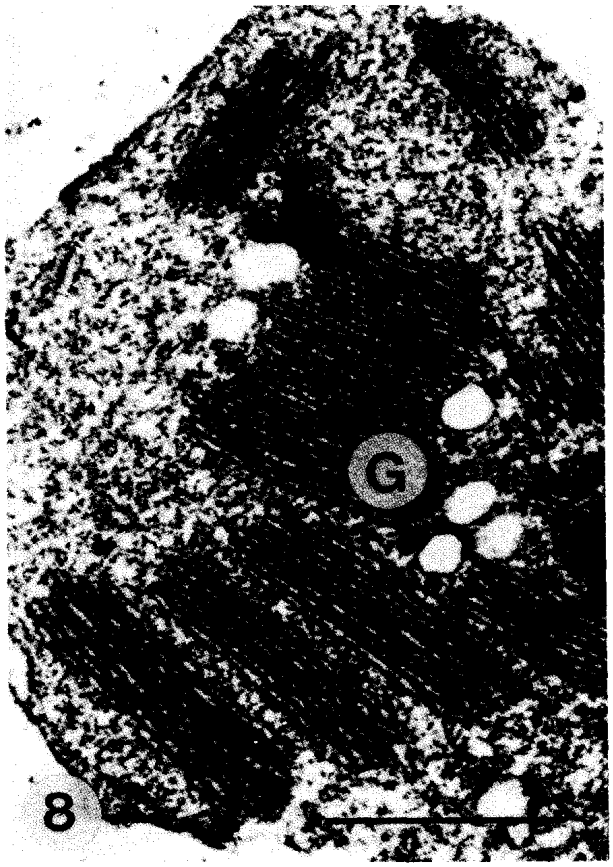

Fig 8 Brown tissue + DOPA. Chloroplast grana (G) to show absence of PPO positive reaction product deposits in the thylakoids. (Marker = 0,5 μm).