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HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON TUBERIZING ROOTS IN SWEET POTATO (*Ipomoea batatas* L., CV 049)

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ABSTRACT

Histochemical studies on roots of sweet potato at different stages of development revealed the presence of guaiacol oxidase and 3,3-diaminobenzidine (DAB) oxidase activities in all cells except for xylem vessels. In roots at primary and secondary stages of development similar activities were observed with highest activities in the endodermal and companion cells. In primary roots high activities were also observed in the hypodermal cells. In roots showing secondary growth cells of the periderm, which developed from the hypodermis, as well as xylem tracheids continued to show high activities. Syringaldazine oxidase showed a lag phase with weak activity in the cortical cells and high activities in all cells undergoing lignification in roots at all stages of development. Biochemical assays of guaiacol oxidase indicated high activities in both the soluble cell free (CF) and the solubilized ionically wall bound (I) enzyme fractions with the I fraction consistently showing higher activities in all stages of tuberizing roots. Qualitative tests showed that syringaldazine oxidase activity was restricted to the CF fraction. Both cofactor dependent and independent indole acetic acid (IAA) oxidase were assayed with only the I fraction in all stages of tuberizing roots showing cofactor dependent IAA oxidase activity. The implications of these results are discussed in relation to the anatomical changes and physiological processes taking place in the developing tuberous root.

INTRODUCTION

Wilson (1970) defined the sweet potato tuberous root as the localized lateral swelling that develops in certain roots of the sweet potato root system. He also reported that tuberization of the sweet potato root necessitated the suppression of lignin biosynthesis and the formation of secondary cambia leading to cell division and expansion. This suppression of lignin biosynthesis was suggested by Artschwager (1924) who noted that the activity of the vascular cambium in the sweet potato root produced mostly thin-walled parenchyma. Some of these non-lignified cells or xylem parenchyma later display secondary anomalous cambial activity producing a large number of parenchyma storage cells that greatly contribute to lateral

expansion of the root through pith development (Esau, 1977; Wilson and Lowe, 1973).

The suppression of lignin biosynthesis and formation of secondary anomalous cambia are most likely controlled by endogenous IAA levels (Sirju-Charran, 1983). IAA levels in turn may be regulated through the activities of IAA oxidase and/or peroxidase (Meudt and Stecher, 1972). The literature has shown an inverse relationship between IAA oxidase and/or peroxidase activities and IAA content and growth rate (Akita et al., 1962; Mills and Crowden, 1968; Birecka and Galston, 1970; Gardiner and Cleland, 1974). Peroxidases have also been implicated in the final stage of lignin biosynthesis (Mader et al, 1977). Duncan and Wilson (1968, unpublished data quoted in Wilson and Lowe, 1973) demonstrated the absence of peroxidase activity in the cortex and pith of tuberous sweet potato roots. This would imply that lignification of these tissues would not be possible, thus enhancing the tuberization process.

The ability of peroxidase to catalyze the destruction of IAA and to take part in the final stage of lignin biosynthesis, their widespread distribution in the sweet potato roots and their possible involvement in other roles that would enhance root tuberization and development such as primary wall formation (Fry, 1986), wound healing (Thomas and Delincee, 1979) and disease resistance (Fehrmann and Dimond, 1966; Johnson and Cunningham, 1972) were the reasons for studying this enzyme during the tuberization of roots.

Peroxidases may be studied by the simultaneous use of histochemical and biochemical techniques. A histochemical study, coupled with the use of proper control experiments, gives accurate information on the localization and relative activity of peroxidase *in situ* while a biochemical study is an *in vitro* study that can only indicate what might be occurring *in vivo*.

The aim of this study was to compare *in situ* and *in vitro* the activities of peroxidases and observe their distribution in the developing roots of sweet potato at different stages leading to the tuberization of the mature root.

MATERIALS AND METHODS

Sweet potato plants of cultivar 049 were established singly in 40 L tins containing a mixture of soil, sand and rotted pen manure in the ratio 5:2:2. Whole mature tuberized roots were used as planting material. Plants were harvested after 16 weeks of growth and adventitious roots were selected at different stages of development from late primary growth to root tuberization. These stages were identified as stages A, B, C and D. Stage A roots were in late primary growth. All other roots were at various stages of secondary growth. Roots at stage B contained a central stele just prior to pith formation and they

showed activities of the vascular cambium and phellogen only. In stage C roots the pith was recently initiated and only activities of the vascular cambium and phellogen were observed. Roots at stage D would be described as tuberized roots. These possessed a large pith and activities of the vascular cambium, phellogen and secondary anomalous cambia were observed. Histochemical and biochemical assays were then performed as soon as possible after harvesting. Biochemical assays were only performed on roots at stages B, C and D, while histochemical assays were performed at all stages.

Histochemical Assays

Transverse sections 20 - 30 um thick were cut on a precooled microtome and collected either in cold 0.05 M tris-HCL buffer at pH 7.6 or in cold 2.5 per cent glutaraldehyde buffered with the tris-HCL buffer. Sections collected in the buffered glutaraldehyde were fixed for 30 minutes and this was followed by several post-fixation washes in the tris-HCL buffer. Unfixed and fixed sections were then incubated at room temperature in solutions of 3,3-diaminobenzidine (Graham and Karnovsky, 1966) for 10-15 minutes and guaiacol (Chance and Maehly, 1951) for 5-10 minutes.

Control experiments were performed to show that the observed activities were due to peroxidase and not as a result of catalase activity. These experiments were performed by incubating sections in media lacking H_2O_2 or in complete media containing a final concentration 0.02 M 3-amino-1,2, 4-triazole, a specific inhibitor of catalase activity (Margoliash and Novogrodsky, 1958). Lignification of cells was observed by staining sections with HCL and phloroglucinol.

Sections were examined directly under a light microscope. The amount of reaction product was visually estimated for different tissues and cell types and photograpns were taken of the sections. However, good contrast between stained and unstained areas was not always obtained on the black and white film used. This was more a problem for sections incubated in the syringaldazine medium since the oxidized end product was a relatively unstable pink color. Hence no photographs were taken for sections displaying syringaldazine oxidase activity.

Preparation of Enzyme Fractions

Ten g fresh weight of root tissue at a specific stage of root development were completely macerated using 20 g hydrated PVP and 10 cm^3 0.05 M phosphate-citrate buffer pH 6.0 using a cold mortar and pestle. Except for an increased number of washes of the cell wall material in deionized, distilled water extraction of the various enzyme fractions was performed according to Ridge and Osborne (1970) and Parish (1975). Two fractions were successively solubilized in 0.05 M phosphate-

citrate buffer pH 6.0 and 1 M NaCl to yield the cell-free fraction (CFf) and ionically wall bound fraction (If) respectively. The two fractions were dialyzed against two changes of the phosphate-citrate buffer over a period of 15-20 hours at 5°C using pretreated dialyzing membranes (Stenesh, 1984). The dialyzed extracts were centrifuged and the supernatants collected were used in all enzyme assays.

Enzyme Assays

All enzyme assays were performed at 25°C. Guaiacol oxidase was assayed according to Chance and Maehly (1951). Reaction mixtures for IAA oxidase contained 0.5 umol IAA, 8 x 10^{-4} umol 2,4-Dichlorophenol (DCP), 85 umol phosphatecitrate buffer pH 6.0 and 1.0 ml enzyme in a total volume of 4.0 ml. The reaction mixture was constantly agitated in a 25 ml flask shaker. After 5, 10 and 15 minutes of incubation 1 ml of the reaction mixture was removed and incubated with 2 ml of the $FeCl_3$-$HClO_4$ reagent (Gordon and Weber, 1951). This was left to stand for 30 minutes and then the absorbance was determined at 530 nm. The IAA destroyed was determined from a standard curve. Syringaldazine oxidase was initially quantitatively assayed according to Imberty et al. (1985) but because of extended lag phases associated with the CF fractions a simple qualitative test was performed instead, where the amount of reaction product was visually estimated. The assay used was the same for the spectrophotometric assay used by Goldberg et al. (1983). Protein content was determined according to Bradford (1976).

RESULTS AND DISCUSSIONS

Histochemical studies revealed that the activities and distribution of guaiacol oxidase and DAB oxidase were similar for roots at all stages of development. However, when guaiacol was used as the substrate the colored oxidized end product was unstable and there were many diffusion artifacts. Consequently DAB was the preferred substrate and was used in all further experiments.

Roots in the primary stage of development (stage A) showed high wall bound DAB oxidase activity in hypodermal (Plate 1), endodermal and companion cells and on the walls of articulated laticifers. All other cell walls showed lower activity with the exception of the primary xylem vessels which showed none. Cytoplasmic activity was observed in all cells but this was always less than that observed for wall bound activity. This was supported by the results of the biochemical assays for roots which were examined (Table 1). These results revealed that both the CF and I fractions of these roots possessed guaiacol oxidase activity with the activity of the I fraction being greater than that of the CF fraction.

The widespread distribution of DAB oxidase in the cytoplasm and free space of different cell types of these roots is not surprising given the large number of roles in which peroxidase has been implicated. Furthermore, in accordance with the idea of a ribosomal synthesis of peroxidase and a transfer to the Golgi apparatus for the attachment of carbohydrate molecules followed by transport to either the vacuole(s) or cell wall (Alberts et al., 1983), it seems reasonable to find the enzyme present in the cytoplasm of most cells.

Wall bound peroxidases have been implicated in many roles (Higuchi, 1957; Elstner and Heupel, 1976; Mader et al., 1975; Gross, 1977; Imberty et al., 1985) and these may account for the presence of such high activity on most cell walls in these roots.

At the onset of secondary growth in roots described as Stage B, the vascular cambium produced secondary xylem parenchyma, tracheids and vessels in a centripetal direction, with the tracheids surrounding individual vessels or groups of vessels. The tracheids showed high wall bound DAB oxidase activity and the secondary vessels, as observed for primary vessels, showed none (Plates 2 and 3). Weak wall bound and cytoplasmic DAB oxidase activities were observed in secondary xylem parenchyma (Plates 2 and 3).

With histochemical assays syringaldazine oxidase activity was observed after a lag phase anywhere between 10 to 30 seconds in these developing roots. In the biochemical assays, a lag phase was also observed but this was extended by as much as 45 seconds over that observed in the histochemical assays. A lag phase was also observed by Goldberg et al (1983) when the enzyme was assayed in the presence of sodium diethyldithiocarbamate.

The authors suggest that the lag phase was due to the formation of an inhibitor-enzyme complex that eventually broke down to release the active enzyme. It is quite possible that the cell regulates syringaldazine oxidase activity through the formation of such complexes. Endogenous inhibitors that might have been present but separated from the enzyme could have been brought together during the experimental procedure. This could have occurred upon tissue sectioning for histochemical assays and even more so upon tissue maceration and hence cellular and organellar disruption for biochemical assays and hence the longer lag phase observed.

In roots at stage B the highly defined endodermis showed very high wall bound syringaldazine oxidase activity. The extra-vascular cambial parenchyma cells, however, showed weak activity in the cytoplasm and free space while the xylem parenchyma showed very little to none. Unlike the case for DAB oxidase and guaiacol oxidase, all lignified tracheary elements or both tracheids and vessels showed equally high syringaldazine

oxidase activity. Despite these histochemical findings only the CF fractions showed syringaldazine oxidase activity (Table 1). The apparent disimilarity observed between the histochemical data which indicated syringaldazine oxidase might also be wall bound in cells undergoing lignification and biochemical data that indicated it to be part of the CF fraction only, may be explained by one of the following possible suggestions:

(1) There were both CF and I peroxidases present but activity of the I fraction was lost during its lengthy extraction procedure and subsequent preparation leaving activity in the easily prepared CF fraction only.

(2) The dilute buffer used for the extraction of the CF fraction enzymes could have solubilized the enzyme (Mader et al., 1975). This may suggest that syringaldazine oxidase is not a wall bound enzyme but only concentrated in the free space of the wall.

(3) Syringaldazine oxidase may in fact be a wall bound enzyme, but attached by strong covalent bonds. This possibility was tested by assaying cell wall material prepared for extracting the I fraction, but the presence of covalently wall bound syringaldazine oxidase was also shown to be absent.

Syringaldazine oxidase is the peroxidase specifically involved in the final stage of lignin biosynthesis (Harkin and Obst, 1973; Mader et al., 1977) so its presence in the walls of both vessels and tracheids is to be expected. Its presence in the cytoplasm and free space of some parenchyma cells, though relatively weak, is however not easily explained since these cells do not undergo lignification. An alternative explanation may be due to its synthesis in the cytoplasm of these cells and subsequently a symplastic and apoplastic transport to cells where they are needed, thus also accounting for their presence in the free space of the cortical parenchyma cells.

The fact that both vessels and tracheids show syringaldazine oxidase activity while only tracheids show DAB oxidase activity (Plates 2, 3, 4 and 5) may suggest a specific role for DAB oxidase in producing the final wall structure in tracheids. This is quite possible since both tracheids and vessels are non-living cells performing the same function but differ somewhat in anatomy. It is possible that the tracheids which evolved prior to vessels (Esau, 1977) may have contained an additional wall material not required for vessel wall synthesis. This additional wall material may have required the use of a specific isoperoxidase to incorporate it into the polymeric wall structure of the tracheids and hence accounting for the presence of the enzyme in these walls. Analyses of lignin from various higher plants showed large variations in their alcohol residual content (Gross, 1980). Though these

studies did not measure the lignin alcohol residual content of tracheids versus vessels, it was observed that the nonvessel vascular plants generally had guaiacyl lignins with very small amounts of syringyl residues incorporated into it. The vessel vascular plants, however, showed a significant increase in the syringyl residual content with a corresponding decrease in the guaiacyl residual content to produce an almost equally ratioed guaiacyl-syringyl lignin. This seems to suggest that guaiacyl residues have been highly associated with the tracheid wall structure rather than that of the vessel. Though this suggestion would now require an actual lignin residual analysis of tracheids versus vessels, it still lends some support to the idea of having a DAB or guaiacol type oxidase highly associated with tracheid walls and not with vessel walls.

Plates 2, 3, 4 and 6 show in the extra-vascular cambial position radial files of secondary sieve elements and their associated companion cells were laid down alternating with files of secondary phloem parenchyma. All companion cells showed high wall bound DAB oxidase activity comparable to that of the tracheids, but their cytoplasmic activity was very weak. All other cells of the secondary phloem showed very little to almost no activity in both the cytoplasm and walls. Articulated laticifers differentiated among the secondary phloem and secondary xylem, and they showed high wall bound DAB oxidase activity (Plate 7). It is possible that since peroxidases have been implicated in other types of polymerization reactions they may also have some similar type of role to play in the production or modification of the latex macromolecule. This may then account for its presence on the walls of the articulated laticifers

The very high wall bound DAB oxidase activity found in companion cells would suggest a specific role for phloem peroxidases. Catesson (1980) observed very high activity of DAB oxidase in the companion cells and differentiating sieve tubes of carnation (Dianthus caryohyllus) and suggested a role through IAA oxidation in IAA transport or in phloem differentiation. Whether this suggestion is also applicable to the high DAB oxidase activity observed in the companion cells in the developing sweet potato root requires further experimental investigation.

Periderm development was initiated from the hypodermal layer shortly after some degree of radial expansion of the secondary stelar tissues had occurred. The hypodermal cells which showed very high DAB oxidase activity (Plate 1) assumed meristematic activity and divided periclinally to produce phellem on the outside, which became lignified and suberized, and a wide multiseriate phelloderm on the inside (Plates 8, 9 and 10). Living cells of the developing periderm showed high wall bound and cytoplasmic DAB oxidase activity in contrast to the underlying parenchyma cells of the cortex which continued to show weak activity (Plates 8 and 9).

High levels of peroxidase were observed in the endodermal cell walls (Plates 3, 8 and 9). The endodermal cells in the sweet potato root contain casparian strips which are both suberized and lignified. The presence of suberin and lignin may therefore account for the presence of peroxidase in these walls since peroxidase has also been implicated in suberin biosynthesis (Cottle and Kolattukudy, 1982) and in the already discussed role of lignin biosynthesis (Mader et al., 1977). Light microscopy was unable to reveal the presence of a casparian strip in the morphologically distinct outer layer of cortical cells (Plate 1) and hence the layer was identified as a hypodermis (Peterson, 1988). The absence of lignin and suberin in the hypodermal cell walls may then suggest some other specific role for DAB oxidase in these cells. Or it may in fact have a similar role since the hypodermis is the precursor of the phellogen which gives rise to the outer lignified/suberized phellem. It is therefore possible that peroxidases remain on the hypodermal and then phellogen cell walls. From this position all subsequent suberization and lignification of developing phellem cell walls, which themselves show minimal activity if any (Plates 6, 8, 9 and 10), take place.

The inner derivatives of the phellogen which develop into the phelloderm never become suberized nor lignified and hence the high levels of wall bound and cytoplasmic DAB oxidase (Plates 3, 4, 6 and 9) cannot be related to the biosynthesis of these compounds. A possible role, however, for the high levels of activity observed here might be in wound healing (Thomas and Delincee, 1979) and disease resistance (Fehrmann and Dimond, 1967; Johnson and Cunningham, 1972). This is a fair assumption since the phelloderm forms a major part of the external tissues that may first be exposed to wounding and pathogen invasion. Furthermore, since the sweet potato root, unlike many other roots, undergoes such rapid radial expansion during the tuberization process, the periderm often shows a lag in development leading to the formation of small fissures in its structure that make it quite susceptible to pathogen invasion. Hence, if DAB oxidase is functioning in wound healing and disease resistance, it would display high activity in these cells and this was observed in all roots as well as in the cortex of older roots.

Further activity of the vascular cambium produced a much larger proportion of xylem parenchyma compared to tracheary elements (Plate 4). The xylem parenchyma were displaced towards the centre of the root leading to the formation of a small pith through the separation of the solid central stele into smaller groups of tracheary elements. This is stage C, the stage of tuberizing initiation. These secondary xylem parenchyma continued to show very weak, if any, DAB oxidase activity like the radial files of secondary phloem parenchyma. With continued root development and tuber formation stage D (Plate 11) of development was attained, where some of these secondary xylem

parenchyma showed anomalous cambial activity producing tracheids, vessels, sieve elements, companion cells and a large number of parenchyma storage cells that further contributed to pith development. These parenchyma cells continued to show very weak DAB oxidase activity, with the vessels showing none. The pattern established for DAB oxidase did not change as the mature tuberizing root developed, except for parenchyma cells of the narrow cortex showing slightly greater DAB oxidase activity than in earlier stages of root development.

If the combined guaiacol oxidase activities of the CF and I fractions are compared for roots at different stages of development (Table 1) it is observed that there was over a 50 per cent decline in activity from Stage B to C but the activity remained fairly constant from Stage C to D. An examination of the individual fractions shows a similar pattern. These results are supported by the histochemical data since Stages C and D compared to Stage B show a much greater ratio in the production of non-lignified cells to lignified cells by the vascular cambium. Since these thin-walled parenchyma show very little DAB oxidase or guaiacol oxidase activity in both their walls and cytoplasm, a reduction in guaiacol oxidase activity was expected. Any further decline in activity from Stage C to D was probably offset by the increase in activity observed in the cortical cells as the tuber developed.

Histochemical assays showed that syringaldazine oxidase activity was fairly constant throughout all stages of root development. Since only qualitative biochemical tests were performed to show either the presence of, or absence of, its activity in the different fractions, no conclusions can be made with respect to quantitative changes in activity over the period of root tuberization.

Cofactor (2,4-DCP) dependent IAA oxidase activity was observed in the I fraction only (Table 1). There was very little variation *in vitro* in the cofactor dependent IAA oxidase activity at different stages of root development. There was no cofactor dependent activity in the CF fraction and neither was there any cofactor independent activities in the CF and I fractions.

The polar movement of auxins in stems and roots has been shown to involve both the apoplast and symplast (Goldsmith, 1977). As a result of this the wall bound IAA oxidase can therefore but indirectly still regulate IAA levels in the cytoplasm. Certain clear advantages can be derived by oxidizing IAA in the free space instead of the cytoplasm, where so many metabolic processes are occurring. These include the following:

(1) The chances of the end products of IAA oxidation capable of inhibiting any enzyme catalyzed reactions in the cytoplasm are greatly reduced.

(2) The chances of side reactions in the cytoplasm resulting from possible non-specificity of the enzyme are eliminated.

Though the biochemical data (Table 1) did not show a significant decline in IAA oxidase activity *in vitro*, activity may be controlled by reducing, in the free space, the phenolic cofactor concentration needed for optimal enzyme activity (Sirju and Wilson, 1974). The fact that enzyme activity shows a definite dependence on the phenolic cofactor, 2,4-DCP, would suggest that this may be a way to regulate activity *in vitro* and hence IAA oxidase activity may be lowered within the roots during the tuberization phase. One way of examining this would be to monitor the endogenous levels of phenolic cofactors that have been shown to stimulate IAA oxidase activity *in vitro*. So it is therefore possible that IAA oxidase activity declines *in vivo* during the tuberization phase, provided that enzyme activity is indeed under regulation by endogenous cofactor levels.

The biochemical and histochemical data were generally very similar for the observed activities of DAB oxidase in the cytoplasm and on the cell walls of sweet potato roots at different stages of tuberization. The histochemical data enhanced the biochemical findings by being able to suggest more specific roles for peroxidase in these tuberizing roots based on their location *in situ*. Though there was not a good correlation in results for the syringaldazine oxidase, the suggestions proposed may be able to specifically determine the nature of this isoperoxidase with further experimental investigation.

All of these results nevertheless support Wilson's (1970) suggestion that tuberization in sweet potato roots proceeds with reduced lignification and formation of secondary cambia which produce a large number of parenchyma cells for storage of starch. The observed decline in peroxidase activity with tuber initiation also supports previous data obtained by Sirju-Charran (1978). Whether this decline is a reflection of a decline in the total IAA oxidizing potential of the root, where peroxidase may be acting as an IAA oxidase, and which has been suggested as a requirement for greater growth (Akita et al., 1962; Mills and Crowden, 1968; Birecka and Galston, 1970; Gardiner and Cleland, 1974) and hence tuberous root formation, requires further investigation. It is difficult to interpret the results of the biochemical investigation of IAA oxidase activity *in vitro* unless other additional studies of factors are made that may also be influencing IAA oxidase activity *in vivo*. When endogenous control of IAA oxidase is better understood, then we will be able to determine the changes in IAA oxidase activity and IAA levels, if any, during the tuberization process.

Table 1. Results of the activities of guaiacol oxidase, syringaldazine oxidase and indole acetic acid oxidase from the CF and I fractions of sweet potato roots at three different stages of development.

Stage[1]	Fraction	Guaiacol oxidase ($\Delta A_{480} min^{-1} \mu g^{-1}$ protein)	Syringaldazine oxidase (Qualitative test)	Indole acetic acid oxidase μmoles IAA destroyed $min^{-1} \mu g^{-1}$ protein	
				Cofactor dependent	Cofactor independent
B	CF	$9.9 \times 10^{-3} \pm 1.1$	Present	-	-
	I	$2.5 \times 10^{-1} \pm 1.8$	-	$4.8 \times 10^{-2} \pm 0.9$	-
C	CF	$4.0 \times 10^{-3} \pm 1.3$	Present	-	-
	I	$9.0 \times 10^{-2} \pm 1.7$	-	$3.8 \times 10^{-2} \pm 1.2$	-
D	CF	$1.6 \times 10^{-3} \pm 1.5$	Present	-	-
	I	$9.9 \times 10^{-2} \pm 1.8$	-	$2.8 \times 10^{-2} \pm 1.5$	-

[1]B : Roots with a solid central stele. Activities of the vascular cambium and phellogen observed only.

C: Roots with a recently initiated pith. Activities of the vascular cambium and phellogen observed only.

D: Roots at the stage described as a tuberous root with a well developed pith. Activities of the vascular cambium, phellogen and secondary anomalous cambia observed.

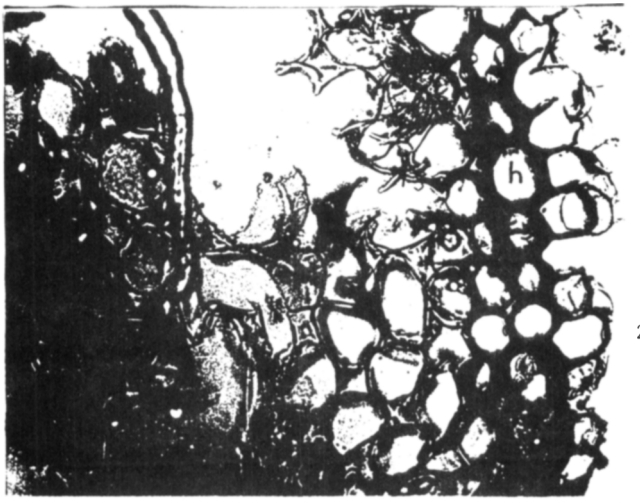

PLATE 1

25 µm

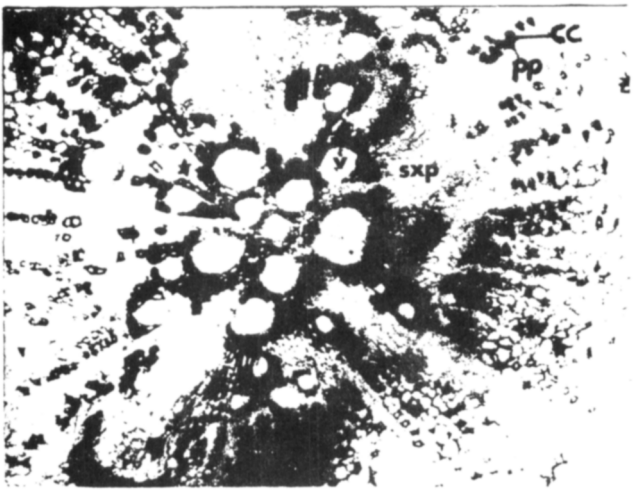

PLATE 2

100 µm

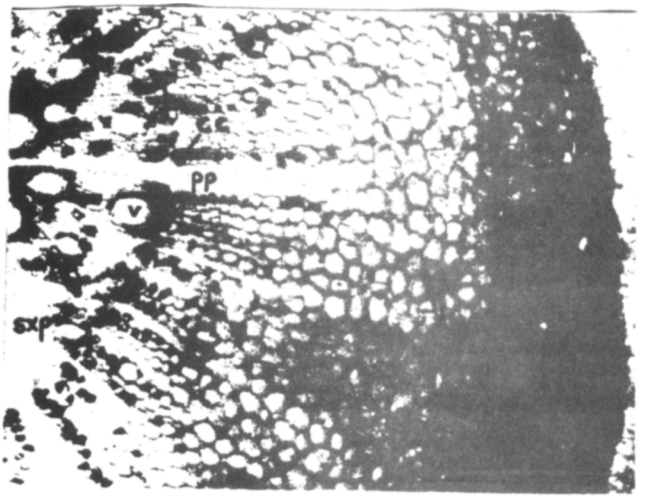

PLATE 3

100 µm

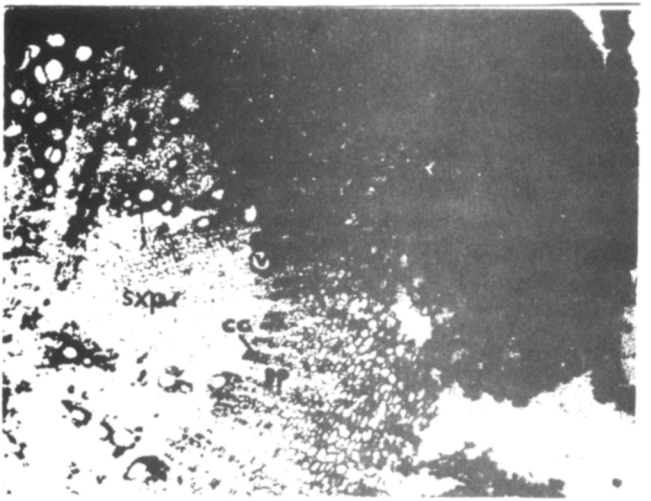

PLATE 4

300 µm

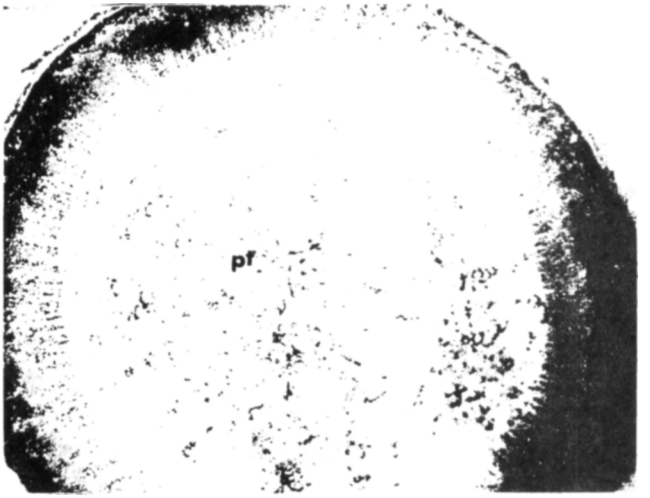

PLATE 11

1 mm

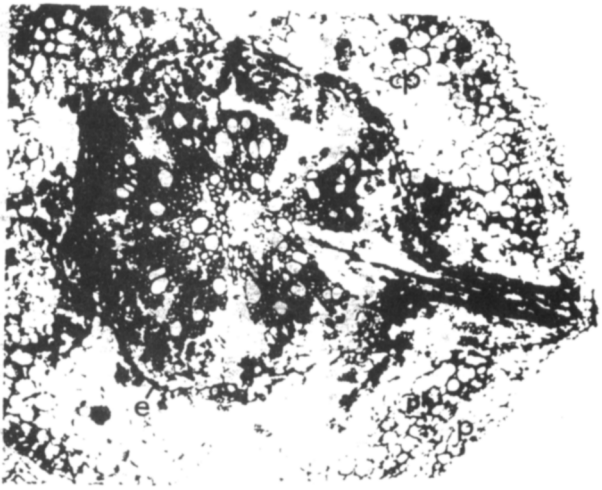

PLATE 9

200 µm

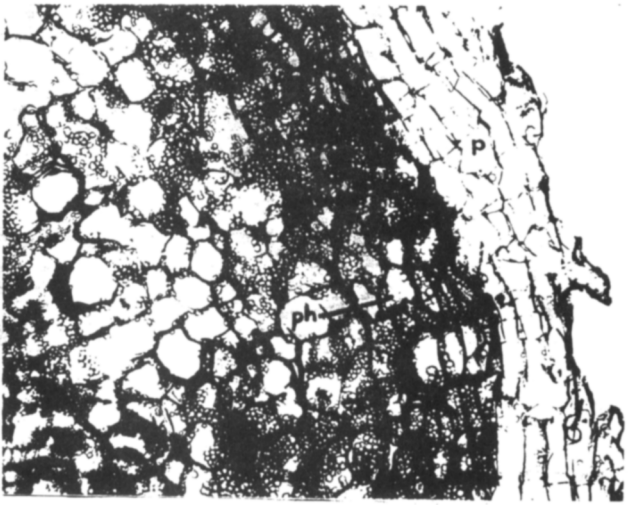

PLATE 10

50 µm

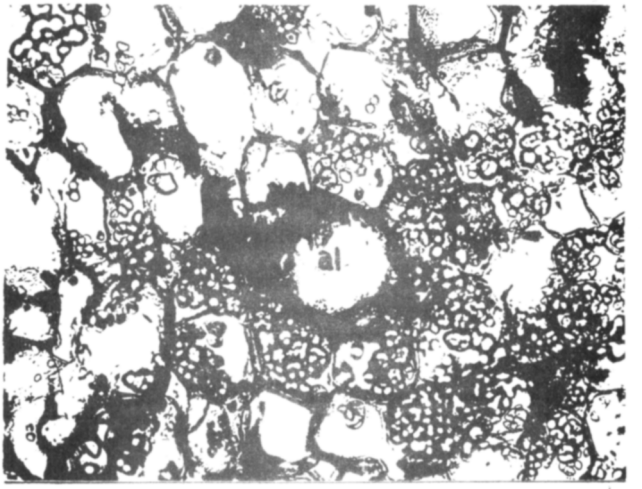

PLATE 7

25 µm

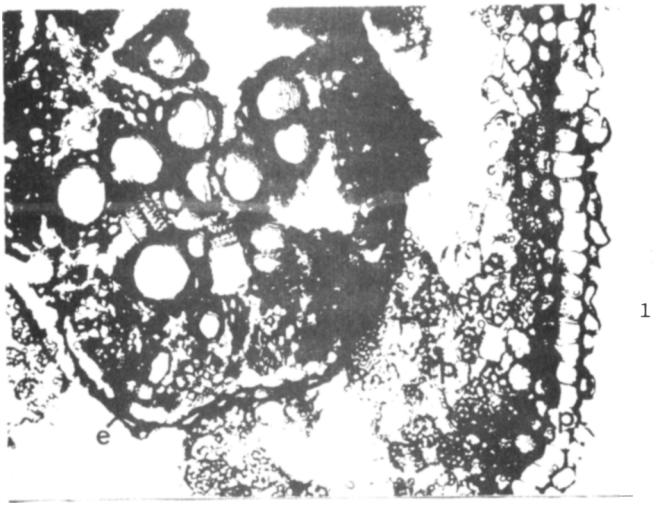

PLATE 8

100 µm

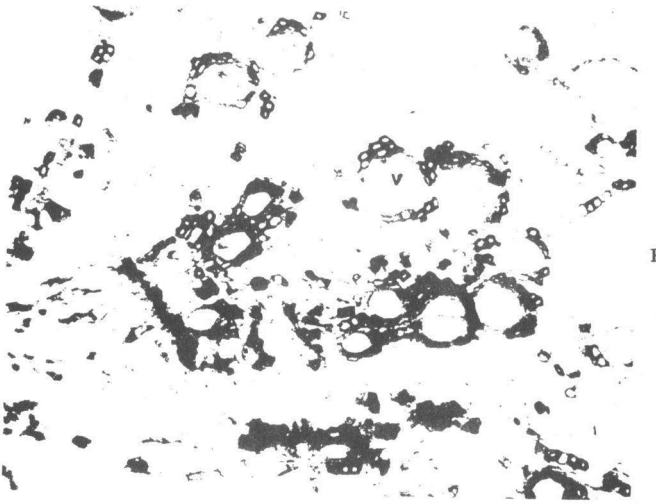

PLATE 5

100 µm

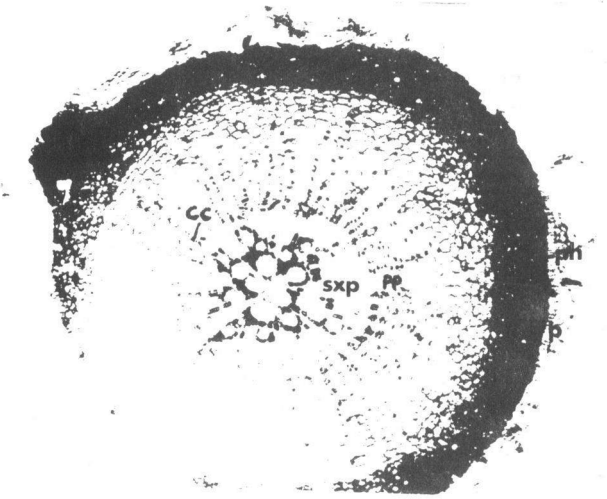

PLATE 6

300 µm

EXPLANATION OF PLATES

All plates are transverse sections of sweet potato roots of cultivar 049 at different stages of tuberization. All sections show staining patterns as a result of incubation in DAB and H_2O_2. Labels and anatomical descriptions explained in one plate are not repeated in subsequent plates. Staining patterns related to the histochemical data are described in Results and Discussions.

Plate 1

Stage A root: Late primary growth, showing the highly defined hypodermis and other cortical cells.

h: hypodermis
cp: cortical parenchyma

Plate 2

Stage B root: Secondary growth, showing a central stele made up of xylem parenchyma and tracheids mostly surrounding the vessels. In the extra-cambial position, radial files of sieve elements and their associated companion cells alternating with radial files of phloem parenchyma are observed.

v: vessels
t: tracheids
sxp: secondary xylem parenchyma
pp: (secondary) phloem parenchyma
cc: companion cells

Plate 3

Stage B root: Peripheral tissues made up of a relatively young periderm outside a narrow cortical region. The endodermis is still a well defined structure.

e: endodermis
ph: phellodern

Plate 4

Stage C root: An increased production of secondary xylem parenchyma has led to the dispersion of the central stele into smaller groups of tracheary elements resulting in the initiation of a pith.

PLATE 5

Stage C root: This shows the general arrangement of tracheids relative to the vessels.

PLATE 6

Stage B root : This shows the general distribution of tissues and their relative abundance at this stage of development.

P : Phellem

PLATE 7

An articulated laticifer found among the pericyclic parenchyma of a stage C root.

al : articulated laticifer

PLATE 8

Stage B root : The spaces observed in the cortex are cortical lacunae. The hypodermis has recently undergone a single periclinal division. This is the initiation of the periderm.

PLATE 9

Stage B root : This shows the development of a lateral root arising from the pericyclic region opposite a primary xylem arm.

PLATE 10

Stage B root : High power magnification of the peripheral tissues.

PLATE 11

Stage D root: This shows the general distribution of tissues and their relative abundance at this stage of development. The pith is a highly prominent structure.

Pt : pith

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