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THE DISTRIBUTION OF PEROXIDASES IN EXTREME DWARF AND NORMAL TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Abstract—Studies of the peroxidases in various parts of dwarf (d^x) and normal (d^+) tomato plants showed that the peroxidase activities of the pith, cortex and leaf of the dwarf are about 3 times greater than those of respective normal tissues. The dwarf and normal roots, however, have essentially the same peroxidase activity. In both the dwarf and normal plants, the peroxidase activity is lowest in the leaf tissue.

Electrophoretic studies indicate that different tissues and organs possess different peroxidases. It is suggested that these various peroxidases have different substrate (hydrogen donor) requirements and carry out different reactions *in vivo*. No qualitative electrophoretic differences between dwarf and normal peroxidases were found.

INTRODUCTION

MANY attempts have been made to establish a physiological or biochemical basis of genetic dwarfing in higher plants. Numerous investigations have shown that the peroxidase activity in dwarf plants is, in general, higher than the peroxidase activity in normal plants.[1–3] As a result, peroxidases have been considered as one possible cause of the dwarf type of growth.

Even though peroxidases from many different plants have been shown to exist as isoenzymes,[4–8] little is known about the distribution of these enzymes in different plant tissues and organs. This paper presents data on the qualitative and quantitative distribution of peroxidases in various parts of the extreme dwarf (d^x) and normal (d^+) tomato plants.

RESULTS AND DISCUSSION

Table 1 shows the specific activities of various parts of dwarf and normal tomato plants using pyrogallol and guaiacol as substrates (hydrogen donors). The peroxidase activities of the dwarf pith, cortex and leaf are about 3 times greater than the activities of the normal pith, cortex and leaf, respectively. The dwarf and normal roots, however, have essentially the same peroxidase activity. In both the dwarf and normal plants, the peroxidase activity is lowest in the leaf tissue.

The dwarf and normal roots have different relative activities when compared with the

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other plant parts. Using pyrogallol as the substrate, the peroxidase activity of the normal pith, cortex and root are essentially the same, but the dwarf root has about one-half the peroxidase activity of the dwarf pith and cortex. With guaiacol as the substrate, the normal root activity is twice as great as the activity found in the normal pith and cortex while peroxidase activities in the dwarf pith, cortex and root are equal. The different relative activities of the dwarf and normal root peroxidases with the two substrates suggest differences in substrate specificity.

Since 2 moles of pyrogallol and 4 moles of guaiacol are required to form 1 mole of purpurogallin and 1 mole of tetraguaiacol,[9] respectively, the amount of purpurogallin formed should be twice as great as the tetraguaiacol formed. Tables 1 and 2 show that this is not the case. The peroxidase has a higher turnover number with pyrogallol than with guaiacol. Eight to ten times more purpurogallin is formed than tetraguaiacol in the pith, cortex and

TABLE 1. SPECIFIC ACTIVITIES OF PEROXIDASE IN VARIOUS PARTS OF NORMAL AND DWARF TOMATO PLANTS USING PYROGALLOL* AND GUAIACOL† AS THE HYDROGEN DONOR

Donor	Plant	Tissue			
		Pith	Cortex	Leaf	Root
Pyrogallol	Normal	9·5±2·3	8·0±1·4	0·6 ±0·3	10·2±4·3
	Dwarf	25·5±6·0	23·5±1·8	1·9 ±0·7	11·9±5·6
Guaiacol	Normal	1·1±0·5	1·0±0·3	0·04±0·02	2·0±0·5
	Dwarf	2·7±0·4	3·0±0·3	0·24±0·11	2·8±1·3

* Results expressed as micromoles of purpurogallin produced/mg protein/min and are averages of duplicate determinations from 5 plants.

† Results expressed as micromoles of tetraguaiacol produced/mg protein/min and are averages of duplicate determinations from 5 plants.

leaf in both dwarf and normal plants. The dwarf and normal root peroxidase does not show so great a difference in its turnover number but the peroxidase is still more "efficient" when pyrogallol is used as the substrate.

Figure 1 shows the electrophoretic patterns of peroxidases from dwarf and normal tissues and organs using pyrogallol and guaiacol as substrates (hydrogen donors). The bands moving to the anode are lettered *A* through *D* depending on their electrophoretic mobilities, the *A* band being the fastest moving component. In most cases, the *D* band is followed by continuous streaking back to the origin. Because of this streaking, any additional bands found in this streak are lettered D_2, D_3, etc. The single band moving to the cathode is designated as *A'*.

There are no qualitative differences in the isoenzymes of dwarf and normal plant parts, but the specific tissues or organs apparently have their own complement of peroxidases. The same six peroxidase bands may be present in all of the tissues but in a concentration too low to be detected. Nevertheless, certain isoenzymes predominate in the different tissues and

[9] A. C. MAEHLY and B. CHANCE, *Methods of Biochem. Anal.* **1**, 357 (1954).

organs. The *B* band is the only band common to all plant parts and tissues. The *C* band is found only in the cortex and root. The D_2 band is discernible only in the pith but may be present in other parts. The *A'* band is found in roots and flowers. The D_1 band is found in all parts except the pith and fruit while the *A* band is found in the leaf, cortex, flower and roots.

There are indications of substrate specificity with these isoenzymes. For example, leaves have the *A*, *B* and D_1 bands with the *A* band staining most intensely with pyrogallol and the *B* band staining most intensely with guaiacol. The *A* band in all of the tissues studied does not noticeably stain with guaiacol. This lack of staining or possible substrate specificity of the *A* band may be partially responsible for the lower turnover number observed in the specific

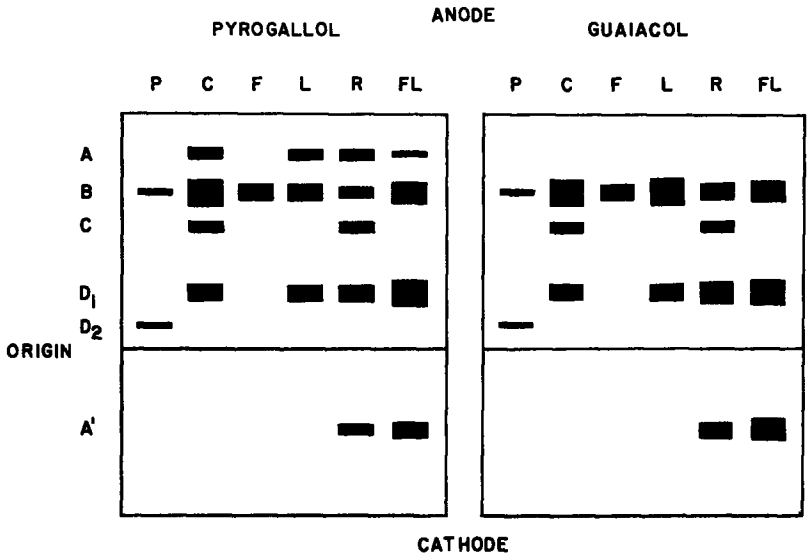

FIG. 1. ELECTROPHORETIC PATTERNS OF PEROXIDASES LOCATED IN DWARF OR NORMAL TOMATO TISSUES AND ORGANS USING PYROGALLOL AND GUAIACOL AS SUBSTRATES (HYDROGEN DONORS).
P, pith; C, cortex; F, fruit; L, leaf; R, root; FL, flower.

activity studies with guaiacol as the substrate. It also indicates that a peroxidase is present but that the substrate (hydrogen donor) used is inadequate to measure the true activity of the enzyme.

The staining intensities of the bands vary with the plant part suggesting that different activities or concentrations of the same isoenzyme may be present in different parts of the plant. For example, the *B* band stains most intensely in the cortex while the D_1 band stains most intensely in the flower. The flower has a very light *A* band while the leaf has a heavy *A* band.

From these results, there appears to be no direct relationship between specific activities and isoenzyme complements in the various tissues and organs studied. The pith and cortex have the same specific activities even though the cortex has four isoenzymes and the pith has only two isoenzymes.

Peroxidase has been shown to catalyze certain steps in lignin synthesis[10,11] and may also

[10] S. M. SIEGEL, *J. Am. Chem. Soc.* **78**, 1753 (1956).
[11] S. M. SIEGEL, *J. Am. Chem. Soc.* **79**, 1628 (1957).

be involved in indole-3-acetic acid[12, 13] and anthocyanin degradation.[14] These varied catalytic properties of peroxidase coupled with the different turnover numbers and possible substrate specificity observed in these experiments, suggest that these isoenzymes represent peroxidases having different substrate (hydrogen donor) requirements *in vivo*, with the common requirement of H_2O_2. The different peroxidases located in the different parts of the plant may reflect the concentrations or presence of specific substrates in these various tissues. The various tissues and organs would be capable of catalyzing different reactions with "peroxidase" and have common reactions differing only in rate. Assuming this to be possible, the cortex could have as many as four different substrates for its "peroxidase" while the fruit would have one substrate. Because both have the *B* band, this reaction would be common qualitatively but the cortex could, in addition, catalyze three different reactions with its *A*, *C* and D_1 bands.

The existence of these different substrates and peroxidases may be related to the changing function of the tissues and organs in development and maturity.

EXPERIMENTAL

The extreme dwarf (d^5) tomato plant was originally described by Rick and Butler[15] and the plants used in this study were descendents of Rick's stock.

Preparation of Tissues

Fully expanded, mature tissues of the shoot were used for preparations of dwarf and normal tissues unless otherwise noted. Tissue from leaf, cortex, pith, root, flower and ripe and unripe fruit was used. Leaf tissue was prepared by excising the midvein of the leaflets. Pith and cortex were excised from the stem. Root "tissue" consisted of the entire root. The roots were excised, washed and blotted dry. Flower "tissue" included the entire open flower. The fruit tissue consisted entirely of ovary wall.

Specific Activity

The leaf, cortex, pith and root of dwarf and normal plants were used for this study. One gram, wet weight, of each tissue was ground in a Waring blendor with 25 ml of 0·025 M phosphate buffer, pH 6·7, for 15 sec. The resulting slurry was filtered through filter discs and centrifuged at 25,000 *g* for 20 min. The supernatant was used for peroxidase determinations. All procedures were performed at room temp.

Peroxidase activity was determined by following the formation of the colored reaction products in a Beckman Model DU spectrophotometer at room temp. Pyrogallol and guaiacol were used as substrates (hydrogen donors). The reaction mixture, after McCune and Galston,[3] consisted of 30 μmoles of pyrogallol or guaiacol, 57·5 μmoles of phosphate buffer, pH 6·7, 26 μmoles of H_2O_2 and 0·12 ml of the enzyme preparation. The total volume of the reaction mixture was 3·02 ml. The spectrophotometer was set at zero absorbance with the reaction mixture minus the H_2O_2. Addition of the H_2O_2 was used to start the reaction and optical density readings were taken at 20 sec intervals. The formation of purpurogallin from pyrogallol and tetraguaiacol from guaiacol was followed in the spectrophotometer at

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[15] C. M. RICK and L. BUTLER, *Adv. in Genet.* **8**, 267 (1956).

wave lengths of 430 and 470 mμ, respectively. The amount of purpurogallin and tetraguaiacol produced was calculated using extinction coefficients of $\epsilon_{430} = 2{\cdot}47\ cm^{-1}\ mM^{-1}$ and $\epsilon_{470} = 26{\cdot}6\ cm^{-1}mM^{-1}$, respectively.[16]

Protein was determined by the method of Lowry *et al.*[17] Peroxidase activity was expressed as micromoles of product produced/mg protein/min.

Starch-gel Electrophoresis

Tissue samples for starch-gel electrophoresis were prepared in two ways: (1) the tissue was squashed between 2 glass plates and the expressed sap was applied to 3 mm square, Whatman No. 1 or 3 filter paper, (2) tissue extracts of dwarf and normal cortex, pith, leaf and root were made and then applied to the filter paper. The extracts were made by grinding the tissues with sand in a mortar and pestle. The resulting mash was squeezed through a filter disc and the cloudy filtrate was centrifuged at 4600 *g* for 5 min. The supernatant was dialyzed for 12 hr against two changes of 1000 vol of water at 2°. The extract was centrifuged at 1600 *g* for 3 min and the supernatant was stored at 2°. All procedures, other than dialysis, were performed at room temp. The peroxidases in these extracts are stable for more than 1 month.

Horizontal starch-gel electrophoresis was run at room temp. according to the method of Smithies[18] using borate buffer of pH 8·5. The gels were run for 3 hr with a potential of 2–2·3 V/cm.

After electrophoresis, the gel was sliced in half horizontally and one half was stained with 0·1 M pyrogallol and the other half with 0·1 M guaiacol. 0·5 ml of 3% H_2O_2 was used to start the reaction. The reaction was allowed to proceed for 10 min and then stopped with 10% acetic acid. The gels were visually compared within 10 min after the addition of the acetic acid because the bands faded rapidly.

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