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SHORT COMMUNICATION

ISOPEROXIDASES IN ORGANS OF TWO SPECIES OF THE GENUS *DATURA* (SOLANACEAE)

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Abstract-Molecular heterogeneity of peroxidases in organs of two species of the genus *Datura (D. ferox* and *D. innoxia)* was tested by means of starch gel electrophoresis. Enzymatic activity was determined using benzidine and hydrogen peroxide. Each organ shows a characteristic isoenzymatic pattern, that of the root having the highest molecular heterogeneity. Compared with the other organs tested, the root showed a highest total enzymatic activity.

INTRODUCTION

THE IMPORTANT roles peroxidases play in plant oxidation processes have been shown by several investigators. Among these auxin degradation,' respiration and lignification[2] should be noted. Although their action in the cell is not absolutely clear it has been shown that peroxidase activity is related with the processes of tissue differentiation[3] and growth.[4,5] Variations in peroxidase patterns in healthy plants and in those in which disease had been induced,[6] have been examined by some investigators.

These enzymes require both a hydrogen donor and oxygen donor' and show different activities with different hydrogen donors. Recent investigations suggest that this is due to the fact that the peroxidase system is formed by several active fractions which differ from each other in biochemical characteristics.[1,8] The more commonly studied hydrogen donors have been: pyrogallol, guaiacol, p-phenylendiamine, p-methyl aminophenol sulphate,[9] and benzidine which has been used in the present investigations.[10,11]

RESULTS AND DISCUSSION

Electrophoresis

The electrophoretic runs are presented in Fig. 1, where the isoperoxidase bands are numbered according to international nomenclature.[12] As can be seen, *D. ferox* roots have up to seven peroxidase forms: three cathodic bands and four anodic ones. In other

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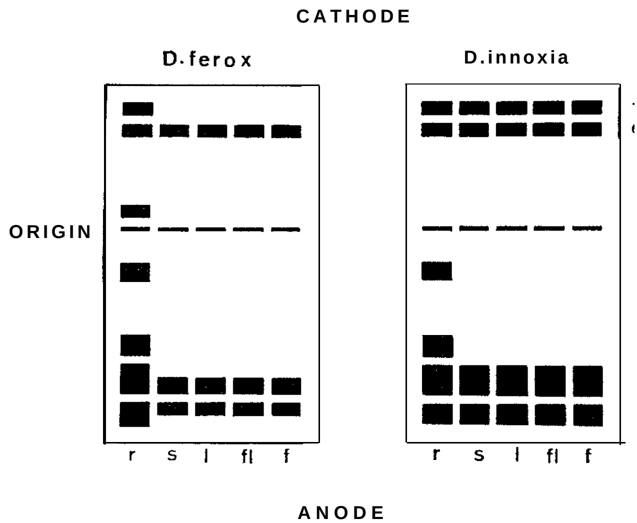

FIG. 1. ELECTROPHORETIC PATTERNS OF PEROXIDASES FROM DIFFERENT ORGANS OF TWO SPECIES OF THE GENUS *Datura*.

r, root; s, stem; 1, leaf; fl, flower; f, fruit.

organs only three bands appeared: one cathodic and two anodic bands. *D. innoxia* roots showed six peroxidase forms: a peroxidase form comparable with the slow running cathodic band of *D. ferox* was absent. The other organs showed four forms, two cathodic and two anodic bands. Bands 1, 2 and 6 appear to be common to all the organs of the two species studied.

Enzymatic Activity

The effects of pH were studied in relation to enzymatic activity, and it was found that acetate buffer 0·02 M pH 4·5 was optimum for the enzyme. Under such conditions, the roots showed the highest enzymatic activity when compared with the other organs. The leaves showed the highest enzymatic activity among aerial parts. The remaining organs were rather similar.

The presence of common bands (l-4, 6 and 7 for roots; 1, 2 and 6 in the remaining organs tested, Fig. 1), as well as the existence peroxidase bands specific for each species, shows that the study of the composition of peroxidase may be useful in determining relationship among different plant species. This might be a useful auxiliary technique in taxonomic studies.[13]

The particular pattern for different organs confirms the observations of other authors.[14–16] The synthesis of enzymes is determined not only by the genes but also by regulations mechanisms. This is supported by the fact that organs with distinct differences

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TABLE 1. ACTIVITY OF PEROXIDASE FROM ORGANS OF *Datura* SPECIES

	Absorptivity/min/mg protein	
	D. ferox	*D. innoxia*
Root	207	213
stem	36	38
Leaf	96	98
Flower	30	43
Fruit	29	45

in isoenzymic composition show the same pattern when cultivated *in* vitro under identical conditions.' [7–19] Enzymatic activity values are shown in Table 1.

EXPERIMENTAL

Material. Normal plants, collected in Córdoba, Argentina in March 1968, were used.

Homogenization. The material was washed (H_2O) and homogenized with a Wemir disintegrator cooled to $-5°$. An equal quantity of H_2O (v/w) was added to roots and stems and less for the other tissues. The homogenized material was filtered through cheese cloth and centrifuged at 2000 *g* at 2".

Preparation of acetone powder. The supernatant was precipitated with acetone (10:1) at $-10°$. This precipitate was dried, and kept at 4". The powder was dissolved in H_2O and the determinations were carried out with this solution.

Starch gel electrophoresis. Vertical electrophoresis was performed according to Smithies technique[20] using 6 V/cm in 0·3 M pH 8·6 borate buffer. Samples were run for 12 hr. The enzymes were developed using 0·01 M benzidine in 50% alcoholic solution,[11] 0·1 M pH 4·5 acetate buffer and 0·1 M H_2O_2 for 5 min. The gels were then washed with H_2O and fixed with MeOH–H_2O–HOAc (5:5:1).

Electrophoretic runs were also done using the crude extract. The same results were obtained.

Enzymatic activity. The order of reagents was as follows: pH 4·5 acetate buffer 0·02 M 1 ml, enzyme preparation 0·5 ml H_2O 1·3 ml, 0·01 M benzidine 0·1 ml, 0·1 M H_2O_2 0·1 ml. The increase inabsorptivity measured at 530 nm and 20–25°; the value obtained over the 90 sec was used for calculation. Proteins were determined according to the method of Kalckar.[21]

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