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ISOPEROXIDASE PATTERNS IN PETALS OF *PLUMERIA ACUTIFOLIA* POIR[1].

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Introduction

Increased activity of hydrolytic enzymes in senescing plant organs have been reported by Kessler & Engellberg (1962), Udvardy *et al* (1969), De Leo & Sacher (1970 a & b) and Shannon *et al* (1971). The present investigation was undertaken to study the isoperoxidase patterns in petals of *Plumeria acutifolia* Poir. during ageing and senescence.

Materials and Methods

Flowers were used from a single plant of *Plumeria acutifolia* Poir., growing adjascent to Karachi University Botany building for the qualitative and quantitative studies of peroxidases. Petals from young (flower buds just before opening), mature (fully opened flowers) and senesced (flowers about to fall from the tree) flowers were excised, their fresh weight recorded and used for the extraction and estimation of peroxidases.

The tissue was extracted with 5 ml of 0.02 M Tris-HCl buffer, pH 7.2 in an ice jacketed mortar. Solid material was separated by centirifugation at 1000 x rpm for 5 min., and the supernatent was used as a crude soluble enzyme extract. The solid material containing cell wall was washed three times with distilled water, followed by extraction of bound peroxidase with 3 ml of 10 mM Ca $(NO)_2$ solution for 24 hr at 10°C (Parish & Miller, 1969). Calcium nitrate treatment released most of the bound enzyme but still some activity was found in the cell-wall fraction. Since this extract was used for pH optima study only, complete extraction of the enzyme was not attempted.

Peroxidase (E.C. 1-11.1.7) activity was measured in the soluble and wall-bound extracts by measuring the increase in optical density (O.D.) at 470 nm during the initial reaction of 30 seconds. The reaction mixture contained 1 ml each of 5 mM guaicol and H_2O_2 in 0.2 M citrate-phosphate buffer, pH 6.0 or at an appropriate pH, to which was added 0.5 ml of the enzyme solution. Specific activity of the enzyme is expressed

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as O.D. at 470 nm/mg protein/30 seconds. Protein was measured by the method of Lowry *et al* (1951).

Peroxidase isozymes, of soluble enzyme extract, was separated by vertical gel electrophoresis. Fifty µl of the dialysed soluble enzyme extracts were placed in 10 mm slots in a 3 x 54 x 270 mm gel of 0.8% agar made up in 25 mM Tris-Glycine buffer (pH 9.0). The gels were connected with electrode buffer of 500 mM Tris-Glycine (pH 9.5) and were run in a refrigerator (10°C) for 6 hr at 400 Volts. Isozymes were detected by flooding the surface of the plate with 120 mM guaicol and 5 mM H_2O_2 in 0.2 M Citrate-phosphate buffer (pH 6.0).

Results and Discussion

pH optima:

The optimal pH of soluble and wall-bound peroxidases were determined using 0.2 M Citrate-phosphate buffer at pH values ranging from 3.6 to 7.8 and 0.2 M Tris-HCl buffer, (pH 8.4). The results, presented in Fig. 1. indicates an optimal value

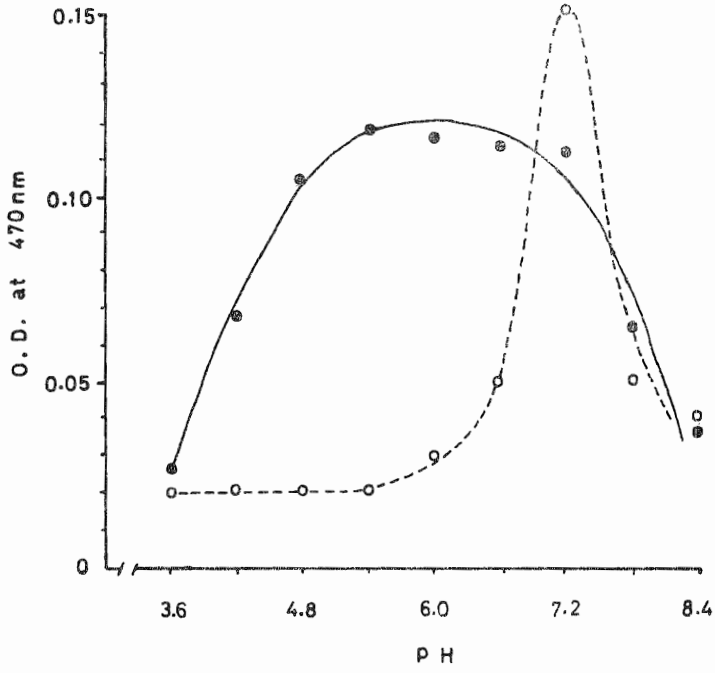

Fig. 1. pH optima of soluble (●—●) and wall bound (o---o) peroxidase from *Plumeria acutifolia* petals.

between pH 5.4 and 6.6 of soluble enzyme while a pH optima at 7.2 was obtained in the wall-bound enzyme. This experiment was repeated twice with similar results.

Total peroxidases in ageing and senescing petals:

The estimation of peroxidase activity in crude extracts from petals of *P. acutifolia* Poir. at various stages of their development has shown a remarkable increase in its activity (Table 1). During ageing from young to mature petals, a correlation between fresh weight and enzyme activity was observed. However, the fresh weight of wilting petals decreased while the enzyme activity increased to about 3-fold the activity of young petals. Recently Shannon *et al* (1971) reported a 100-fold increase in the activity of the enzyme peroxidase in ageing slices of *Ipomea batatas* in an air atmosphere containing ethylene. Similar results have also been reported for other enzymes as well. Kessler & Engleman (1962) found an increase in the activity of RN ase and acid phosphatase during ageing of *Rheo* leaf discs. This led them to postulate that the increase in the enzyme activity is dependent on the synthesis of some form of RNA or specific messenger RNAs for both the enzymes.

Table 1. Fresh weight and peroxidase activity of young, mature and senesced petals of *Plumeria acutifolia* Poir.

	Young petals	Mature petals	Senesced petals
Fresh Weight (mg)	127 ± 7.5	213.6 ± 11.2	183.6 ± 8.9
Peroxidase (Sp. activity)	1.19 ± 0.12	1.85 ± 0.18	3.22 ± 0.15

Peroxidase isozymes of ageing and senescing petals:

Peroxidase isozymes of young, mature and senesced petals is presented in Fig. 2. Electrophoresis of soluble enzyme fraction of young petals contained two isozymes (A_1 and A) which moved toward the anode and three more (C , C_3 and C_4) which moved toward the cathode. However, in mature petals, two new isozymes; one anodic (A) and one cathodic (C) appeared in addition to the five previous ones. Senesced petals contained seven isozymes i.e. A_1, A_2, A_3, C_1, C_2, C_3 and C_4, identical to that of mature petals.

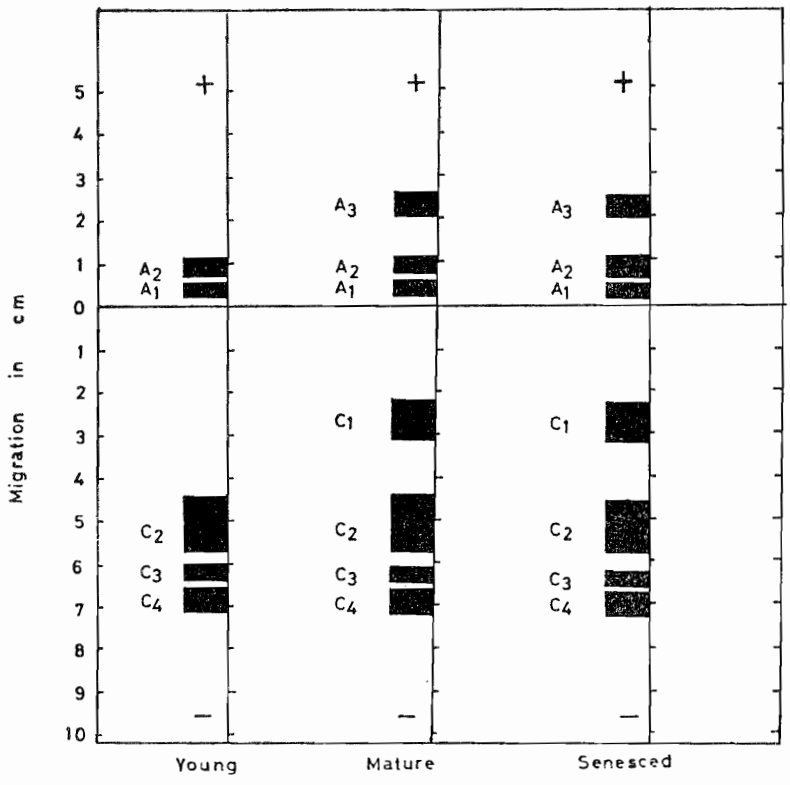

Fig. 2. Isoperoxidase patterns of young, mature and senesced petals of *Plumeria acutifolia*.

It should be noted that the increasing peroxidase activity with increasing age of the *in situ* petals is mainly due to increase in the two isozymes, one anodic and one cathodic. If, as the results suggests, ageing and senescence is in some way related to increased peroxidase activity, then it is these new isoperoxidases which must be involved. As demonstrated by Mc Cune (1962) and Macnicol (1966) that different peroxidases have different relative affinities toward a variety of substrates the appearance of two new isoenzymes in aged and senesced petals of *Plumeria acutifolia* might suggest the onset within the ageing tissues, of new biochemical changes, such as lignin biosynthesis and auxin metabolism (Siegel, 1955, 62; Riddle & Mazelius, 1964; Galston *et al*, 1968).

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