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INVERTASES OF POLLEN OF *HAEMANTHUS ALBIFLOS*

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Abstract—Soluble and insoluble invertase occurs in dormant pollen of *Haemanthus albiflos*, with pH optima of 5·7 and 5·5 respectively. At their pH optima the activity of the soluble enzyme is 3·5-fold higher. After 2 hr germination the pH optimum of the insoluble invertase is increased to 6·0 and the activity is increased 2-fold while the activity of the soluble invertase is decreased by 26%.

THE PRESENCE of invertase (β-fructofuranosidase) in pollen has been described for several plants.[1] The role of this enzyme must be seen in relation to the whole metabolic activity, which is correlated with pollen dormancy, pollen tube growth and fertilization.[2] Each of these steps has its own physiological and morphological characteristics and it is of interest to study the change in their enzymatic constitution. Dickinson[3] found an invertase in germinating pollen of *Lilium longiflorum* which is localized outside the cell membrane and hydrolyses sucrose in the germination medium. This external bound invertase could not be detected in dormant pollen. The germination of pollen must be regarded as the last phase of differentiation. This phase differs from dormancy. We were interested in comparing both phases in respect to the solubility and pH optimum of invertase.

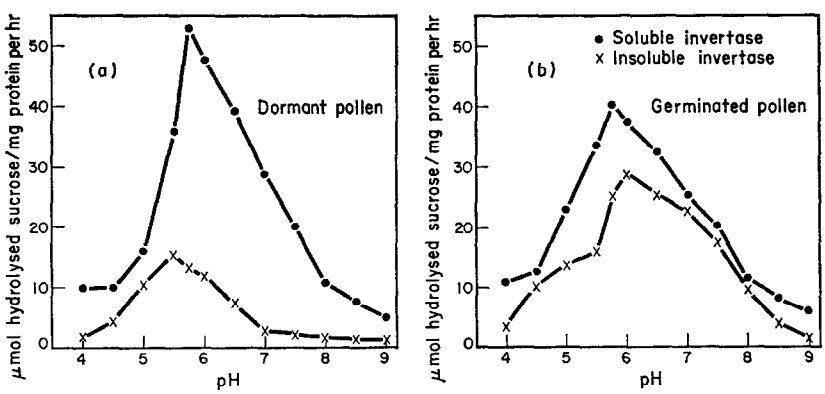

FIG. 1. pH-ACTIVITY CURVE OF SOLUBLE AND INSOLUBLE INVERTASE IN DORMANT POLLEN (a) AND IN POLLEN GERMINATED FOR 2 hr (b).

The activity of invertase was measured in dormant pollen and in pollen germinated for 2 hr. The results are given in Fig. 1(a, b). In dormant pollen both the soluble and the

[1] LINSKENS, H. F. (1967) in *Encyclopedia of Plant Physiology* (RUHLAND, W., ed.), p. 368, Springer, Heidelberg.
[2] HESLOP-HARRISON, J. (1971) *Pollen; Development and Physiology*, Butterworths, London.
[3] DICKINSON, D. B. (1967) *Physiol. Plant.* **20**. 118.

insoluble fractions show invertase activity. The pH optimum lies at pH 5·7 and 5·5 respectively. The pH curve of the soluble invertase has a very sharp peak, unlike the bound invertase. The activity of the soluble enzyme is 3·5-fold higher than that of bound invertase. A peak attributable to neutral invertase could not be detected, but the soluble acid enzyme shows significant activity even in the neutral range.

After 2 hr germination there is a change in the behaviour of the 2 invertases (Fig. 1b). The activity of soluble acid invertase decreased by 26%, but the pH optimum remained at 5·7 with a sharp peak. However the pH optimum of the insoluble enzyme was shifted to pH 6·0; its activity was increased 2-fold. In this experiment, too, a specific neutral invertase could not be detected but the total enzyme activity increased in the neutral pH range.

In the two stages of pollen ageing, in the dormant and germination phases, invertase shows different behaviour with respect to pH. The remarkable fact is that there is a shift of pH optimum of insoluble invertase towards the neutral range. Similar results are described for tissues in stalks of sugar cane.[4] In the juvenile phase of growth the internodes have a high activity of soluble acid invertase with a pH optimum of about 5·3. The enzyme activity decreased rapidly due to the process of ageing, and an insoluble acid invertase appeared, as well as a neutral one.

If the bound invertase is localized outside the pollen tube cell membrane (intine), as described by Dickinson,[3] one can understand the change in the behaviour of enzyme action. The pollen tube is supplied with sugars by the pistil tissue. The invertase may play an important role in the degradation of sucrose from pistil tissue into glucose and fructose. These monosaccharides can easily penetrate through the intine into the pollen tube. There is evidence that the intine is a barrier for oligosaccharides.[3] Here, omission of Ca^{2+} or addition of EDTA to germinating pollen tubes of *Lilium longiflorum* resulted in considerable leakage of carbohydrates into the medium.

EXPERIMENTAL

The pollen was harvested from the anthers on maturation. Pollen (30 mg) was homogenized at 0° in 2 ml glycylglycine buffer (pH 7·0) containing 0·3 M 2-mercaptoethanol till the exine was destroyed mechanically. The resulting suspension was centrifuged at 15000 *g* for 20 min. The supernatant was regarded as soluble enzyme solution. The pellet was washed 2× and resuspended in 0·5 ml buffer. This suspension was designated as insoluble enzyme solution. For germination, 30 mg of pollen were suspended for 2 hr in a germination medium.[5] Then the medium was removed by filtration, the pollen washed 2× with glycylglycine buffer (pH 7 0) and homogenized as before. The activity of invertase was determined by the method of Roe.[6] The reaction mixture (final vol. 0·5 ml) contained: 1 μmol acetate buffer, 0·5 μmol sucrose and 0 01 ml enzyme extract. Protein was determined by the method of Lowry *et al.*[7]

[4] HATCH, M. D. and GLASZIOU, K. T. (1963) *Plant Physiol.* **38,** 344.
[5] DICKINSON, D. B (1965) *Science* **150,** 1818.
[6] ROE, J. H. (1934) *J. Biol. Chem.* **107,** 15.
[7] LOWRY, D. H., ROSEBROUGH, N. J , FARR, A. L. and RANDALL, R. J. (1951) *J Biol. Chem.* **193,** 265.