Experimental

To a mixture of freshly fused and powdered $ZnCl_2$ (10g.) in dry acetic anhydride (14 ml) contained in a conical flask dry resorcinol (10 g.) was added quickly while stirring. The mixture was gently heated on a flame to 142° C (15 mts.). The viscous red solution was allowed to cool to room temperature. 80 ml. of HCl (1 : 1) were added to syrupy mass and stirred. After a few minutes an orange–red crystalline material separated out. The crude product (17 g., 96·5%) was crystallised twice from methanol using norit to give 2 : 4–dihydroxy 5–acetyl acetophenone (resodiacetophenone) as colourless crystalline solid (Yield 90%, m.p. 178–80° C). Found : C, 61·65; H, 5·25. $C_{10}H_{10}O_4$ requires C, 61·80; H, 5·15% Resodiacetophenone diacetate : (Acetic anhydride in pyridine at room temperature for 24 hrs.) m.p. 120° C (Lit[9] m.p. 120° C) Resodiacetophenone dibenzoate : (Benzoyl chloride in Pyridine) m.p. 118° C (Lit[9] m.p. 118° C).

Department of Chemistry, Andhra University, Waltair, *October* 3, 1979.

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ACID PHOSPHATASE ACTIVITY IN COTYLEDONS OF GERMINATING SEEDS OF *VIGNA SINENSIS* (LINN.) SAVI

On the basis of the extensive studies of the developmental patterns of alkaline and acid phosphatases of avian embryos, it was suggested that acid phosphatases which remain at relatively constant level of activity during the growth of the chick embryo, function as constitutive enzymes whereas alkaline phosphatases which show marked fluctuation in the level and locus of activity, function as adaptive enzymes[1,2]. However, the developmental patterns of the phosphatases of growing plants are in contrast with those of the chick embryo. The activity of acid phosphatase at various hours of germination increases with time. The present investigation is mainly concerned with the development of acid phosphatase activity in the cotyledons (half-seeds without embryo) at various times of germiation. Also, we report here the presence of multiple forms of acid phosphatase as separated by polyacrylamide gel electrophoresis and subsequenlty stained by the α-naphthyl phosphate and "fast" blue RR[3].

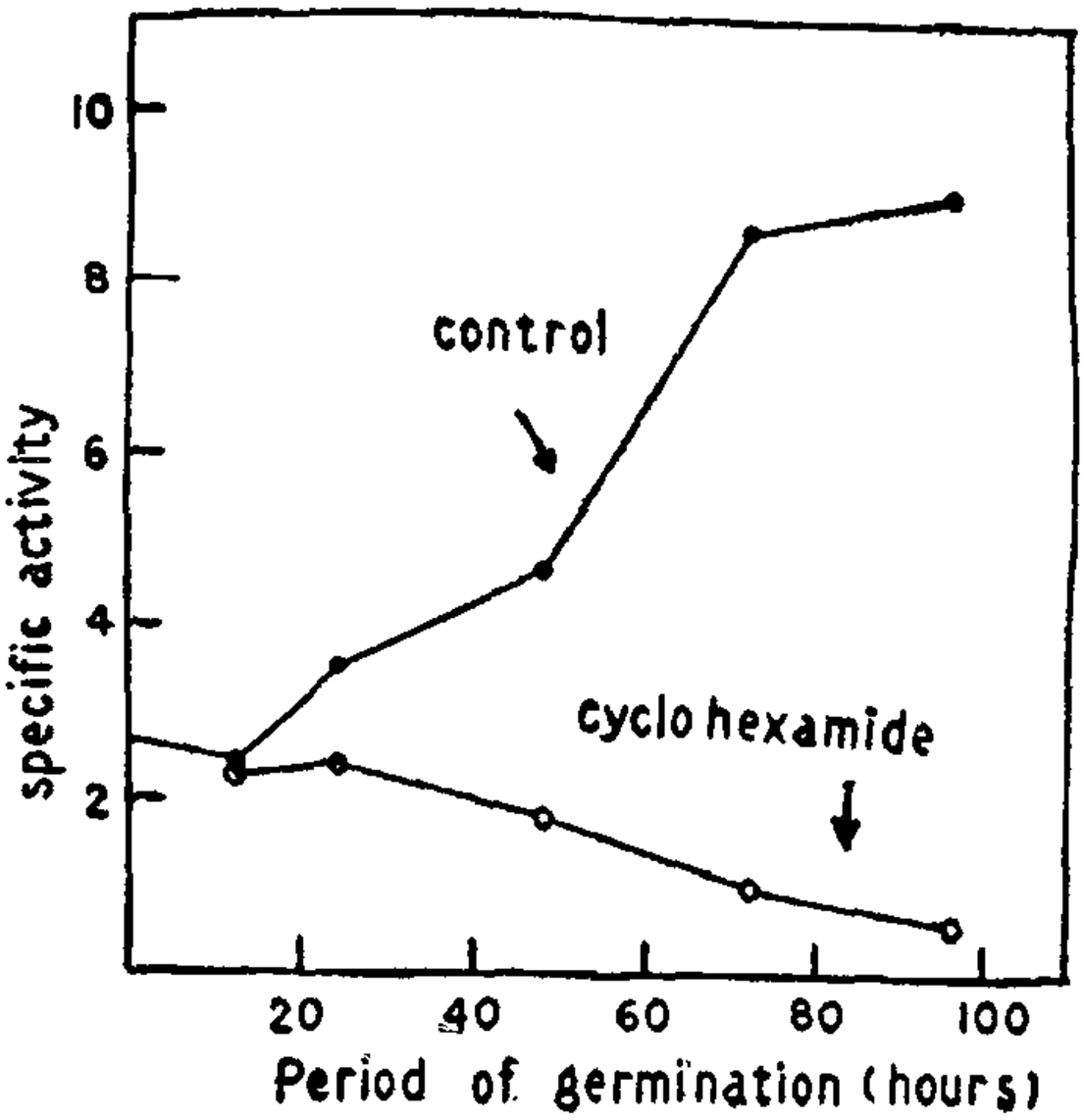

Fig. 1. Acid phosphatase activity as measured by *p*-nitrophenol method (4) at various hours of germination at 28° C. Control: After imbibition whole seeds were broken into half seeds and the half seeds without embryo were kept under germinating conditions at 28° C.

In another set half seeds without embryo were kept in germinating conditions with cyclohexamide solution (100 μg/ml). Cotyledons were homogenized as described in legends of Fig. 2 and the homogenate was then centrifuged at 3,000 × g for 10 min at 4° C. The Cell-free extract was then used as enzyme source. Specific activity has been defined as μ mole of *p*-nitrophenol liberated/mg protein/hr.

The results given in Fig. 1 indicate that the acid phosphatase activity (as measured using *p*-nitrophenyl phosphate as substrate[4]) increases with the increase in time after imbibition of the half seeds (without embryo) at 28° C. The rate of increase of acid phosphatase activity in the half-seeds is maximum in 48-60 hrs. However, the acid phosphatase activity has been found to decrease when the seeds were kept in cyclohexamide solution (100 μg/ml) after 2 hrs of imbibition at 28° C. The results given in Fig. 1 suggest that the increase in the acid phosphatase activity in the cotyledons, kept under germinating condition is mainly due to the *de novo* synthesis of the

enzyme protein but not due to the activation of the already synthesized protein present in the dry seeds.

It has been reported that some plant growth hormones mainly gibberellic acid (GA_3) induces the acid phosphatase activity in half-seeds of barley and wheat[5]. We examined the effects of GA_3 and indole acetic acid (IAA) on the development of acid phosphatase activity in half seeds (without embryo) of *Vigna sinensis* (Linn.) *Savi* kept in germinating condition at 28° C. The results given in Fig. 3 show that both GA_3 and IAA have practically no stimulatory effect on the development of acid phosphatase. On the contrary both GA_3 and IAA, particularly at higher concentration, have been found to have little inhibitory effects.

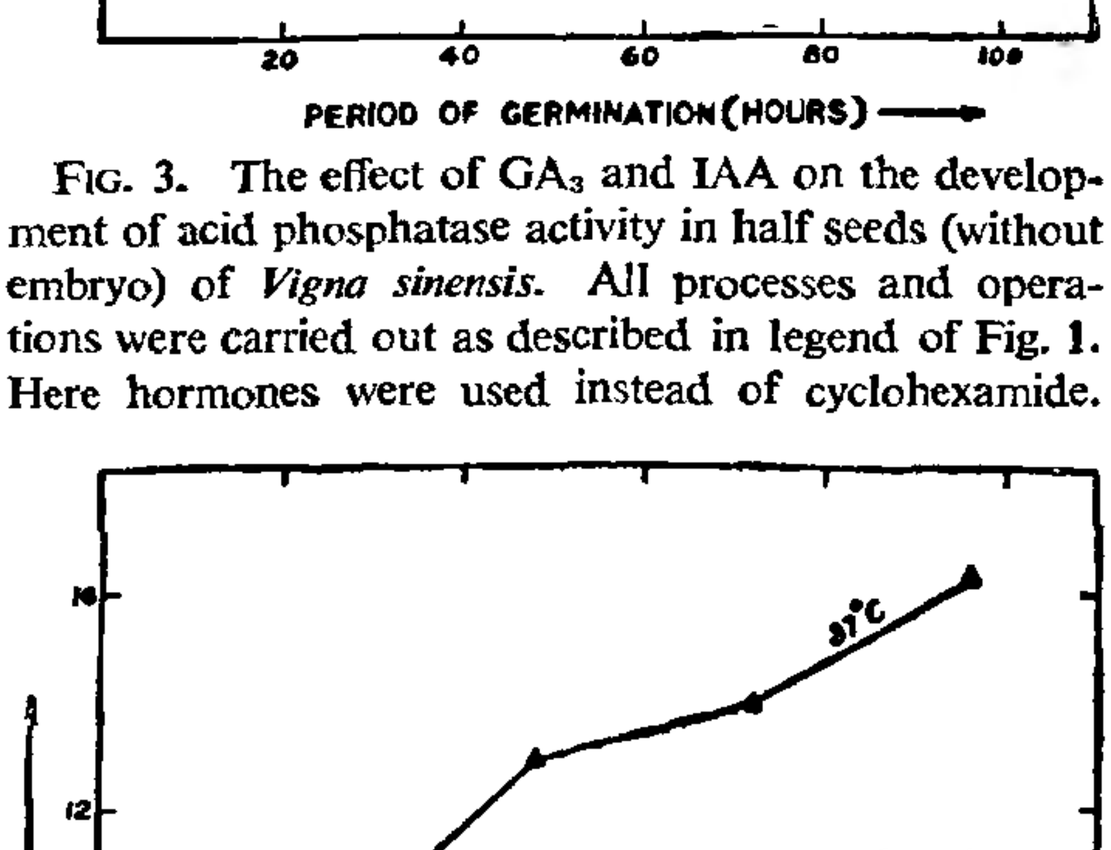

FIG. 3. The effect of GA_3 and IAA on the development of acid phosphatase activity in half seeds (without embryo) of *Vigna sinensis*. All processes and operations were carried out as described in legend of Fig. 1. Here hormones were used instead of cyclohexamide.

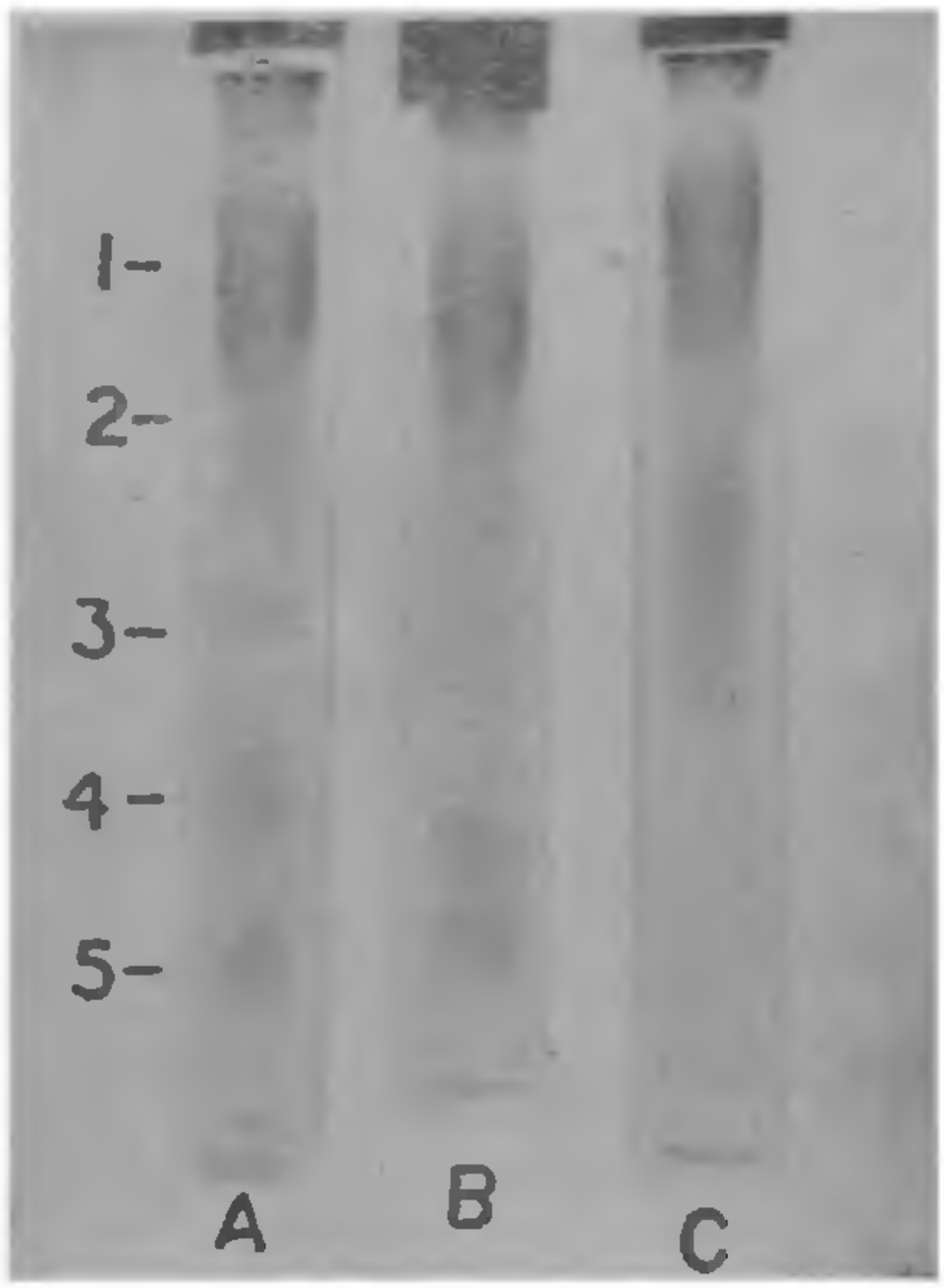

FIG. 2. Zymogram patterns of acid phosphatases using different pH for gel buffer.

A: pH 8·5; B: pH 7·0; C: pH 5·0. After 48 hrs, half seeds were homogenized in a mortar pestle with sea sand in a buffer containing 10 mM Tris–HCl, pH 7·4 and 0·1% Triton X–100. The mixture was kept overnight at 4° C and was then centrifuged at 10,000 × g for 20 min. The sediment was discarded and the supernatant was then centrifuged at 1,05,000 × g for 90 min in an Ultracentrifuge. The supernatant was then used as enzyme source.

Next we studied the effect of temperature on the germination and the development of acid phosphatase activity in the cotyledons (with embryo). The results given in Fig. 4 show that with the increase in temperature of germination, there is increase in acid phos-

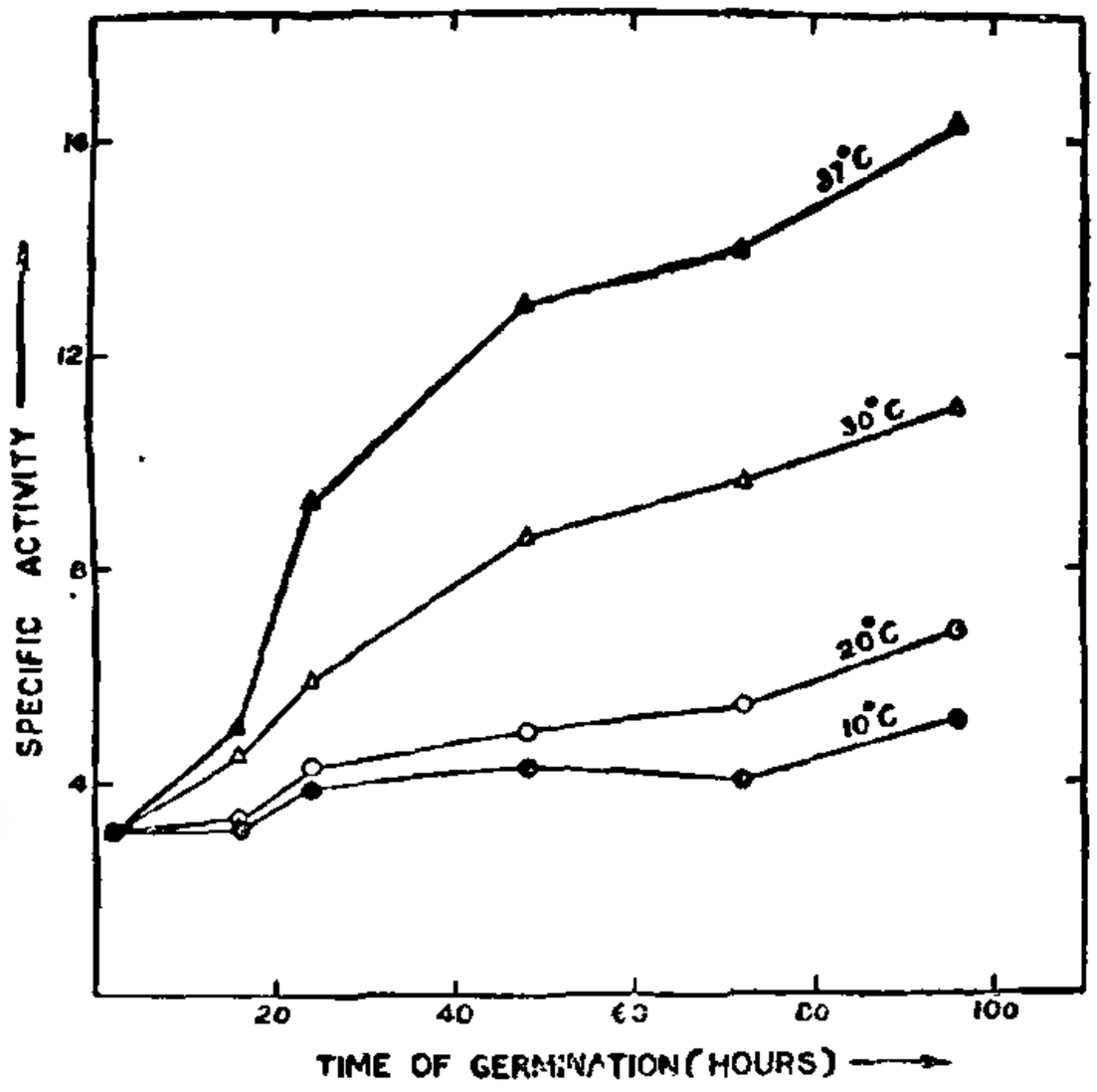

FIG. 4. The effect of temperature on the germination and development of acid phosphatase activity in cotyledon (with embryo). In this experiment 0·001% chloramphenicol, an antibacterial agent, was used to minimize the bacterial growth. It has been found that this low concentration of chloramphenicol has no effect on the germination of seeds and development of acid phosphatases. Preparation of cell free extract and enzyme assay were carried out as described in legend of Fig. 1.

phatase activity. The results also confirm the observation given in Fig. 1.

We also determined the number of multiple forms of acid phosphatase activities as separated by electrophoresis on a polyacrylamide gel, carried out using 7% gel[6] at 10 volt per tube for nearly five hours. After the run was over, the gels were stained for phosphatase activity according to Brewer[3]. The three tubes A, B and C (Fig. 2) represent the three gels with different pH of the gel buffer (pH 8·5, 7·0 and 5·0). Acid phosphatases are separated best on the gels having pH 8·5. The results indicate that at pH 8·5, there are at least 5 different forms of acid phosphatase activity. However, one cannot rule out the possibility of the presence of more species of acid phosphatase activity in No. 1 and 4 bands in tube A of Fig. 2.

It is now well established that a large number of enzymes exist in multiple forms. Isozymes are the examples of these multiple forms. According to Shaw[7] isozymes may be classified into two major categories: (*a*) distinctly different molecules, presumably produced from different genetic sites, and (*b*) those which result from secondary alteration in the structure of a single polypeptide species. For classification of different isozymes of acid phosphatase found to be present in the cotyledons of *Vigna sinensis*, detailed immunological studies are required because of the difficulty of getting conditional mutants in eucaryotic systems. For detailed immunological studies, the separation and purification of different isozymes to homogeneity is a prerequisite. The work on the purification of different isozymes present in the cotyledons of germinating seeds of *Vigna sinensis* (Linn.) Savi are in progress.

Our preliminary results indicate that there is distinct change in the zymogram pattern of acid phosphatase activities present in cotyledons of the seeds of *V. sinensis* with the change in germination period and environmental conditions (Results not given). This change in zymogram may be exploited to monitor the gene expression during germination.

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SOME NOVEL SPRAY REAGENTS FOR NATURALLY OCCURRING HIGHER FATTY ACIDS AND THEIR METHYL AND ETHYL ESTERS

A NUMBER of spray reagents are used for detecting the presence of fatty acids and their methyl and ethyl esters[1-3]. These reagents very often do not produce pronounced colour. With a view to find out some new spray reagents which could produce pronounced colours with fatty acids and their esters, the present investigation was carried out. The fatty acds and their esters (20 μg each) were app'ied on Whatman No. 1 filter-paper strips (30 × 10cm) impregnated with 2% olive oil solution in benzene[4] and the chromatograms were developed by descending technique using a mixture of ethanol and water (9 : 1 v/v) as irrigating solvent. The developed chromatograms were dried in air and treated with the spray reagents (1) 0·5% fluorescene in ammonium hydroxide and then with 0·5% aqueous uranyl acetate (or uranyl nitrate) or (2) 1% aqueous cupric acetate and then with 0·1% aqueous rhodmine B solution or (3) 0·5% aqueous uranyl acetate (or uranyl nitrate) and then with 1% aqueous bismuth iodide solution or (4) 0·5% aqueous uranyl acetate or uranyl nitrate and then with 1% aqueous potassium ferrocyanide solution or (5) 0·1% aqueous rhodamine B solution only. The coloured spots appear instantaneously. In visible light the reagents 1 & 3 gave yellow colour, 2 gave violet colour, 4 gave greenish brown colour and 5 gave bright pink colour whereas in UV light 1 gave shining yellow colour, 2 gave bluish pink colour, 3 gave violet colour, 4 gave brown colour and 5 gave pinkish orange colour. The R_f values of the acids and their esters were oleic acid, 0·04; stearic acid, 0·16; palmitic acid, 0·21; myristic acid, 0·52; ethyl stearate, 0·87; ethyl oleate, 0·28; methyl palmitate, 0·85; and ethyl myristate, 0·32.

The above paper chromatographic procedure has also been extended successfully to quantitative estimation of fatty acids and their methyl and ethyl esters