DE NOVO SYNTHESIS OF PROTEASE DURING GERMINATION OF PEARL MILLET SEEDS

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ABSTRACT

Protease activity in the endosperm of pearl millet (*Pennisetum typhoides*) seeds increased gradually during germination. The enzyme activity was slightly greater when the seeds were germinated at 28°C in diffused daylight than in dark. The protease activity increased rapidly only after 2 days of germination and this increase was effectively inhibited by inhibitors of protein synthesis such as chloramphenicol (1.5×10^{-3}M), cycloheximide (2×10^{-4}M) and ethionine (5×10^{-3}M). Cycloheximide has been found to be a more potent inhibitor than ethionine. Results obtained indicate the *de novo* synthesis of the enzyme during germination rather than activation of a zymogen.

INTRODUCTION

DURING the period of imbibition, the seed develops the metabolic systems necessary for the growth and also the enzymic components of these systems. In the germinating seed, the enzymes may be released or activated from the existing proteins, or they may be synthesized *de novo* through the nucleic acid directed, protein synthesis. Amylopectin glucosidase of pea seedlings[1], β-amylase of wheat[2] and phosphatase of mung bean[3] are released from the pre-existing latent forms, whereas α-amylase in germinating barley seeds[4], isocitratase in peanut cotyledons[5] and lipase of cotton seedlings[6] originate through *de novo* synthesis. Earlier we have reported the presence of an acid protease in germinating pearl millet seeds[7]. The increase in protease activity during germination of pearl millet seeds is closely correlated with the breakdown of proteins[8]. It is of interest to investigate the origin of protease during the germination of pearl millet seeds and in this paper we present the results obtained to indicate the *de novo* synthesis of this enzyme during germination.

MATERIALS AND METHODS

Pearl millet (*Pennisetum typhoides*) seeds (variety Vijai compositae) were obtained from the Millet Research Station, Vizianagaram, Andhra Pradesh. Bovine serum albumin (BSA), chloramphenicol and cycloheximide were obtained from Sigma Chemical Co., USA. DL-Ethionine was purchased from BDH, England. All other chemicals used were of analytical reagent grade.

Enzyme preparation

Pearl millet seeds were soaked either in tapwater or in an appropriate inhibitor solution at 28°C for 18 hr and spread over a moist filter paper for germination in the dark. Seedlings were harvested at 24 hr intervals and hand excised to remove axis from the endosperm (seed coat intact). The enzyme extracts (25%) from the endosperms were prepared by grinding the tissue with acid washed sand in distilled water at 4°C. The homogenates were centrifuged at 6000 g for 15 min at 4°C and the supernatants were used as the enzyme source.

Protease assay

Protease activity was assayed using BSA as substrate. The reaction mixture contained 0.8 ml of 0.36% BSA in 0.1 M citric acid phosphate buffer, pH 3.6 and 0.2 ml of 25% enzyme extract. After arrested by the addition of 1.5 ml of 10% trichloroacetic acid. The mixture was heated briefly in a oacetic acid. The mixture was heated briefly in a boiling water bath for complete precipitation of protein and filtered. Suitable aliquots of acid filtrates were used to develop the colour with Folin-Ciocalteau reagent. In all assays, appropriate control experiments were carried out and the readings obtained for controls were subtracted from the experimental values. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μg of tyrosine equivalent per 2 hr under assay conditions.

Protein concentration in the enzyme extract was estimated by the procedure of Lowry *et al*[9] using

crystalline BSA as the reference protein. Specific activity refers to units of enzyme activity per mg protein.

RESULTS AND DISCUSSION

When the pearl millet seeds were allowed to germinate at 28°C separately in diffused daylight and in dark, it was noticed that the light had no significant effect on the growth of the seedling, though the formation of green-coloured pigment in the axis was affected. It is clear from figure 1 that the protease activity is slightly greater in the seedlings germinated in diffused daylight than those germinated in the dark. It is also evident that the protease activity of pearl millet seedlings increased rapidly only after 2 days of germination. The enzymes which are observed relatively in the later periods of germination may be formed due to *de novo* synthesis[1].

Some enzymes like amylopectin glucosidase of germinating peas, increase in activity during the initial stages of germination and appear promptly after imbibition of the seed and hence may be released from a latent form. An attempt has been made to test whether the increase in protease activity in the endosperm of germinated seedlings of pearl millet is due to the activation of any zymogen present in the dormant seeds. The extract of soaked seeds (25% w/v) (as source of zymogen) was mixed with the enzyme extract obtained from the endosperm of 6-day-old germinated seedlings (as source of activator) in the ratio of 10 : 1. The results obtained indicate that there is no increase in the protease activity of the extracts of soaked seeds when incubated with the enzyme extract either at pH 7.6 or at pH 3.6. Treatment of the soaked seed extract with trypsin also did not enhance the protease activity. Hence it may be concluded that *the protease of pearl millet is not formed by the* activation of any zymogen present in the dormant seeds.

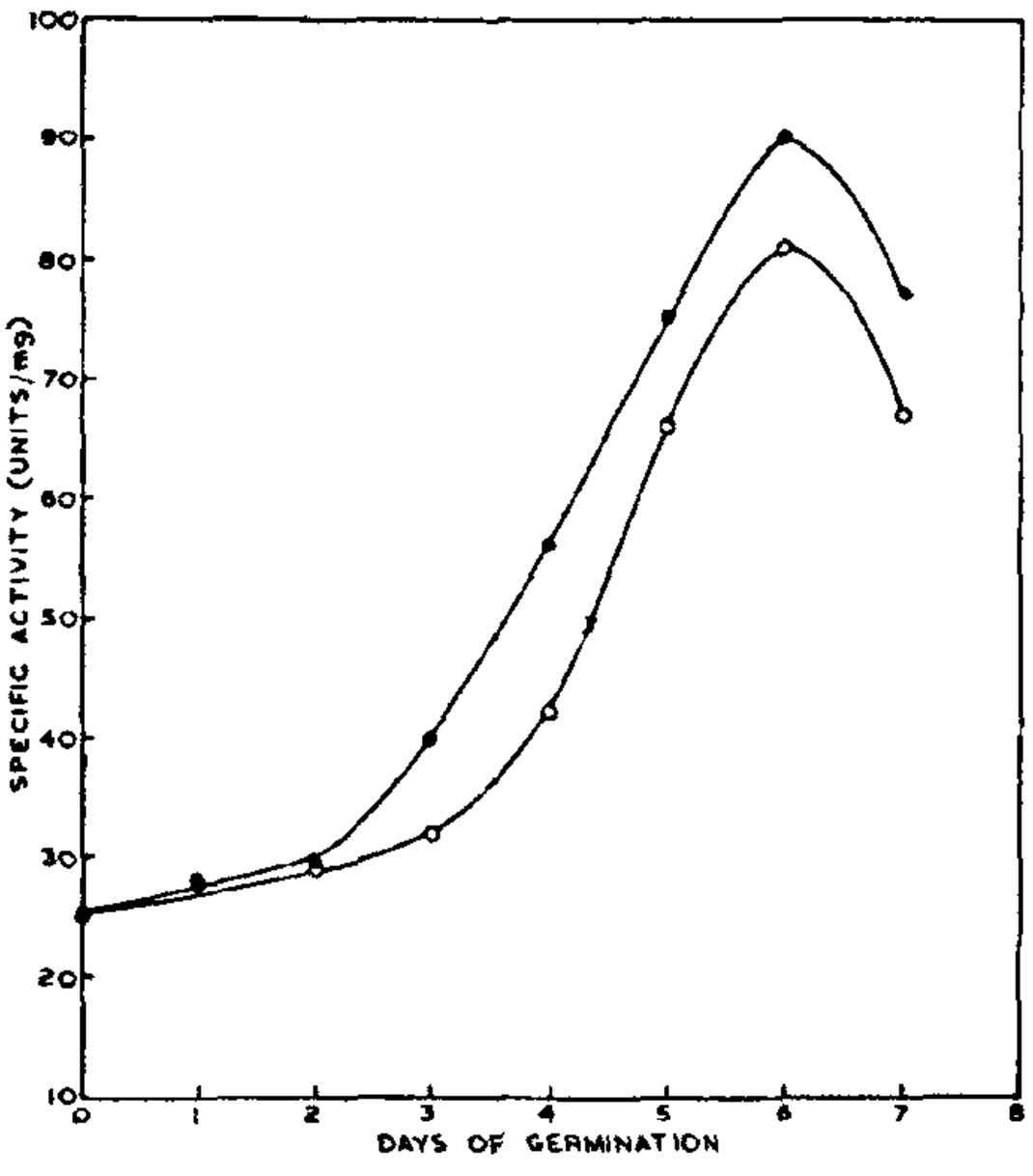

Figure 1. Effect of light on protease activity during germination of pearl millet seeds. Germination in diffused daylight (●) and in dark (○).

Further, the effect of various inhibitors of protein synthesis on the appearance of protease activity during germination of pearl millet seeds was studied and the results are presented in table 1. Streptomycin and chloramphenicol inhibited the protease activity markedly at higher concentrations. At levels of 1000 μg/ml these inhibitors caused about 60% inhibition of the protease activity on the third day of germination. Cycloheximide inhibited the germination of seeds at all the concentrations (1000, 100, 10 μg/ml) tested. However the growth of the seedlings was decreased by streptomycin and chloramphenicol, only at levels of 1000 μg/ml.

The effect of imbibition of cycloheximide, ethionine and chloramphenicol by pearl millet seeds on the appearance of protease activity during

Table 1 *Effect of streptomycin and chloramphenicol on the protease activity during germination of pearl millet seeds*

Inhibitor	Concentration μg/ml	Protease activity units/mg	
		Third day	Sixth day
None	–	45	100
Streptomycin	1000	16	75
	100	40	84
	10	45	95
Chloramphenicol	1000	15	55
	100	35	78
	10	44	89

Seeds were soaked separately in 100 ml of inhibitor solution of specified concentration at 28°C for 18 hr and germinated in dark. The appropriate inhibitor solution (15 ml) was sprayed daily on the seedlings.

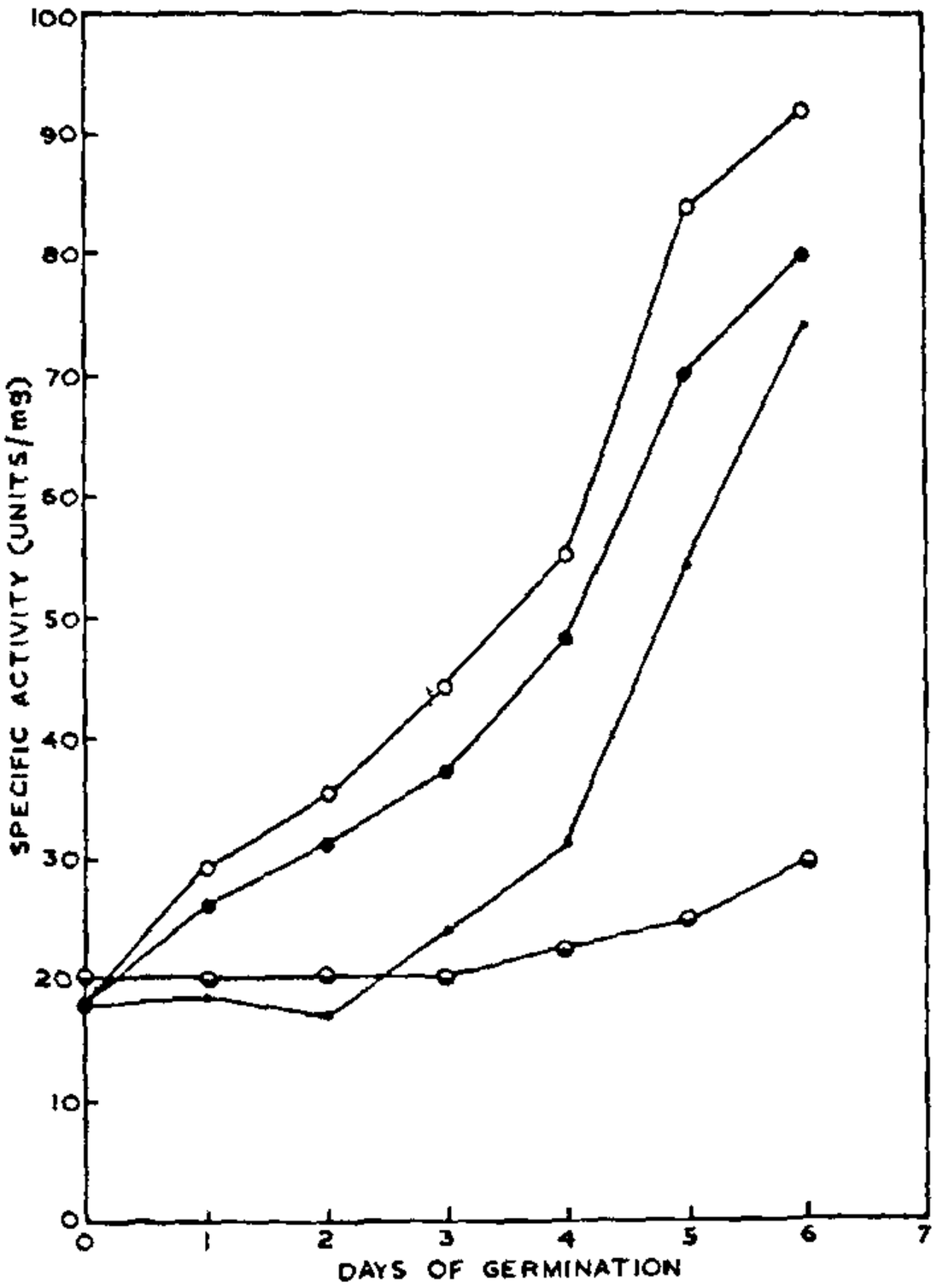

Figure 2. Effect of imbibition of inhibitors of protein synthesis on protease activity during germination of pearl millet seeds. Seeds were separately soaked in 50 ml of distilled water (○), or 1.5×10^{-3}M chloramphenicol (●), or 2×10^{-4}M cycloheximide (⊖), or 5×10^{-3}M DL-ethionine (◐) for 18 hr at 28°C and germinated in dark. Distilled water was sprinkled on the seedlings periodically.

germination is shown in figure 2. It is clear that all the compounds used inhibited the protease activity to different extents during germination. On the sixth day of germination about 65% inhibition of protease activity was caused by cycloheximide, a well-known inhibitor of protein synthesis in eukaryotes.

The effect of cycloheximide and ethionine was further studied by spraying the solutions of these inhibitors separately on the 48 and 72 hr germinated pearl millet seedlings respectively. Figure 3 indicates that the protease activity of the seedlings is significantly inhibited when they were sprayed with cycloheximide and ethionine. Cycloheximide appears to be a greater potent inhibitor of protease synthesis than ethionine.

The protease activity in the extracts of endosperms of 6-day-old germinated seedlings was assayed separately in the presence of chloramphenicol (1.5×10^{-3}M), cycloheximide (2×10^{-4}M) and ethionine (5×10^{-3}M) to investigate whether these compounds have any direct effect on the enzyme activity. It is evident from the data presented in table 2 that there is no direct inhibition of protease activity in the extracts by the inhibitors of protein synthesis.

The observations reported in this communication clearly indicate the *de novo* synthesis of protease in the endosperm during germination rather than activation of a zymogen. Similar *de novo* synthesis of a protease in the cotyledons during germination of squash was also reported earlier[10].

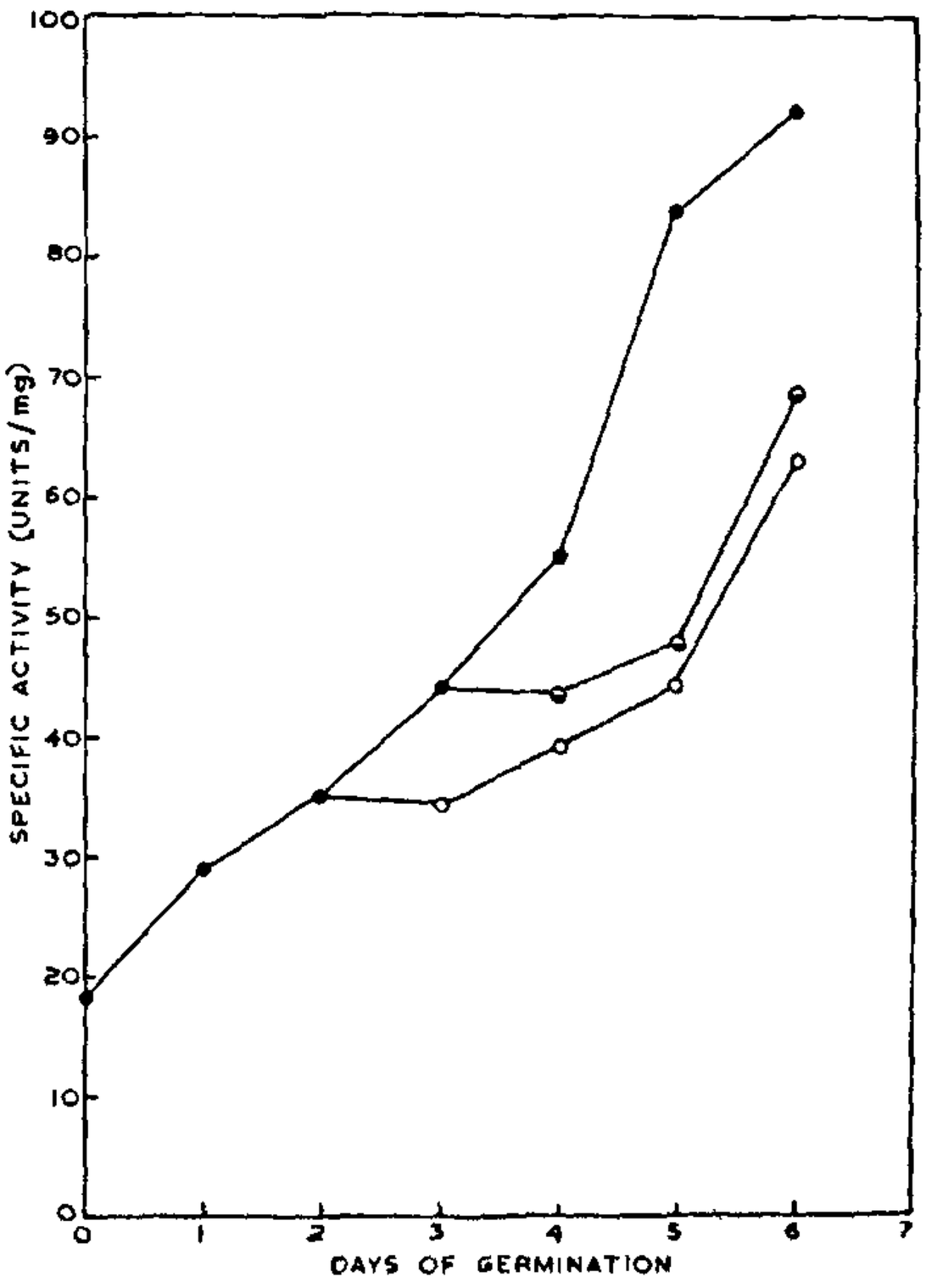

Figure 3. Effect of spraying of inhibitors of protein synthesis on protease activity of pearl millet seedlings during germination. Seedlings grown only in distilled water served as control (●). 5 ml solutions of 2×10^{-4}M cycloheximide (○) or 5×10^{-3}M DL-ethionine (◒) were sprayed at 24 h intervals on the seedlings grown in distilled water for 2 and 3 days respectively.

Table 2 *Effect of inhibitors of protein synthesis on the protease activity of pearl millet extracts*

Inhibitor	Concentration	Protease activity (% control)
None		100
Chloramphenicol	1.5×10^{-3} M	105
Cycloheximide	2×10^{-4} M	100
Ethionine	5×10^{-3} M	95

Extracts obtained from the endosperms of 6-day-old germinated seedlings were used to study the protease activity.

ACKNOWLEDGEMENT

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NEWS

AIR POLLUTION IN THE MODERN OFFICE

. . . "Few work environments would appear safer than the modern office, its sealed windows and carpeted floors filtering out all the harshness of the outside world. In fact, though, it can harbor an array of pollutants ranging from cancer-causing hydrocarbons and various microbes to radioactive radon gas. The reason is often as simple, or, rather, as complicated, as a lack of ventilation. . . . Buildings simply are not inspected for adequate ventilation, as they are for, say, fire safety. As a result, few landlords have any notion of either the actual or the required ventilation rates for their buildings, and no one knows how often the standards are being met. What we do know is that many modern buildings recirculate 80% of all indoor air and that the amount of available fresh air often varies widely within a structure. . . . The energy crisis also spawned significant changes in construction materials and architectural designs. New insulation products, including particleboard that contained formaldehyde and foams made with polyurethane and urea-formaldehyde, were approved for general use, despite their tendency to give off toxic gases and residues. At the same time, new, and noxious, chemical sealants were introduced to stem the loss of heated or cooled air, and, in new office towers, windows that open were eliminated in favour of mechanical ventilation systems. The result, for many buildings, was an atmosphere two to five times more polluted than even the dirtiest outdoor urban air."

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