

## Partial purification and some biochemical properties of acid phosphatase in germinating chickpea (*Cicer arietinum*) seeds

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Angosto, T., González, F. and Matilla, A. 1988. Partial purification and some biochemical properties of acid phosphatase in germinating chickpea (*Cicer arietinum*) seeds. – *Physiol. Plant.* 74: 715–719.

An acid phosphatase (EC 3.1.3.2.) from the embryonic axes of chickpea seeds (*Cicer arietinum* L. cv. Castellana) was purified by ammonium sulphate precipitation, chromatography on Sephacryl S-200 and polyacrylamide gel electrophoresis. The preparation has an apparent molecular weight of 39 kDa, pH optimum for *p*-nitrophenylphosphate hydrolysis of 5.25, and  $K_m$  of 0.57 mM. The enzyme hydrolyzed all the mono- and di-phosphorylated sugars tested, but had no effect on ATP, ADP, AMP and phosphoenolpyruvate. Phosphate was a competitive inhibitor.  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Hg^{2+}$ ,  $Fe^{3+}$ , arsenate,  $K^+$  and  $Zn^{2+}$  were inhibitory.  $Mn^{2+}$ , dithiothreitol and EDTA had no effect, and polyamines were activators.

**Key words** – Acid phosphatase, *Cicer arietinum*, germination, inorganic phosphorus, nucleotides, phosphate sugars, seeds.

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### Introduction

Acid phosphatases (EC 3.1.3.2) are a group of enzymes that non-specifically catalyze the hydrolysis of phosphate monoesters and oxygen exchange from water to inorganic phosphate in an acid environment (Hollander 1971). They are widely found in small quantities in microorganisms as well as in plants and mammals, but unlike the alkaline phosphatases their molecular structure and properties are not clear. They are subject to surface denaturation in their pure state and are unstable in diluted solutions (Hye-Shin and Van-Etten 1986), which makes their isolation and purification difficult. In microorganisms, animals and plants, phosphatases, particularly the acidic ones, play an important role in the production and transport of inorganic phosphate, which is essential to a large number of metabolic reactions (Schlosnagle et al. 1974, Bielecki and Ferguson 1983).

Acid phosphatases play a major role in the mobilization of phytate, an organic phosphate reserve in the cotyledon of leguminous seeds, and thus contribute to the growth of the embryonic axes (Sugiura and Sunobe

1962, Mandal and Biswas 1970, Guardiola and Sutcliffe 1971, Tamura et al. 1982). In the present paper we describe a method for the partial purification of a soluble acid phosphatase in *Cicer arietinum* seeds and report on some of its biochemical properties, prior to the publication of our findings on its physiological involvement in the germination of this seed.

**Abbreviations** – BSA, bovine serum albumin; cyt *c*, cytochrome *c*; DTT, dithiothreitol; *p*-NPP, *p*-nitrophenylphosphate; PVP, polyvinylpyrrolidone; Tris, tris(hydroxymethyl)aminomethane.

### Materials and methods

#### Plant material

The experiments were made with embryonic axes excised from 1985 seeds of *Cicer arietinum* L. cv. Castellana, after 24 h of germination. The seeds were germinated in darkness at 25°C, in plastic trays (50 seeds per tray) with 125 ml of water and a relative humidity of 70% (Hernandez-Nistal et al. 1983).

Received 3 June, 1988; revised 7 September, 1988