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A Novel Comparative Assessment of Extracted Amylase Activity in germinating and germinated seeds of *Cicer arietinum, Ceci neri,* and *Pisum sativum*

Kirti Rani*, Sunidhi Kant, Shreya Goyal, Akshay Saini and Chandrika Gupta

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida (UP), India

*Corresponding Author E-mail: krsharma@amity.edu

ABSTRACT

Amylase is an enzyme that occurs naturally in humans, mammals, bacteria, fungi and plants which converts starch into maltose by cleaving off starch from the non-reducing end by catalyzing the hydrolysis of second 1, 4 glycosidic bond of amylose, amylopectin, glycogen and their degradation products. Leguminous seeds are the most important plant food material and they are the rich in concentrated cheap sources of carbohydrate as starch and protein for the vegetarian population. In our present work, we were selected three sources of leguminous seeds (Cicer arietinum, Ceci neri, and Pisum sativum) which are purchased from local market of New Delhi, India and a study was done on comparative assessment of extracted amylase level in all three selected samples of leguminous seeds. Hence, amylase was extracted from germinating and germinated seeds of Cicer arietinum, Ceci neri, and Pisum sativum. The amylase assay was done by dinitrosalicylic acid method at 570nm. Maximum crude amylase activity was found in germinating seeds of Cicer arietinum followed by Ceci neri and Pisum sativum. While, crude amylase activity was found maximum in germinated seeds of Pisum sativum followed by Cicer arietinum and Ceci neri respectively. That comparative assessment of crude amylase activity was clearly showed that germinated seeds showed lesser activity as compared to germinating seeds in case of Cicer arietinum and Ceci neri which was just opposite in case of Pisum sativum.

Keywords: *Cicer arietinum, Ceci neri , Pisum sativum, amylase.*

INTRODUCTION

Amylase was the first enzyme to be discovered and isolated by Anselme Payen in 1833. There are two types of amylases, α-amylase (a-1,4 glucan glucohydrolase) which hydrolyses a-1,4 linkages in a random manner to by-pass 1,6 branch points and β-amylase (a-1,4 glucan maltohydrolase) which hydrolyses alternate bonds from the non-reducing end of starch into maltose which results in 55% conversion of starch into maltose as well as large limit dextrin[1,11]. Seed development is complex process comprising of a series of events involving cell division, cell differentiation and storage of macromolecules. In legume cotyledons, cell differentiation starts in certain regions and progressively spreads to other parts, thereby building up a developmental gradient too. Seeds accumulate starch, storage proteins and oil in different proportions depending upon the species[13]. Thus, the seeds are good reserve of all these important contents, synthesized and accumulated in the storage organs called endosperm or the cotyledons which have economic importance as well as make it a subject of intensive investigation[13]. The large sized legume seeds are combination of physiological, biochemical and molecular approaches with an analysis of the underlying developmental processes too[6]. During germination, storage constituents starts to used up for seedling growth by hydrolytic enzymes such as amylase, invertase, protease and lipase whose activities are higher in germinating to germinated seeds till root growth, shoot growth, leaves growth and petiole growth are started from seeds to complete plant[7]. Hence, germinating seeds are known to contain starch degrading-enzymes, amylases which digest starch digestion resulting in the formation of sugars which are subsequently used in various metabolic activities[3,8,10].

Starch saccharification or depolymerization by amylases is the basis for running several industrial processes such as preparation of supplementary foods, glucose syrups, brewing, bread making, paper industry, leather industry and detergent industry too[11, 12, 13]. Chickpeas are grown in the Mediterranean, western Asia, the Indian subcontinent, Australia, the north-western United States region, and the Great Plains. India is the world leader in chickpea[3, 8]. Pea is a pulse crop appearing in the Gangetic basin and southern India[6]. In present study, three sources of leguminous seeds were taken, namely *Cicer arietinum*, *Ceci neri* of family *Fabaceae* and *Pisum sativum* of *Leguminosae* family and were purchased from local market of New Delhi, India. Comparative assessment was performed to know the extracted amylase level in all three selected samples of germinating and germinated seeds of *Cicer arietinum*, *Ceci neri*, and *Pisum sativum* by dinitrosalicylic acid method at 570nm[2, 9].

MATERIALS AND METHODS

Extraction of Crude Amylase

Cotyledons from 3-5day old seedlings from germinating and germinated seeds of *Cicer aerietinum*, *Ceci neri and Pisum sativum* were homogenized using pestle mortar in 1M sodium phosphate buffer (pH 7.0) in the ratio of 6 ml of buffer: per gram of fresh weight of seeds. Crude amylase extract was filtered through two layers of muslin cloth and centrifuged at 8000 rpm for 20min at 4°C. The supernatant was collected which contained crude enzyme and stored at 4°C and used for entrapment by emulsification and chemical modification of bovine serum albumin[8,9,13].

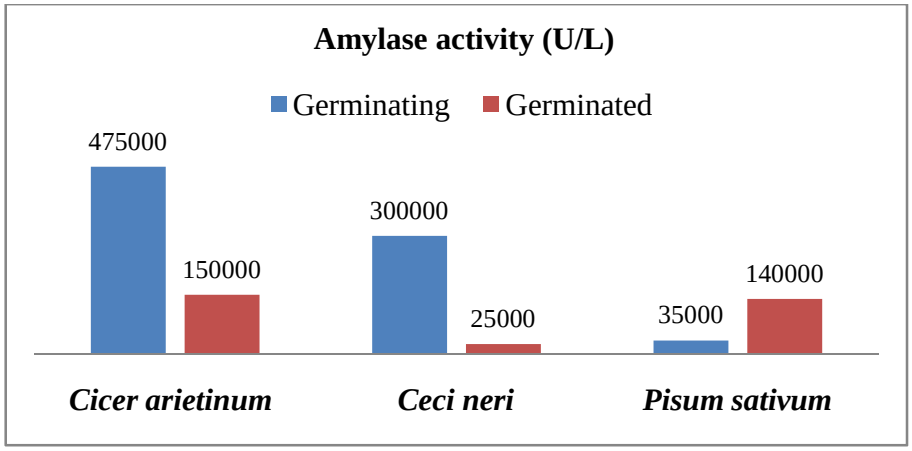
Amylase assay by Dinitrosalicylic acid method

Amylase was assayed by determining the amount of reducing sugars released from used starch as substrate. The starch solution was prepared from 1% (w/v) soluble starch in distilled water. 0.5 ml of crude amylase extracts were added to 1 ml of the starch solution and the mixture incubated at 37ºC for 20 min. After that 2 ml of Dinitrosalicylic acid was added into incubating reaction mixture and boiled for 5 minutes[2,5]. The amount of reducing sugars in the final reaction mixture was determined spectrophotometrically at 570 nm [1,2,8,9]. One unit of enzymatic activity is defined as the amount of enzyme that produces 1 µg of maltose per minute[1, 2,8,13].

RESULTS AND DISCUSSION

Maximum amylase activity has been estimated in crude enzyme extract of germinating seeds of *Cicer aerietinum* with 4,75,000U/L followed by *Ceci neri* with 3,00,000U/L and *Pisum sativum* with 1,40,000U/L whose results were comparable to earlier reports[6, 7, 13]. While, germinated seeds of *Cicer aerietinum* had highest level of crude amylase with its measured activity of 1,50,000U/L followed by *Pisum sativum* with 35,000U/L and *Ceci neri* with 25,000U/L whose results were pretty comparable with previous estimated amylase activity in leguminous seeds (Fig 1) [6,7,13]. Hence, it was clear from this comparative study that germinating seeds of *Cicer aerietinum* and germinated seeds of *Pisum sativum* had highest crude amylase activity (Fig 1).

Fig. 1: Comparative level of extracted amylase in germinating and germinated seeds of *Cicer arietinum, Ceci neri and Pisum sativum*



CONCLUSION

By this comparative assessment of measured crude amylase activity of germinating and germinated seeds of *Cicer aerietinum* and *Ceci neri* and *Pisum sativum*, it was concluded that germinating seeds of *Cicer aerietinum* and *Ceci neri* had highest level crude amylase amylase activity as compared to *Pisum sativum*[7, 13]. It was due to that seed storage substances were found to decrease gradually with the increase of germination time in seeds of *Cicer aerietinum* and *Ceci neri* which lead to degradation of reserve seed nutrients accelerate the development of seedling growth during germination and the highest amylase activity was found in during germination done at 45 hours in water[4, 7]. On the other hand, germinated seeds of *Pisum sativum* had appreciable amount of crude amylase activity as compared to *Cicer aerietinum* and *Ceci neri* [6] (Fig 1). The starch degradation was very slow during the phenological growth stages as termed germinating seeds suggesting that the active mobilization of starch in *Pisum sativum* cotyledons takes place after the radicle has started to

elongate. In general, the pattern of starch degradation in germinating and germinated seeds of *Pisum sativum* was in conformity with earlier reports[6]. Hence, *in vitro* amylase present in germinating and germinated seeds of leguminous plants may have industrial application as *In vitro* carbohydrate digestibility of incorporated whole-chickpea and chickpea bread products was reported earlier too[4] which are cost effective too as compared to its microbial or biochemical production of amylase[3,8,10] and in turn, may be helpful to run several industrial processes such as preparation of supplementary foods, glucose syrups, brewing, bread making, paper industry, leather industry and detergent industry too[11, 12].

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