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ENZYME ACTIVITIES AND DEGRADATION OF NUTRIENTS IN CHICKPEA (*CICER ARIETINUM* L.) SEEDS DURING GERMINATION

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Abstract

Enzyme activities and degradation of seed storage substances were investigated in BARI-1, BARI-2 and BARI-3 varieties of chickpea (*Cicer arietinum* L.) seed at different germinating periods. Among the varieties, the highest amylase activity was found in BARI-2 and lowest in BARI-3 during germination at 45 hours in water. The maximum activity of invertase was found in BARI-2 and minimum in BARI-1 at 72 hours of germination. Lipase activity was highest in BARI-2 and lowest in BARI-1. The highest protease activity was found in BARI-2 and lowest in BARI-3. The amount of total protein and water-soluble protein were found to be highest in BARI-1 and BARI-3, respectively. During light germination, carbohydrate depletion starts after initial imbibitions, and was completed in 120-144 hours. The variety BARI-1 was found to contain the highest amount of free sugar while BARI-3 had the lowest amount. BARI-2 was found to contain highest amount of reducing sugar and BARI-1 contained the lowest amount. The highest amount of starch was found in BARI-3 and lowest in BARI-1. The starch content in chickpea seed decreased gradually during germination. Among the varieties, BARI-1 was found to contain the highest amount of lipid while BARI-3 contained the lowest amount. The seed storage substances were found to decrease gradually with the increase of germination time. The results indicate that degradation of reserve seed nutrients accelerate the development of seedling growth during germination.

Key words: Chickpea (*Cicer arietinum* L.) seed, Carbohydrate, Protein, Lipid, Hydrolytic Enzymes.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in Bangladesh occupying second position after lentil. It is the world's third most important pulse crop. Bangladesh and India produce about 75% of the world's supply (Ignacimuthu and Prakash 2006). It is grown in over 40 countries representing all the continents of which over 95% of the area. Seeds of chickpea are valuable source of protein (Gupta *et al*. 2007). It is also an important source of carbohydrates, B-group vitamins, and certain minerals, particularly to the populations of developing nation and it is mostly consumed as dhal, whole seeds, and several types of traditional, fermented, deep fried, sweetened, and puffed products (Chavan *et al.* 1986). There was no difference in the essential amino acid contents and in chemical scores of desi and kabuli (Viveros *et al*. 2001) chickpeas. During germination the seed storage substances used up for seedling growth and some hydrolytic enzymes such as amylase, invertase, protease and lipase are activated. Chickpea contains insoluble and soluble fiber: the former promotes regular bowel movements, so helping to guard against constipation, as well as possibly lessening the risk of cancers of the colon and rectum; the latter has been connected with lowering blood cholesterol levels, thereby lessening the risk of heart disease and stroke (Geervani 1991). It is mainly used for human consumption and only a small proportion as feed. Chickpeas are used for making various delicious food items such as butter substitute, sprouting, salads, soups and stews. It is also known for its use in herbal medicine and cosmetics**.** Some researchers studied amino acids content (Khan *et al.*1995, Gupta *et*

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al. 2007), its chemical composition (Viveros *et al.* 2001, El-Adawy 2002, Suhasini and Malleshi 2003), carbohydrates (Hawkins and Johnson 2005) and mineral content (Nestares *et al.* 1999) in ungerminated chickpea seeds.

There have been very few reports on the enzyme activities and degradation of seed storage substances particularly on germinated chickpea seeds. The aim of this report is to study the enzyme activities and degradation of nutrients in different varieties of chickpea at different germinating periods.

Materials and Methods

Material: The three varieties BARI-1, BARI-2 and BARI-3 of chickpea seeds (*Cicer arietinum* L.) were collected from Bangladesh Agriculture Research Institute (BARI), Iswardi, Pabna; in the month of October-November. After collection, the seeds were cleaned, dried in the sunlight and kept in a polyethylene bag and stored separately in deep freeze (-10°C) for biochemical analysis. Glucose, BSA, Dinitrosalicylic acid (DNS) and Sodium tungstate were purchased from Sigma Chemicals Ltd., USA. Chloroform, Ethanol and Trichloro acetic acid (TCA) were purchased from Pharmacia Fine Chemicals Ltd., Sweden. All other chemicals were of analytical grade and were used without further purification.

Germination of seed: Good and mature seeds of chickpea were sorted out for germination. The sorted seeds were soaked in distilled water within a glass beaker with potassium permanganate for six hours. Potassium permanganate was used for avoiding the growth of microorganism on seed surfaces during germination. The soaked seeds were then taken out from water and scattered on a filter paper, placed on a plastic tray containing little amount of distilled water. The tray was then placed in a lighted room at 22°C for 24, 36, 40, 45, 72, 96, 120 and 144 hours including soaking time. The germinated seeds at different hours were separated from seedling, rinsed with distilled water and stored separately in a deep freeze (-10°C) for analysis.

Preparation of the crude extract: 100 grams of germinated seeds were crushed in a mortar with a pestle and then suspended in 40 ml of 30% acetone. After occasional gentle stirring for 3 hours at 4°C the suspension was filtered through double layer of muslin cloth. The filtrate was collected and centrifuged in a refrigerated centrifuge at 5500 rpm for 15 minutes at 4°C. The supernatant was used as “crude extract”.

Ammonium sulphate fractionation: The crude extract was saturated to 30-50% by the addition of solid ammonium sulphate under constant and gentle stirring at 4°C. The resulting precipitate was collected by centrifugation, dissolved in minimum volume of pre-cold distilled water and dialyzed against distilled water for 24 hours at 4°C. The dialyzed solution was then centrifuged in a refrigerated centrifuge machine at 5500 rpm for 15 minutes to remove the insoluble materials. The clear supernatant thus obtained was designated as “crude enzyme solution”.

Measurement of enzyme activity: The amylase activity was assayed following the method as described by Jayaraman (1985), the invertase activity was assayed following the method of Mahadevan and Sridhar (1982), the protease activity was measured following the method of Kunitz (1947) and the lipase activity was assayed essentially as described by Sugihara *et al.* (1990).

Degradation of nutrients during germination of chickpea seed: Total protein content of chickpea seeds was determined by the method of Micro-Kjeldahl (Wong 1923) and the water-soluble protein content by the method of Lowry *et al*. (1951). Free sugar content of chickpea seeds was determined calorimetrically by the anthrone method (Morse 1947). Reducing sugar content of chickpea seeds was estimated by DNS (Dinitrosalicylic acid) method (Miller 1972). The starch content of chickpea seeds was determined by the anthrone method (Morse 1947, Jayaraman 1985) and lipid content of chickpea seeds was determined by the method of Bligh and Dyer (1959).

Results

The activities of different enzymes in the three varieties of chickpea seeds are shown in Table 1. Activity of amylase in the three varieties of chickpea was determined during various germinating periods. The activities varied from 9.85 to 40.25 unit/ml. After 45 hours germination, the variety BARI-2 and BARI-3 showed the highest (40.25 unit/ml) and the lowest (9.85 unit/ml) amount of amylase activities, respectively. During germinating stage, invertase activities in the three different varieties ranged from 2.30 to 7.21 unit/ml. The highest invertase activity was found in BARI-2 (7.32 unit/ml) and the lowest in BARI-1 (5.32 unit/ml) at 72 hours. At 40 hours of germination, BARI-2 showed the highest protease activities (38.45 unit/ml) while lowest in BARI-3 (37.01 unit/ml) variety. The lipase activities in different varieties varied from 2.72 to 11.32 unit/ml. Among the varieties, BARI-2 and BARI-1 showed the highest (11.32 unit/ml) and the lowest (9.55 unit/ml) lipase activities at 40 hours, respectively.

Total protein and water-soluble protein contents of the germinated chickpea seeds at different periods of germination are presented in Table 2. The protein content determined by the Micro Kjeldahl method showed considerably higher amount than that given by Lowry method.

Free sugar content at different periods of germination of chickpea seeds is represented in Table 2. As shown in table, the free sugar content in the three varieties (BARI-1, BARI-2 and BARI-3) were found to be 5.90%, 5.65% and 5.45% respectively. During germination up to 144 hours, free sugar degradation was found to be regular in intact chickpea seeds.

The results about the degradation of storage reducing sugar are shown in Table 2. The reducing sugar contents in BARI-1, BARI-2 and BARI-3 were found to be 0.40%, 0.43% and 0.41% respectively. During germination up to 144 hours reducing, sugar was found to be regularly degraded to 0.05%, 0.10% and 0.05%, respectively.

Degradation of starch during different periods of germination of chickpea seeds is represented in Table 2. It was observed that BARI-1, BARI-2 and BARI-3 contained 56.00%, 56.04% and 56.12% starch, respectively. The starch contents in chickpea seeds were found to decrease gradually to 2.11%, 2.09% and 2.21% respectively during germination at 144 hours.

Amount of lipid at different periods of germination was determined by solvent extract process and is shown in Table 2. The result shows that the chickpea seeds contained 5.00%, 4.96% and 4.90% lipid respectively. Chickpea seeds lipid was found to be 0.17%, 0.11% and 0.10% respectively at 144 hours during germination in water.

Table 1. Activities of amylase, invertase, protease and lipase in three varieties of chickpea seed.

Varieties	Germinating time hours	Activity of enzymes (Units/ml)			
		Amylase	Invertase	Protease	Lipase
BARI- 1	36	9.95±0.03	2.55±0.02	20.04±0.01	5.35±0.03
	40	31.52±0.01	3.85±0.01	37.95±0.02	9.55±0.03
	45	38.69±0.02	4.08±0.01	32.51±0.02	5.19 ±0.01
	72	19.82±0.04	5.32±0.02	27.45±0.03	4.35±0.02
	96	14.45±0.03	3.15±0.03	23.35±0.02	3.85±0.03
	120	11.23±0.01	2.30±0.02	21.02±0.01	2.72±0.02
BARI-2	36	10.04±0.02	2.85±0.01	21.15±0.01	5.92±0.02
	40	32.85±0.03	4.15±0.02	38.45±0.04	11.32±0.02
	45	40.25±0.01	5.10±0.01	33.00±0.03	6.94±0.01
	72	21.05±0.01	7.21±0.02	28.25±0.02	5.75±0.03
	96	15.35±0.02	3.45±0.03	23.35±0.03	4.85±0.01
	120	11.88±0.05	3.01±0.01	20.71±0.01	4.02±0.02
BARI- 3	36	9.85±0.01	2.52±0.01	20.01±0.02	5.45±0.01
	40	30.65±0.02	3.95±0.03	37.01±0.02	9.76±0.02
	45	37.11±0.03	4.09±0.02	31.86±0.02	6.27±0.03
	72	19.18±0.02	6.19±0.02	27.35±0.03	4.65±0.01
	96	13.95±0.04	3.55±0.03	22.25±0.01	4.01±0.02
	120	11.02±0.01	2.39±0.01	19.96±0.02	3.35±0.03

Table 2. Protein, sugar, starch and lipid content of chickpea seed at different periods of germination.

Nutrients	Varieties	Protein (%) at different hours (h)					
		0 (h)	48 (h)	72 (h)	96 (h)	120 (h)	144 (h)
Total protein	BARI-1	22.51±0.01	18.80±0.01	12.09±0.02	8.25±0.03	5.40±0.01	4.30±0.01
	BARI-2	22.05±0.01	18.72±0.02	12.95±0.01	8.65±0.01	5.61±0.01	4.45±0.01
	BARI-3	22.11±0.02	18.87±0.01	12.62±0.02	8.54±0.01	5.36±0.01	4.19±0.01
Water soluble protein	BARI-1	15.55±0.02	12.23±0.01	8.01±0.02	5.68±0.03	3.90±0.04	2.09±0.03
	BARI-2	15.61±0.01	12.95±0.01	8.26±0.02	5.40±0.04	4.01±0.02	2.26±0.03
	BARI-3	15.68±0.02	12.39±0.01	8.21±0.03	5.72±0.01	3.09±0.01	2.17±0.02
Free sugar	BARI-1	5.90±0.02	4.52±0.01	3.19±0.05	2.00±0.01	1.65±0.03	0.99±0.01
	BARI-2	5.65±0.04	4.45±0.01	3.15±0.01	1.85±0.01	1.20±0.01	0.94±0.02
	BARI-3	5.45±0.01	4.36±0.02	3.06±0.02	1.65±0.01	1.05±0.02	0.87±0.01
Reducing sugar	BARI-1	0.40±0.01	0.35±0.02	0.24±0.01	0.14±0.01	0.11±0. 01	0.05±0.01
	BARI-2	0.43±0.01	0.38±0.01	0.29±0.01	0.19±0.01	0.16±0.01	0.10±0.01
	BARI-3	0.41±0.01	0.36±0.01	0.25±0.02	0.15±0.01	0.11±0.02	0.05±0.01
Starch	BARI-1	56.00±0.02	50.44±0.01	40.00±0.02	17.41±0.01	9.32±0.02	2.11±0.02
	BARI-2	56.04±0.04	50.50±0.01	40.23±0.01	17.43±0.01	9.29±0.03	2.09±0.01
	BARI-3	56.12±0.03	50.65±0.03	40.29±0.02	17.50±0.01	9.39±0.03	2.21±0.01
Lipid	BARI-1	5.00±0.01	4.17±0.03	3.37±0.01	2.13±0.01	0.98±0.02	0.17±0.01
	BARI-2	4.96±0.01	4.09±0.02	3.28±0.01	2.17±0.01	0.98±0.04	0.11±0.01
	BARI-3	4.90±0.02	4.06±0.02	3.31±0.02	2.14±0.01	0.90±0.01	0.10±0.01

Discussion

Amylase and invertase play a major role in carbohydrate metabolism in several plant tissues (WHO 1985) and starch is the major component of most of the world's crop yield and the degradation of starch is essential in the germination of these plants (Yamasaki 2003). Starch degradation in seeds requires the action of α- and β- amylase. The amylase activities were found to be highest in BARI-2 and lowest in BARI-3 at 45 hours of germination. It was observed that amylase activity increased considerably during germination. The finding is in good agreement with those reported by Evans *et al*. (1997) and Sopanen and Lauriere (1989).

Invertase, which hydrolyzes sucrose into glucose and fructose, occurs in many plants and microorganisms. The expression and distribution of plant invertases has been especially well documented, because these are considered to play an important role in sugar metabolism (Kastle and Clark 1903, Krishan *et al*. 1885). Among the varieties, the maximum activity of invertase was found in BARI-2 and minimum in BARI-1 at 72 hours of germination.

Upon seed germination, storage proteins are degraded by protease enzymes to provide nutrients for embryo/seedling growth (Bing *et al*. 2003). The highest protease activity was found in BARI-2 and lowest in BARI-3 at 40 hours of germination while BARI-1 showed the value between the ranges.

Lipases are lipolytic enzymes catalyze the hydrolysis of fats as well as esters of fatty acids with alcohols (Sarda and Desnuelle 1957, Wills 1965). During germination the rapid mobilization of storage triacylglycerols (TAGs) in the cotyledons of seedling require the action of lipases. Of the varieties, BARI-2 should the highest lipase activity and BARI-1 the lowest activity.

From the above result it seems that BARI-2 is a suitable source of amylase, invertase, protease and lipase for purification and characterization.

Total protein and water-soluble protein content of the different varieties of chickpea seeds are presented in Table 2. From the tables, it is found that the chickpea seeds contain a significant amount of protein (total 22.05-22.51% and water-soluble 15.55-15.68%). The results clearly demonstrate that the percentage of both

types of proteins present in different varieties of chickpea seeds decrease gradually up to 48 hours and then sharply declined up to 144 hours of germination. This indicates that after 96 hours of germination, the proteolytic enzymes may vigorously involve for hydrolysis of seed storage proteins.

Free sugars, particularly glucose, are important in the nervous systems, muscles and many other tissues. Combined with proteins as glycoproteins, the sugars play a role in secretion and external recognition properties of cell membranes. The free sugar contents in three varieties of chickpea seeds are shown in Table 1. During germination, degradation of free sugar in chickpea seed was observed to be rapid.

Reducing sugar contents in three varieties of chickpea seeds are represented in Table 1.. From the table, it was observed that reducing sugar contents in the three varieties of chickpea seed are different. Degradation of reducing sugar at different periods of germination was significant.

One of the nutritional reservoirs in plants, which are used as a fuel, is starch. The starch contents of different varieties of chickpea seeds are shown in Table 1. Starch is the most important polysaccharide in the storage form of carbohydrate in plant. It was found that chickpea seeds contain a large amount of starch 56.00-56.12%. The starch contents of chickpea seeds were found to decrease gradually during germination. In light germination, probably starch first degrades for embryo growth and latter other enzymes utilize seed's storage substance, for the energy supply, required for seedling growth.

The lipid contents of different varieties of chickpea seeds are shown in Table 1. The lipid content was found to degrade quickly up to 48 hours and then gradually up to 144 hours of germination. The decrease in lipid content in the germinating seeds probably caused by the involvement of a lypolytic enzyme, which is responsible for hydrolysis of triacylglycerol that ultimately generate sugars for the growth of germinating embryo. This finding is in good agreement with those reported by Ben *et al*. (2000).

The seed storage substances gradually decrease with the increase of germination time. The decrease in different types of nutrient content in germinating seeds probably caused by involvement of the hydrolytic enzymes, which hydrolyses seed storage nutrients, a process that generates amino acids and sugars for the development of embryo and seedling growth.

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