ISSN : 0974 - 7508 Volume 7 Issue 6

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 7(6), 2011 [325-330]

Evaluation of changes in enzyme activities and nutrient degradation of chickpea seed (*Cicer arietinum* L.) during germination

M.Shaela Pervin, M.G.Sarowar Jahan*

Department of Biochemistry & Molecular Biology, University of Rajshahi, Rajshahi-6205, (BANGLADESH)

E-mail: sjahan.biochem@ru.ac.bd

Received: 8th October, 2011 ; Accepted: 8th November, 2011

ABSTRACT

Enzyme activities and mobilization of seed storage substances were investigated in BARI-4, BARI-5, and BARI-6 varieties of Chickpea seed (*Cicer arietinum* L.) at different germination periods. Among the varieties, the highest urease activity was found in BARI-4 and lowest in BARI-5 during germination at 192 hours in water. Of the varieties, BARI-4 contained the highest and BARI-6 contained the lowest amylase activity during 144 hours. Among the varieties, the maximum activity of invertase was found in BARI-5 and minimum in BARI-6 at 144 hours of germination. The activity of lipase was highest in BARI-4 and lowest in BARI-5. The highest protease activity was found in BARI-5 and lowest in BARI-4. The amount of total protein and water soluble protein were found to be highest in BARI-5 and lowest in BARI-4. During germination, protein depletion starts after initial imbibitions, and is completed in 240-288 hours. The variety BARI-4 was found to contain the highest amount of free sugar while BARI-6 contained the lowest amount. Of the varieties, BARI-5 was found to contain the highest amount of reducing sugar and BARI-4 contained the lowest amount. The highest amount of starch was found in BARI-6 and lowest in BARI-4. The starch content in chickpea seed decreased gradually during germination. Among the varieties the BARI-4 was found to contain the highest amount of lipid while the BARI-5 contained the lowest amount. The seed storage substances decreased gradually with the increase of germination time. The results suggest that degradation of seed reserve nutrients accelerate the development of seedling growth during germination.

KEYWORDS

Chickpea;
Enzyme activity;
Germination;
Hydrolytic enzymes;
Nutrients.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops in Bangladesh occupying a prominent position. It is one of the most important human and domestic animal foods in South Asia and is thought to be the third most important pulse crop after dry beans, *Phaseolus vulgaris* L. and dry peas, *Pisum sativum* L.[1]. Seeds of chickpea are valuable source of protein[2]. 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[3].

Full Paper

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[4]. 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[2,5], its chemical composition[6-8], carbohydrates[9] and mineral content[10] 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. Therefore, 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 & METHODS

Material

The three varieties (BARI-4, BARI-5, and BARI-6) of chickpea seeds (*Cicer arietinum*) 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 polythene bag and stored in an airtight 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. Then 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 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 covered by a glass lid and placed in a dark room at 22^0C for 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours including soaking time. The germinated seeds at different hours were separated from seedling, rinsed with distilled water and stored separately in the deep freeze (-10^0C) for further biochemical analysis.

Preparation of the crude enzyme extract

100 grams of germinated seeds were crushed in a mortar and pestle and then suspended in 40 ml of 30% acetone (chilled to -20^0C). After occasional gentle stirring for 3 hours at 4^0C, the suspension was filtered through double layer of muslin cloth. The filtrate was collected and centrifuged in a refrigerated centrifuge at 6000 r.p.m. for 20 minutes at 4^0C. The supernatant was used as "crude extract".

Acetone fractionation

The "crude extract" was collected and adjusted to 50% saturation by the addition of acetone (chilled to -20^0 C) under constant and gentle stirring at 4^0C. The resulting precipitate was collected by centrifugation at 4^0C, dissolved in minimum volume of pre-cold 50 mM phosphate buffer pH 7.0 and dialyzed against same buffer for 24 hours at 4^0C. The dialyzed solution was then centrifuged in a refrigerated centrifuge at 8000 r.p.m. for 5 minutes to remove the insoluble materials. The clear supernatant thus obtained was designated as "crude enzyme solution".

Enzyme activities in chickpea seed during germination

Urease and amylase activities were assayed following the method as described by Jayaraman[11]. Invertase activity was determined by the method of Mahadevan and Sridhar[12]. Protease activity was measured following the method of Kunitz[13] and lipase activity was assayed essentially as described by Sugihara *et al.*[14].

Degradation of nutrients during germination of chickpea seed

Total protein content of chickpea seed was deter-

mined by the method of Micro-Kjeldahl[15] and water-soluble protein content of chickpea seeds was determined by the method of Lowry[16]. The starch and free sugar content of chickpea seeds was determined colorimetrically by the anthrone method[11,17]. Reducing sugar content of chickpea seeds was estimated by DNS (Dinitrosalicylic acid) method[18]. Lipid content of chickpea seeds were determined by the method of Bligh and Dyer[19].

RESULTS & DISCUSSION

Activity of urease in chickpea seed

The primary role of urease is to allow the organism to use external or internally generated urea as a nitrogen source[20,21]. Significant amounts of plant nitrogen flow through urea. It may function coordinately with arginase in the utilization of seed protein reserves during germination[22] Immobilization of urease has been carried out in several matrices for clinical/analytical applications[23,24] and has also been used for the treatment of urea-containing effluents[25,26]. Urease activities in the three different varieties of chickpea at different germinating periods are given in the TABLE 1. During germinating stage urease activities in different varieties ranged from 8.93-60.14 U/ml. Among the varieties the highest urease activity was found in BARI-4 (60.14 U/ml) at 192 hour and lowest in BARI-5 (58.26 U/ml) at 192 hour. BARI-6 gave the value between these ranges.

TABLE 1 : Activities of urease, amylase, invertase, protease and lipase in the three different varieties of chickpea seed.

Variety	Germinating Hour	Activity of enzymes (Units/ml)				
		Urease	Amylase	Invertase	Protease	Lipase
BARI-4	48	6.05±0.02	8.37±0.01	1.53±0.01	13.39±0.03	2.95±0.02
	96	10.85±0.01	20.25±0.03	4.06±0.02	32.51±0.01	6.43±0.02
	144	25.73±0.01	40.11±0.01	6.27±0.03	37.23±0.05	10.45±0.01
	192	60.14±0.02	12.38±0.02	2.45±0.03	20.17±0.03	3.81±0.03
	240	21.67±0.02	6.44±0.01	0.95±0.02	7.81±0.02	1.87±0.01
BARI-5	48	5.72±0.03	7.85±0.01	2.22±0.02	14.31±0.02	2.55±0.03
	96	8.93±0.02	20.02±0.01	4.70±0.02	33.46±0.01	6.07±0.02
	144	21.37±0.02	39.55±0.03	7.59±0.01	37.95±0.03	10.15±0.03
	192	58.26±0.01	11.98±.02	2.54±0.04	20.34±0.02	3.58±0.03
	240	19.49±0.01	5.67±0.04	1.25±0.02	7.46±0.05	1.22±0.01
BARI-6	48	4.38±0.01	7.55±0.02	1.77±0.01	14.43±0.02	2.45±0.01
	96	9.47±0.01	19.74±0.03	4.00±0.01	32.62±0.02	6.13±0.01
	144	23.04±0.03	39.03±0.02	6.20±0.03	37.74±0.01	10.22±.02
	192	59.33±0.02	11.52±0.01	2.30±0.03	20.46±0.03	3.69±0.03
	240	18.21±0.02	5.13±0.01	1.05±0.02	7.47±0.01	1.10±0.01

Activity of amylase in chickpea seed

Amylolytic enzymes are widely distributed in plant tissues, e.g. in storage tissues such as seeds and tubers and in vegetative organs such as leaves. Starch is one of the major components of pulses and is an important carbon source for the growth of germinating seedlings. Amylase activities in different varieties of chickpea during germination were varied between 5.13-40.11 U/ml. The variety BARI-4 contained the highest activity (40.11U/ml) at 144 hour followed by BARI-5 (39.55 U/ml). BARI-6 contained the lowest amylase activity (39.03 U/ml) at 144 hour. From the results it was found that amylase activity increased considerably during germination. The finding is in good agreement with those reported by Evans *et al.*[27] and Sopanen and Lauriere[28].

Activity of invertase in chickpea seed

Invertase occurs widely in plant, microbial and animal sources. It plays an important role in the hydrolysis of sucrose to glucose and fructose in higher plants, especially in the storage organs. During germination, sucrose is a readily degradation source of energy[29]. Invertase activities in three different varieties of chickpea

Full Paper

at different germinating periods are given in the TABLE 1. During germinating stage invertase activities in different varieties ranged from 1.05-7.59 U/ml. Among the varieties the highest invertase activity was found in BARI-5 (7.59 U/ml) at 144 hours and lowest in BARI-6 (6.20 U/ml) at 144 hours. BARI-4 gave the value between these ranges.

Activity of protease in chickpea seed

Seeds are the main source of protein in plant. The protein stored in seeds is used chiefly in the formation of protoplasm in new cells when seeds germinate[30]. The most well characterized proteases are associated with seed germination, and are employed to mobilize stored reserve to provide amino acids and amides for embryogenesis and/or early seedling development[31]. The protease activities of the three different varieties of chickpea were studied during germination. At 144 hours of germination, BARI-5 contained the highest protease activities (37.95 U/ml) while lowest in BARI-4 (37.23 U/ml). The protease activity of BARI-6 placed between these ranges.

Activity of lipase in chickpea seed

The storage nutrient substances in plant seeds are used up for seedling growth during germination[30]. During mobilization of storage lipids in seeds in post terminative growth, the triacylglycerol is converted to sugars and other metabolites for the growth of the embryonic axis[32,33]. Lipase activities in different varieties of chickpea during germination were varied between 1.10-10.45 U/ml. The variety BARI-4 contained the highest activity (10.45 U/ml) at 144 hours followed by BARI-6 (10.22 U/ml). BARI-5 contained the lowest lipase activity (10.15 U/ml) at 144 hour.

From the above result it seems that BARI-4 is a suitable source of urease, amylase, and lipase for purification and characterization, while BARI-5 is a suitable source of invertase and protease.

Amount of protein in chickpea seed during different periods of germination

Total protein and water soluble protein content of the three different varieties of chickpea seeds are presented in TABLE 2. It was found that the chickpea seeds contained a significant amount of protein (Total 19.40-21.07 gm %, water soluble 14.35-15.72 gm %). BARI-5 contained the highest amount of protein (Total protein 21.07 gm %, water soluble 15.72 gm %), while BARI-4 contained the lowest amount of protein (Total 19.40 gm %, water soluble 14.35 gm %). The present results clearly demonstrated that the percentage of both types of proteins present in different varieties of chickpea seeds decreased gradually up to 144 hours and then sharply declined up to 240 hours of germination. This indicated that after 144 hours of germination, the proteolytic enzymes may vigorously involve for hydrolysis of seed storage proteins.

Free sugar content in chickpea seed

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. Free sugar content at different periods of germination of chickpea seeds are shown in TABLE 2. As shown in table, the free sugar content in the three varieties (BARI-4, BARI-5, and BARI-6) of chickpea seeds were found to 5.95 gm %, 5.72 gm %, and 5.43 gm %, respectively. During germination, degradation of free sugar in chickpea seed was rapid.

Reducing sugar content in chickpea seed

The results about the degradation of storage reducing sugar are shown in TABLE 2. From the table, it was observed that reducing sugar contents in the varieties of chickpea seed are different. Degradation of reducing sugar at different periods of germination was significant. Of the varieties BARI-5 contained the highest amount (0.037 gm %) of reducing sugar and BARI-4 contained the lowest amount (0.035 gm %).

Starch content in chickpea seed

One of the nutritional reservoirs in plants, which are used as a fuel, is starch. Starch is the most important polysaccharide in the storage form of carbohydrate in plant. It was found that, chickpea seeds contained a large amount of starch (55.0-55.08) gm %. BARI-6 contained the highest amount of starch while BARI-4 contained the lowest amount. The starch contents of chickpea seeds decreased 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

Full Paper

seedling growth. Starch in chickpea seeds degraded to 15.06, 15.09, and 15.14 gm %, respectively during germination at 240 hour.

Amount of lipid in chickpea seed

Amount of lipid in different period of germination was shown in TABLE 2. The result shows that the chickpea seeds contained 4.90, 4.87, and 4.89 gm of lipid per 100 gm of seed. Chickpea seeds lipid found to be 0.10, 0.08, and 0.09 gm % at 240 hour during germination in water. The decrease in lipid content in the germinating seeds is 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*.[34].

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

Nutrient	Variety	Amount (gm %) at different germinating hours (h)					
		0 (h)	48 (h)	96 (h)	144 (h)	192 (h)	240 (h)
Total Protein	BARI-4	19.40±0.01	17.02±0.03	14.53±0.01	7.15±0.02	3.02±0.02	2.07±0.01
	BARI-5	21.07±0.01	19.33±0.01	16.07±0.01	9.21±0.02	4.16±0.03	2.37±0.03
	BARI-6	19.42±0.02	17.06±0.01	14.60±0.02	7.34±0.01	3.08±0.03	2.14±0.01
Water Soluble Protein	BARI-4	14.35±0.04	11.07±0.01	8.98±0.03	6.13±0.03	2.82±0.01	1.03±0.02
	BARI-5	15.72±0.01	12.31±0.02	9.07±0.01	7.20±0.03	3.16±0.02	1.37±0.02
	BARI-6	14.43±0.02	11.26±0.03	9.06±0.02	6.31±0.01	2.15±0.01	1.12±0.01
Free Sugar	BARI-4	5.95±0.03	4.57±0.02	3.24±0.02	2.01±0.01	1.72±0.01	1.0±0.03
	BARI-5	5.72±0.03	4.50±0.02	3.21±0.01	1.95±0.03	1.26±0.01	0.97±0.01
	BARI-6	5.43±0.01	4.42±0.01	3.16±0.03	1.71±0.01	1.15±0.03	0.92±0.01
Reducing Sugar	BARI-4	0.035±0.01	0.03±0.01	0.021±0.01	0.011±0.01	0.008±0.0	0.006±0.0
	BARI-5	0.037±0.01	0.032±0.01	0.023±0.01	0.013±0.01	0.009±0.0	0.007±0.0
	BARI-6	0.036±0.01	0.031±0.01	0.022±0.01	0.012±0.01	0.008±0.0	0.006±0.0
Starch	BARI-4	55.00±0.03	52.43±0.02	41.09±0.04	26.31±0.03	20.12±0.02	15.06±0.02
	BARI-5	55.02±.02	52.41±0.03	41.13±0.04	26.33±0.04	20.13±0.02	15.09±0.03
	BARI-6	55.08±0.02	52.47±.03	41.15±0.02	26.36±0.02	20.15±0.03	15.14±0.04
Lipid	BARI-4	4.90±0.01	4.87±0.01	3.21±0.01	2.00±0.01	0.90±0.01	0.10±0.01
	BARI-5	4.87±0.03	4.85±0.03	3.20±0.01	2.01±0.03	0.87±0.02	0.08±0.01
	BARI-6	4.89±0.03	4.86±0.01	3.21±0.02	2.00±0.03	0.91±0.01	0.09±0.01

The seed storage substances gradually decreased 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.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the research facilities provided by the Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

REFERENCES

[1] M.C.Saxena; Problems and Potential of Chickpea Production in Nineties, in Chickpea in the Nineties, 'Proceedings of the Second International Workshop on the Chickpea Improvement', 4-8 Dec. 1989, ICRISAT Center, Patancheru, India, 13-25 **(1990)**.

[2] D.K.Gupta, R.D.Tripathi, U.N.Rai, S.Dwivedi, S.Mishra, S.Srivastava, M.Inouhe; Chemosphere, **66(7)**, 1382 **(2007)**.

[3] J.K.Chavan, S.S.Kadam, D.K.Salunkhe; Crit.Rev. Food Sci.Nutr., **25(2)**, 107-158 **(1986)**.

[4] P.Geervani; Utilization of Chickpea in India and Scope for Novel Alternative Uses, in Uses of Tropical Grain Legume, 'Proceedings of Consultants' Meeting', 27-30 Mar. 1989. ICRISAT Center, Patancheru, Andhra Pradesh, India, 45-47 **(1991)**.

Full Paper

[5] M.A.Khan, N.Akhtar, I.Ullah, S.Jaffery; Int.J.Food Sci.Nutr., **46(3)**, 215-223 **(1995)**.

[6] A.Viveros, A.Brenes, R.Elices, I.Arija, R.Canales; British Poult.Sci., **42(2)**, 242-251 **(2001)**.

[7] T.A.El-Adawy; Plant Foods Hum.Nutr., **57(1)**, 83-97 **(2002)**.

[8] A.W.Suhasini, N.G.Malleshi; Int.J.Food Sci.Nutr., **54(3)**, 181-187 **(2003)**.

[9] A.Hawkins, S.K.Johnson; Int.J.Food Sci.Nutr., **56(3)**, 147-55 **(2005)**.

[10] T.Nestares, M.Barrionuevo, G.Urbano, M.López-Frías; J.Agric.Food Chem., **47(7)**, 2807-2812 **(1999)**.

[11] J.Jayaraman; 'Laboratory Manual in Biochemistry', 1st Edition, Wiley Eastern Ltd., New Delhi, India, **(1981)**.

[12] A.Mahavdevan, R.Sridhar; 'Method in Physiological Plant Pathology', Sivakami Publication, Madras, 316 **(1982)**.

[13] M.Kuntiz; J.Gen.Physiol., **30**, 291-310 **(1947)**.

[14] A.Sugihara, Y.Shimada, Y.Tominaga; J.Biochem., **107**, 426-430 **(1990)**.

[15] T.Wong; J.Biol.Chem., **55**, 427 **(1923)**.

[16] O.H.Lowry, N.J.Rosenbrough, A.L.Fann, R.J.Randall; Biol.Chem., **183**, 265-275 **(1951)**.

[17] E.E.Morse; Anal.Chem., **19**, 1012-1013 **(1947)**.

[18] G.L.Miller; Anal.Chem., **31**, 426-428 **(1972)**.

[19] E.G.Bligh, W.J.Dyer; Can.J.Biochem.Physiol., **37**, 911-917 **(1959)**.

[20] H.L.T.Mobley, R.P.Housinger; Microbiol.Rev., **53**, 85-108 **(1989)**.

[21] H.L.T.Mobley, M.D.Island, R.P.Hausinger; Microbiol.Rev., **59**, 451-480 **(1995)**.

[22] J.C.Polacco, M.A.Holland; Int.Rev.Cytol., **145**, 65-103 **(1993)**.

[23] N.Das, A.M.Kayastha, O.P.Malhotra; Biotechnol. Appl.Biochem., **27**, 25-29 **(1998)**.

[24] A.M.Kayastha, P.K.Srivastava; Appl.Biochem. Biotechnol., **96(1-3)**, 41-53 **(2001)**.

[25] N.Kamath, S.F.D'Souza; Enzyme Microb.Technol., **13**, 935-938 **(1991)**.

[26] P.Thavarungkul, H.Hakanson, O.Holst, B.Mattiasson; Biosensors and Bioelectronics, **6**, 101-107 **(1991)**.

[27] D.E.Evans, W.Wallace, R.C.M.Lance, L.C.MacLeod; J.Cereal Sci., **26**, 241-250 **(1997)**.

[28] T.Sopanen, C.Lauriere; Plant Physiol., **134**, 678-684 **(1989)**.

[29] G.E.Bracho, J.R.Whitaker; Plant Physiol., **92**, 386-394 **(1990)**.

[30] J.F.Harry; The Plants Words, 4th Edition, USA, 23-25 **(1989)**.

[31] K.Muntz; J.Exp.Bot., **47(298)**, 605-622 **(1996)**.

[32] H.Beevers; Ann NY Acad Sci., **168**, 313-324 **(1969)**.

[33] D.Hutton, P.K.Stumpf; Plant Physiol., **44**, 508-516 **(1969)**.

[34] D.D.Ben Miled, M.Zarrouk, A.Cherif; Biochem. Soc.Trans., **28**, 899-902 **(2000)**.