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In vivo Reduction the Phytic Acid Content of Mung Bean (*Phaseolus aureus* L) Cultivars During Germination

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Abstract: Nine cultivars of Mung bean (*Phaseolus aureus* .L) representing a broad range of varietal characteristics were analyzed for their phytic acid and phytase activity. The level of phytic acid was found to be in the range of 6.17-9.90mg/g. Cultivars ALM-1, ALM-2 and ALM-3 found to contain lower levels of phytate than other tested cultivars. Soaking and germination reduced phytate content considerably. Soaking for 12 hrs reduced the phytic acid content only by 13- 41% while soaking for 12 hrs followed by germination for 48 hrs reduced 60-73% of phytic acid. Soaking triggered the phytase synthesis and maximum phytase activity was observed after soaking and germination has been found to be associated positively with the activity of phytase. Upon germination, a pronounced increase in phytase activity accompanied with a decrease in phytic acid content was observed. Mung beans and bean sprouts blends could be useful for various food preparations.

Key words: Mung bean · Phytic acid · Soaking · Germination · Phytase

INTRODUCTION

Legumes are good and relatively cheaper sources of proteins and carbohydrates for developing countries including India. In addition to being an important source of proteins and carbohydrates, legumes also reported to be a good source of minerals [1]. But the biological utilization of existing nutrients of these legumes is limited by the presence of various antinutritional substances [2]. The mineral content of legumes is generally high, but the bioavilibility is poor due to the presence of phytate, which is a main inhibitor of iron and zinc absorption [3]. Phytate not only decreases the bioavailability of essential minerals, it also decreases the bioavailability of proteins by forming insoluble phytate-mineral and phytate-protien complexes [4, 5]. However, remarkable improvements in the nutritive value of legume seeds have been achieved by adopting various processing techniques, such as dehulling, soaking, heat treatment and germination [6].

Phytate, myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) is a major storage form of phosphorous in the mature seeds of both monocot and dicot plants and typically represents approximately 75% of the total phosphorous and >80% of soluble myo-inosotol phosphate in seeds [7]. Phytic acid occurs in most of the legumes and oil seeds to the extent of 1-5% of dry weight [2]. Animal and human feeds are comprised primarily of plant seed components, seed phytic acid is largely unavailable to monogastric animals, including humans due to the lack of phytases and it is excreated in to manure [8]. Excreation of undigested phytic acid in manure leads to the eutrophication and water quality issues [9]. Phytase (myo-inositol hexakis phosphate phophohydrolase, E.C. 3.1.3.8) is a phosphatase that hydrolyses phytate to inositol and free orthophosphate [10].

India is the major pulse producing country. Mung bean is the most important legume due to its high proteins and carbohydarates and its protein quality is similar to or better than other legumes such as chickpea, black gram, peas, pigeonpea etc. [11, 12]. In India, among various pulses, mung bean stands third [13] and is the principal crop from which edible bean sprouts, noodles and weaning foods are prepared [14, 15].

Available literature indicates that information on phytate content of mung bean cultivars differing in seed coat color is scarce to best of our knowledge. In this context, the study was carried out to evaluate the phytate content of mung bean cultivars. The effect of soaking, soaking followed by germination on phytic acid content and phytase activity has been evaluated and correlated.

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MATERIALS AND METHODS

The mung bean cultivars, PS-16, Pusabaisaki, TAP-7, Vaibhav, Hum-1 and China mung were procured from the Agriculture research station, Aland road, Gulbarga, India. The three yellow coloured cultivars were procured from farmers and designated as ALM-1, ALM-2 and ALM-3 respectively. The samples were cleaned and stored in the laboratory at 4°C. All chemicals used were of analytical grade.

Phytic Acid Determination: Phytic acid was determined according to the method described by Wheeler and Ferrel [16] using $Fe(NO_3)_3.9H_2O$ standard. The phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 (iron:phosphorus) molar ratio. The phytic acid content was calculated on the assumption that it contains 28.20 % phosphorus by weight [17].

Soaking and Germination Procedure

Soaking: The seeds were first surface sterilized by treating with 0.1% mercuric chloride. The sterilized seeds were rinsed and soaked in distilled water (1:10; w/v) for a period of 12 hours at room temperature (28±2°C). The soaked seeds were rinsed with distilled water and taken for phytase assay and phytic acid determination.

Germination: Surface sterilized seeds were soaked in distilled water for 12 hours at 28±2°C. The soaked seeds were placed on moist filter paper bed in petri plates and incubated at 37°C in the dark. The seeds were moistened with distilled water at regular intervals. The germinated seeds were sampled at intervals of 12, 24 and 48 hours for phytase assay and phytic acid determination.

Isolation of Phytase: The 12 hours soaked and germinated for 12, 24 and 48 hours seeds of 5 gm weight were homogenized in prechilled pestle and mortar in ice cold conditions, with chilled Tris-HCl buffer of 10 ml. The homogenate was filtered through muslin cloth and centrifuged for 20 min at 20,000g at 4°C. The supernatant was used for enzyme assay.

Assay of Phytase: Phytase assay was carried out by the method of Mandal *et al,* [18]. Assay mixture contained 0.4 ml of Tris-HCl maleate-NaOH buffer, pH 7.5, 100 µmol; 0.1 ml of phytic acid, 1.3 µmol; and 0.5 ml of enzyme extract in a total volume of 1 ml. The additions were made in cold and mixed thoroughly. After incubation for 1 hr at 57°C the reaction was stopped by adding an equal volume (1 ml) of 0.4 M trichloroacetic acid (cold) and the mixture was chilled in ice. The precipitated protein was removed by centrifugation. The supernatant was adjusted to pH 4.0 by adding 0.7 ml of 0.4 M sodium acetate and an aliquot from this mixture was used for the estimation of inorganic phosphorus by the method of Fiske and Subba Row [19].

Optimization of pH and Temperature: For phytase determination of pH optimum, reactions mixture were incubated at 57° C for pH range between 5.5 and 8.5.

For temperature studies, reaction mixtures were incubated at different temperatures ranging from 30 to 60° C at pH 7.5.

RESULTS AND DISCUSSION

The phytate content of yellow and green colored mung bean cultivars varied significantly (Table 1). The phytate–P and phytic acid contents of mung bean cultivars were found to be in the range of 1.74 and 2.79 mg/g and 6.17 and 9.90 mg/g respectively. The percentage of phytic acid was found to be in the range from 0.61 to 0.99% of dry weight, which is in consistent with the range 0.40% to 2.06% reported for legumes [20]. Among the analysed cultivars, TAP-7 showed highest phytic acid content (9.90 mg/g), while the ALM-3 contained lowest phytate (6.17 mg/g). The average phytate content of yellow colored mung bean cultivars was found to be low (7.38 mg/g) when compared to pea green cultivars (9.02 mg/g). Phytate content of various legume seeds including mung bean have been reported earlier (Table 2). In many cases, phytic acid content is not considered to be absolute and varied depending upon the cultivar, climatic conditions, locations, irrigation conditions, type of soil and year during which they are grown [31]. The level of phytic acid in tested mung bean cultivars seemed to be lower than that reported for various legumes so far, thus suggesting that nutritive value of mung bean seeds would be impaired to a comparatively lesser extent, which is relevant for selection of low phytate cultivars to improve mineral bioavailability and also for preparation of weaning foods.

Soaking and germination is an integral part of traditional methods of processing mung bean seeds in India, thus offers the dual advantage of saving energy costs by shortening cooking time as well as removing certain antinutritional factors like phytic acid. In house hold situations legumes are typically soaked in water for

Table 1: Phytate-P and Phytic acid content of mung bean cultivars.

Sl.No.	Cultivar	Phytate-P (mg/g)	Phytic acid (mg/g)	% of phytic acid Dry wt.
1	ALM-1	2.31±0.09	8.20±0.11	0.82±0.12
2	ALM-2	2.19±0.17	7.77±0.08	0.77±0.07
3	ALM-3	1.74±0.05	6.17±0.19	0.61±0.19
4	PS-16	2.23± 0.34	7.93±0.32	0.79±0.03
5	Pusabaisaki	2.67± 0.12	9.47±0.13	0.94±0.05
6	TAP-7	2.79± 0.19	9.90±0.05	0.99±0.02
7	Hum-1	2.36± 0.06	8.37±0.23	0.83±0.07
8	Vaibhav	2.48± 0.36	8.80±0.36	0.88±0.23
9	Chinamung	2.73±0.15	9.69±0.22	0.96±0.09

* Values are mean ± standard deviation of three independent determinations.

Table 2: Phytic acid content of some common edible legume seeds.

Legume	Phytate content Range	Reference
Mung bean	670-693 mg/100g	[21]
	6.5mg/g	[22]
	1.2g/100g	[23]
	741 mg/100g	[11]
Black gram	645mg/100g	[11]
Tribal pulse	6.32mg/g	[24]
Dolichos lablab	605mg/100g	[25]
Moth bean	852-899mg/100g	[26]
P. vulgaris L	1.16-2.93g/100g	[17]
Soy bean	1.47g/100g	[27]
Cow pea	1.37g/100g	[27]
Lima bean	0.88g/100g	[27]
P. vulgaris L	0.54-1.58g/100g	[28]
Great Northern bean	2.7g/100g	[29]
Cajanus cajan	811mg/100g	[30]

Table 3: Effect of soaking on the phytic acid content (mg/g d.wt) of mung bean cultivars.

		Phytic acid (mg/g dry weight)		
Sl.No.	Cultivar Reduction in	No soaking	12-hrs soaking	phytic acid (%)
1	ALM-1	8.20± 0.11	5.71±0.19	30± 0.04
2	ALM-2	7.77± 0.08	4.54± 0.43	41± 0.13
3	ALM-3	6.17±0.19	3.80± 0.16	38±0.09
4	PS-16	7.93±0.32	4.97±0.03	37±0.32
5	Pusabaisaki	9.47±0.13	5.59±0.22	40±0.12
6	TAP-7	9.90± 0.05	8.59± 0.31	13±0.43
7	Hum-1	8.37± 0.23	6.02±0.29	28±0.56
8	Vaibhav	8.80±0.36	6.17±0.13	29±0.23
9	Chinamung	9.69±0.22	6.86±0.11	28±0.06

* Values are mean ± standard deviation of three independent determinations.

Table 4: Effect of germination on the phytic acid content of mung bean cultivars.

		Phytic acid content (mg/g dry weight)			
			Germinated seeds (sprouts)		
Sl.No.	Cultivar	Raw seeds	12 hrs	24 hrs	48 hrs
1	ALM-1	8.20±0.11	4.17± 0.14	3.30 ± 0.21	2.92±0.20
			(49±0.20)	(59±0.15)	(64±0.31)
2	ALM-2	7.77±0.08	3.88±0.27	2.92±0.03	2.05±0.31
			(50±0.03)	(62±0.13)	(73±0.11)
3	ALM-3	6.17±0.19	3.30±0.14	2.71±0.11	1.75±0.06
			(46±0.20)	56±0.07)	(71±0.14)
4	Vaibhav	8.80±0.36	5.11±0.09	4.26±0.43	3.16±0.11
			(41±0.11)	(51±0.10)	(64±0.13)
5	Hum-1	8.37±0.23	4.75±0.12	3.96±0.25	3.3±0.15
			(43±0.09)	(52±0.37)	(60±0.11

* Values are mean ± standard deviation of three independent determinations.
Values in parenthesis represent % reduction in phytic acid content

Table 5: Phytase activity of mung bean seeds.

	Enzyme activity (?mol/min)				
			Germinated seeds		
Cultivar	Raw seeds	Soaked seeds (12 hrs)	12 hrs	24 hrs	48 hrs
ALM-1	ND	0.0301±0.19	0.0374± 0.02	0.0715±0.05	0.0939±0.11
Vaibhav	ND	0.0309±0.23	0.0387±0.0.9	0.0680±0.12	0.1044±0.04

* Values are mean ± standard deviation of three independent determinations.
ND-- Not detected

overnight (12-14 hrs) at room temperature. Soaking of the seeds contributed significantly towards reducing the phytic acid content in mung bean cultivars (Table 3). Upon 12 hours soaking in water, 13 to 41% reduction in phytic acid in mung bean seeds was observed. The lowest (13%) and highest (41%) loss of phytic acid was observed in TAP-7 and ALM-2 cultivars respectively. Similar results for reduction in phytic acid in the soaked legumes have been reported earlier. Decrease of phytic acid by 2 to 8% and 31 to 37% in black gram and mung beans was observed when soaked for 6 hrs and 18 hrs respectively [11]. Antu Grewal and Sudesh Jood [21] reported 19% reduction in phytic acid in mung bean soaked for 12 hrs. Phytic acid is water soluble and the reduction of the same in seeds during soaking could be attributed to leaching out in soaking water under concentration gradient [11] and appearance of phytase during soaking [32]. Germination is a process widely used in legumes to increase their palatability and nutritional value. Germination also resulted in a significant reduction of phytic acid in mung bean cultivars. Successive reduction of phytic acid content was observed as the period of germination prolonged (Table 4). Reduction of 41-50% and 60-73% of phytic acid resulted after 12 hrs and 48 hrs of germination. The soaking followed by germination for 48 hrs seemed to be having the most pronounced effect on decreasing the phytic acid content of the mung bean seeds. Diminishing effect of germination on phytic acid content in various legume seeds has been reported. Reduction of phytic acid content by 18, 20, 21.1 and 20.8% in mung bean, cow pea, lentil and chick pea was observed after 12 hrs of germination [33]. And 48 hrs germination of pea seeds resulted in loss of 39 to 49% phytic acid [34]. Decrease in phytic acid during germination is attributed due to hydrolysis of the same by enzymes broadly designated as phytases synthesized in germinating seedlings as reported for several plants [28, 18].

Phytases are ubiquitous in legume seeds and are responsible for the breakdown of phytate to inositol and free orthophosphate [32]. Germination was carried out for 12, 24 and 48 hrs to study the changes in the total phytase activity in germinated seedlings of mung beans (Table 5). To optimize conditions for phytase activity, assays were conducted over a range of temperature and pH. Under assay conditions, the highest enzymatic activity was recorded at 57° C and pH at 7.5. In our assays, a marked increase in phytase activities was observed during the germination of mung bean seeds. In ungerminated seeds there was no activity.

The levels of total phytase activity reached a maximum after 48 h of germination i.e. 0.0939 µmol/min and 0.1044 µmol/min enzyme activity in ALM-1 and Vaibhav seedlings respectively. The phytase activity was not found in dry seeds, but showed low activity in soaked seeds i.e. 0.0301 µmol/min and 0.0309 µmol/min enzyme activity in ALM-1 and Vaibhav cultivars respectively. Many researchers also reported phytase activity in germinating legume seeds. In the unsoaked cotyledons of mung bean phytase activity is absent but appears after soaking the seeds for 12 hrs and reaches its maximum between 48 and 72 hrs [32, 18] and Gibson and Ullah [35] reported enhanced phytase activity during germination of soybean seeds.

The hydrolysis of phytic acid in germinating mung bean seeds has been found to be associated with the increased activity of phytase. As the phytase activity increased the phyate content decreased. The break down of phytate content was mainly due to *in vivo* hydrolysis by phytase synthesized during soaking and germination. Our results are in agreement with the many researchers who reported the negative correlation between germination and phytic acid content of different legume seeds. During germination phytase activity increases and phytate levels decreases [36]. The increased rate of disappearance of phytate with germination has been shown to be paralleled by a simultaneous increase in the phytate activity [32]. Similar observations were made in soybean [35].

CONCLUSION

The phytic acid content among mung bean cultivars varied significantly. The yellow cultivars were found to contain less phytic acid. The reduction of phytic acid in soaked and germinated mung bean seeds has been found to be associated with the increased activity of phytase. Maximum phytase activity is found to be after 48 hrs of germination. Phytase is optimally active at pH 7.5 and 57°C. Use of phytases as feed supplements or processing method like soaking and germination of seeds can improve phosphorus availability and reduce the phosphorus content in manure. Once the phytate content is reduced significantly by processes such as soaking and sprouting, the mung bean would become a good source of proteins, carbohydrates and minerals, particularly for infants, pre-school children and pregnant and lactating women. For these people mung bean sprouts, noodles or weaning foods could be an inexpensive source of iron and zinc. More over mung bean sprouts can be consumed in either unprocessed or processed forms. The dried or roasted sprouts can be preserved for several months and can be used in preparation of snacks, noodles, weaning foods, porridges, kheer or payasam, ladoos and pan cake. Bread or chapatti or roti made out of wheat or jowar flour blend with mung bean sprouts-meal supplements proteins and minerals there by increasing nutritive value of formers. Mung beans and sprouts are good source of minerals and proteins especially for children and old age people.

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