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The effect of germination and metallic salts on the stability of enzymes of three high yielding varieties of maize (*Zea mays* L.) in respect of Bangladesh

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Abstract

The study was conducted with a view to determine the effect of germination and metallic salts on nutritional quality, enzymes activity and their stability of three high yielding varieties of maize (*Zea mays* L.). The protein content of BHM-3, BHM-5 and BHM-6 were increased 22.37%, 26.48%, and 20.34% respectively at 48 hours then decreased drastically from 72-96 hours of germination. Starch content was increased maximum 29.19% in BHM-6 at 0 hours (non-germinating seeds) among the three varieties and then decreased gradually from 48-96 hours of germination. Total sugar and reducing sugar contents of BHM-3, BHM-5 and BHM-6 seeds were maximum at 96 hours than 24-72 hours of germination while BHM-3 showed boosting increase of total sugar (336.97%) due to 96 hours of germination. BHM-5 showed a tremendous increase of α-amylase (189.83%) and protease (144.44%) activity whereas BHM-6 showed maximum invertase activity (175.27%) at 48 hours then decreased gradually from 72-96 hours of germination. The activities of enzymes were increased in presence of metallic salts such as Ca^{2+}, Mg^{2+}, and Mn^{2+} while Fe^{2+}, Zn^{2+} and Cu^{2+} inhibited the activities moderately.

1. Introduction

Of three most important food crops in the world are rice, wheat and maize (corn). These cereal seeds directly contribute more than half of all calories consumed by human beings. In terms of total pounds produced *Z. mays* L. is the leading cereal seed in the world (Anon, 2003). Maize is the photo-insensitive crop. It can be grown throughout the year in the subtropical climate of Bangladesh. Area and production of *Z. mays* L. in Bangladesh have been rising fast (Hussain *et al.,* 1999). Soil texture with 30% (clay and clay-loam soil), 10% sandy soils and p^H in the range of 7.5 to 8.5 supports good maize crop production (IITA, 1997). The important maize production countries in the world are South Africa, USA, China, Brazil, Argentina, Mexico, India etc. *Zea mays* L. provides nutrients for humans, animals and serves as basic raw materials for the production of starch, oil, protein, alcoholic beverage, food sweetener. Corn has diverse culinary applications all over the world (Kumar *et al.,* 2013). The importance of maize in the human diet, livestock feed and as raw material for some industries had increased of the 20th century (Badu *et al.,* 2008). From ancient time corn has been used for various medicinal purposes (Kumar *et al.,* 2013). A massive breakdown of reserve substances begins with the help of amylolytic, proteolytic and lipolytic enzymes during the period of germination which leads the activity of the enzyme in boosting condition. Protease, amylase and invertase are the important hydrolytic enzymes which are found in cereals, pulses and fruits etc. Germinating seeds generally exhibit high amylase and protease activities. These enzymes were studied in some cereals, legume seeds (Koshiba and Minamikawa, 1983; Morohasi, 1986; Rahman *et al.,* 2006). It was observed that protease activities present in dry seeds were enhanced during germination (Harvey and Oaks, 1974). Availability of literature, the present research works were planned to observe the effect of germination and metallic salts on nutritional quality, enzymes activity and their stability of three different varieties of maize.

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2. Materials and methods

2.1 *Zea mays* L. seed sample collection

Three *Zea mays* L. seed samples were collected from Bangladesh Agricultural Research Institute (BARI) namely BHM-3, BHM-5 and BHM-6. All the apparatus were provided by the Food Enzymology section of IFST of BCSIR, Dhaka-1205. Chemicals and solvents used in the study were of analytical reagents grade.

2.2 Germination test

For germination test, 0.5% aqueous solution of calcium hypochlorite was used for surface sterilization of *Zea mays* L. seeds. Seeds were surface sterilized to avoid microbial infection by soaking the seeds with 0.5% calcium hypochlorite for two minutes followed by washing 7 to 8 times with distilled water to remove any trace of hypochlorite. Then the seeds were kept on Whatman No.1 (9 cm) soaked filter paper in sterilized petri dishes. Distilled water was applied to the experimental seeds at an interval an of 24 hours, 48 hours, 72 hours and 96 hours germination.

2.3 Determination of proximate analysis

Among proximate analysis, protein content was determined using Macro-Kjeldahl AOAC method (2000). Other proximate analysis includes fat, carbohydrate, ash, fibre and moisture contents of raw and germinated maize seeds evaluated (Ranganna, 1991). Soxhlet apparatus was used for determination of fat by hexane and petroleum benzene (40°C-60°C) extraction for 6 hours. Ash content was calculated by 6 hours of burning in muffle furnace at 600°C. Moisture meter was used for determination of moisture content at 105±5°C temperature. Fiber content was measured by using 0.255N H_2SO_4 and 0.313N NaOH solution. Starch, reducing sugar and total sugar contents of different seeds were determined by following the method of Ranganna (1991). The mineral content (calcium, phosphorus, and iron) were determined by the method of Anon. (1976).

2.4 Assay of enzyme activity

2.4.1 Preparation of crude enzymes extract

The seeds (2 gm) were grinded in a mortar with cold 0.1M phosphate buffer of respective pH, for amylase (6.7) and for protease citrate buffer (5.5) and finally crushed into paste using a homogenizer. The temperature was maintained at 4°C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheesecloth in the cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 r.p.m. for 20 minutes at 4°C and used as crude enzyme extract. Amylase activity was assayed following the method as described by Jayaraman (1981). Protease and invertase activity were assayed following the modified method as described by Mahadevan and Sridhar (1982).

2.5 Effect of metallic salts

Metallic salts at different concentrations were added to 5.0ml of amylase, protease and invertase enzymes extract solutions from BHM-5 variety and incubated for 10 minutes at 20°C. The mixtures were again incubated with the substrate for 15 minutes at 37°C and the enzyme activities were assayed.

3. Results and discussion

Table 1. Proximate composition of different varieties of *Zea mays* L. seed

Parameters	Name of *Zea mays* L. varieties		
	BHM-3	BHM-5	BHM-6
Moisture (%)	9.36±0.02	10.48±0.03	9.62±0.02
Ash (%)	1.36±0.10	1.12±0.15	1.43±0.09
Protein (%)	10.95±0.21	10.50±0.33	11.65±0.28
Fat (%)	5.380±0.42	5.08±0.39	5.21±0.41
Crude Fibre (%)	2.09±0.02	1.81±0.01	1.82±0.02
Carbohydrate (%)	72.95±0.32	72.82±0.29	72.10±0.36
Energy (kcal per 100 gm)	394.02±0.42	388.48±0.38	391.51±0.39
Starch (%)	65.72±0.09	64.88±0.18	65.78±0.21
Total Sugar (%)	3.30±0.10	3.38±0.08	3.29±0.11
Reducing Sugar (%)	1.10±0.04	1.04±0.04	1.08±0.06
Calcium (mg/100gm)	48.30±0.22	44.70±0.30	50.60±0.23
Phosphorus (mg/100gm)	252.0±0.36	244.0±0.41	264.0±0.39
Iron (mg/100gm)	2.54±0.03	2.14±0.02	2.60±0.03

The values are mean ± SD of determinations made in triplicates.

3.1 Proximate analysis

The results of the proximate analysis of three different *Zea mays* L. are shown in Table 1.The moisture content of BHM-3, BHM-5, BHM-6 was found 9.36%, 10.48%, 9.62% respectively (Table 1).Cereal seeds are usually harvested at 20-25% moisture content while 14% or less is considered safe for storing grains, 12% or less for storing seeds (Stefania *et al.*, 2005). The ash content

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of *Zea mays* L. seed BHM-3, BHM-5, BHM-6 was 1.36%, 1.12% and 1.43% respectively (Table 1) which is similar to Peplinski (1889). Maziya-Dixon *et al*. (2000) found results in the range of 1.4-3.3%, which is slightly higher than the values determined in the present study. The second largest chemical component of the *Zea mays* L. seed is protein. The protein content of *Zea mays* L. BHM-3, BHM-5, BHM-6 were found 10.95%, 10.50% and 11.65% respectively (Table 1) which were similar to the value 10.67-11.27% mentioned by Ijabadeniyi and Adebolu (2005) of three *Zea mays* L. varieties grown in Nigeria. The fat content of *Zea mays* L. BHM-3, BHM-5, BHM-6 varieties were 5.38%, 5.08%, and 5.21% respectively (Table 1). Ijabadeniyi and Adebolu (2005) determined the percentage of fat content of three *Zea mays* L. varieties grown in Nigeria in the range of 4.77-5.00% which is in close consistency with the present study. The fibre content of BHM-3 variety was found to be the highest (2.09%) followed by BHM-6 (1.82%) and the lowest amount was found in BHM-5 (1.81%). Ullah *et al*. (2010) mentioned the crude fibre content of different *Zea mays* L. varieties were in the range of 0.80-2.32% which is in close agreement with the present study. Carbohydrate is the major chemical component of the *Zea mays* L. seed. The carbohydrate content of BHM -3, BHM-5 and BHM-6 were found 72.95%, 72.82% and 72.10% respectively (Table 1). These results are also in agreement with other scientific works of Ullah *et al*. (2010) who mentioned the carbohydrate content of different *Zea mays* L. varieties were in the range of 69.66% - 74.55%. The energy content of BHM-3 variety was found to be the highest (394.02%) followed by BHM-6 (391.51%) and BHM-5 (388.48%) which is also similar to Ullah *et al*. (2010) and Kouakou *et al*. (2008). In another study Ejigue *et al*. (2005) found the energy value 447 kcal/100g for yellow *Zea mays* L. which is higher than the values determined in this study. The results of the present studies showed that *Zea mays* L. varieties are a rich source of energy as compared to other crops. The starch content of BHM-6 variety was found to be the highest (65.78%) followed by BHM-3 (65.72%) and BHM-5 (64.88%) presented in Table 1, supported by Guria (2006). According to Rahman *et al.* (2011) the total and reducing sugar contents were varied from 3.29%-3.38% and 1.04%-1.10% (Table 1). Mejía (2003) reported that calcium and phosphorus were found highest in BHM-6 (50.60 mg/100gm and 264.0 mg/100gm) followed by BHM-3 (48.30 mg/100gm and 252.0 mg/100gm) and BHM-5 (44.70 mg/100gm and 244.0 mg/100gm) presented in Table 1. The iron content of BHM-6 variety was found highest (2.60 mg/100gm) followed by BHM-3 (2.54 mg/100gm) and BHM-5 (2.14 mg/100gm) which were similar to Guria (2006). This slight difference might be as a result of fertilizer application, the rate of parboiling and the amounts of soil nutrients which affect the mineral contents.

3.2 Determination of protein content during germination

It was observed that due to germination, the protein content of BHM-3, BHM-5 and BHM-6 were increased maximum 22.37%, 13.28%, and 20.34% respectively at 48 hours of germination then decreased drastically after 72 and 96 hours of germination. Maximum decreases of protein were 20.73%, 24.29% and 19.66% at 96 hours of germination (Table 2). Several studies on the effect of germination on cereals and legumes found that germination can increase protein content and dietary fibre; reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Hussein and Ghanem, 1999). Hahm *et al.* (2008) suggested that protein content of cereals and legumes deceased at a certain period because amino acids are oxidized to carbon dioxide and water to generate energy for germination.

Table 2. Changes of protein content of *Zea mays* L. seed during different germinating period

Variety	Duration of germination (Protein %)				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
BHM-3	10.95± 0.21	12.76± 0.29	13.40± 0.31	11.48± 0.33	8.68± 0.25
BHM-5	10.50± 0.33	12.25± 0.40	13.28± 0.42	11.22± 0.38	7.95± 0.29
BHM-6	11.65± 0.29	13.51± 0.26	14.02± 0.32	12.06± 0.28	9.36± 0.31

The values are mean ± SD of determinations made in triplicates.

3.3 Enzyme activity during germination

Enzyme activities of maize seed during the different germinating period were summarized in Table 3. During the different germination period, the highest α-amylase activity was found at 48 hours in BHM-6 (3.64 unit/gm) followed by BHM-3 (3.48 unit/gm) and BHM-5 (3.42 unit/gm). The present findings indicated that amylase activity increased in BHM-5 (189.83%) and BHM-6 (188.89%) and BHM-3 (185.25%) and the invertase activity increased 175.27%, 174.42% and 170.0% respectively at 48 hours of germination and there after decreased drastically. During the different germination period, the highest invertase activity was found at 48 hours in BHM-3, BHM-5 and BHM-6 (2.36 unit/gm, 2.43 unit/gm and 2.56 unit/gm). The higher protease activity was found at 48 hours in BHM-3, BHM-5 and

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Table 3. α-amylase, invertase and protease activities of *Zea mays* L. seed during the period of germination

Variety	Relative activity	Duration of germination				
		0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
BHM-3	α-amylase (unit/gm)	1.22±0.01	2.74±0.03	3.48±0.11	2.80±0.21	1.95±0.13
BHM-5		1.18±0.05	2.70±0.02	3.42±0.03	2.76±0.04	1.78±0.21
BHM-6		1.26±0.02	2.78±0.10	3.64±0.13	2.88±0.09	1.98±0.11
BHM-3	Invertase (unit/gm)	0.86±0.05	1.56±0.14	2.36±0.13	1.72±0.09	1.08±0.17
BHM-5		0.90±0.02	1.62±0.22	2.43±0.19	1.80±0.08	1.16±0.14
BHM-6		0.93±0.10	1.65±0.23	2.56±0.32	1.92±0.24	1.24±0.09
BHM-3	Protease (unit/gm)	1.92±0.33	2.81±0.25	4.48±0.22	3.72±0.42	2.46±0.32
BHM-5		1.80±0.07	2.64±0.31	4.40±0.36	2.62±0.17	2.18±0.43
BHM-6		2.06±0.31	2.90±0.11	4.52±0.44	3.16±0.32	2.76±0.44

The values are mean ± SD of determinations made in triplicates.

Table 4. Changes of starch, total sugar and reducing sugar content of *Zea mays* L. seed during different germinating period

Parameters	Variety	Duration of germination				
		0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Starch (%)	BHM-3	65.72±0.35	52.71±0.36	38.29±0.41	36.80±0.12	31.03±0.19
	BHM-5	64.88±0.24	51.74±0.31	39.79±0.45	35.80±0.36	29.26±0.43
Total sugar (%)	BHM-3	3.30±0.25	7.86±0.39	10.13±0.41	12.15±0.34	14.42±0.42
	BHM-5	3.38±0.03	7.86±0.36	10.13±0.28	11.92±0.41	14.56±0.37
	BHM-6	3.29±0.05	7.63±0.40	10.13±0.31	11.86±0.39	14.59±0.40
Reducing sugar (%)	BHM-3	1.10±0.03	1.24±0.06	1.53±0.11	1.96±0.09	2.35±0.10
	BHM-5	1.04±0.06	1.18±0.07	1.43±0.09	1.95±0.13	2.24±0.20
	BHM-6	1.08±0.08	1.22±0.06	1.48±0.10	2.06±0.17	2.32±0.21

The values are mean ± SD of determinations made in triplicates.

BHM-6 (4.48 unit/gm, 4.40 unit/ gm and 4.52 unit/gm). Enzyme activities were decreased drastically from 72-96 hours due to germination. Results were supported by Koshiba and Minamikawa (1983). In cereal seeds, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al*., 2003), wheat (Yang *et al*., 2001) and rice (Manna *et al*., 1995). It was established that a number of dry matters reduced in germinated wheat and the content of mineral substances, protein and activity of fermented α-and β-amylases, cellulases, proteases, and maltase were greater than in non-germinated wheat, observed by Kraujutiene *et al.* (2010).

3.4 Determination of starch, total sugar and reducing sugar during germination

Table 4 showed that starch content of three high yielding varieties was increased remarkably 22.19%, 20.25% and 19.79% due to 24 hours of germination then decreased drastically from 48-96 hours of germination. BHM-3, BHM-5, and BHM-6 showed a higher amount of starch 52.71%, 51.74% and 51.18% at 24 hours of germination and maximum decrease were 52.78%, 54.90% and 47.07% respectively at 96 hours of germination. Results were similar to the findings of Kashem *et al.* (1995). Stored starch plays an important role in the development of embryo during germination of seeds. The increase in metabolic activity in germinating seeds is due to the induction of some of the hydrolytic enzymes. It was observed that due to germination total sugar content of BHM-3 increased remarkably 336.97% at 96 hours of germination, whereas it was decreased drastically from 24-72 hours of germination (Table 4). Followed by Ali (1992) and Jood *et al.* 1998 it was observed that due to germination reducing sugar content increased significantly at 96 hours of germination whereas it was decreased drastically from 24-72 hours of germination. During germination, there was a decrease in storage carbohydrates and an increase in total soluble sugar. This might be due to the requirement of energy by

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growing utilization and rapid translocation of amino acids to the growing axes.

3.5 Metallic salts

Table 5. Effect of various metallic salts on the activities of α-amylase, invertase and protease

Test Salts	Concentration (Molar)	Relative activities (%)		
		α-amylase	Invertase	Protease
None	-	100.00	100.00	100.00
$MgCl_2$	0.001	104.72	103.54	105.00
	0.002	107.34	106.36	108.12
$ZnCl_2$	0.001	84.24	86.80	92.25
	0.002	72.20	74.32	88.52
$CuCl_2$	0.001	85.70	64.62	70.84
	0.002	75.35	50.28	52.64
$MnCl_2$	0.001	116.74	110.26	112.46
	0.002	126.62	118.42	120.66
$NaCl_2$	0.001	100.00	99.32	99.68
	0.002	99.74	97.46	98.78
KCl_2	0.001	100.00	100.00	100.00
	0.002	100.00	100.00	100.00
$FeCl_2$	0.001	72.16	64.46	66.28
	0.002	58.46	50.74	52.24

Table 5 represents the effect of various metallic salts on the activities of three enzymes. The activities of enzymes were increased remarkably in the presence of Mn^{2+} salts while that was increased slightly in the presence of Mg^{2+} salts. Other metallic salts such as K^{2+} and Na^{2+} produce little or no inhibitory effects on the activities of the enzymes but the activities of all the enzymes reduce significantly in the presence of Zn^{2+},Cu^{2+} and Fe^{2+}. The activities of the enzymes increased significantly in the presence of divalent cations Ca^{2+} and Mn^{2+} suggesting the involvement of these divalent ions in maintaining the active conformation of the enzymes which are partially in accordance with the results of Akand *et al.* 2003.

4. Conclusion

The test results represent that among three high yielding varieties of *Zea mays L.*, BHM-3 was rich in energy and BHM-6 was higher in protein, starch, and mineral. This study could be concluded that germination is a more effective process for improving nutritional value, enzyme activities and functional qualities of *Zea mays L.* seeds. Protein and enzyme activities were increased tremendously during 48 hours of germination. On the other hand, starch content decreased but reducing sugar and total sugar content were increased gradually from 0-96 hours of germination. The activities of enzymes were increased in presence of metallic salts such as Ca^{2+}, Mg^{2+}, and Mn^{2+} while Fe^{2+}, Zn^{2+} and Cu^{2+} inhibited the activities moderately.

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