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# Effect of germination on biochemical changes in high yielding barley (*Hordium vulgare* L.) varieties

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**Abstract**

The effect of germination was evaluated on the nutritional properties and enzyme activities of three barley (*Hordium vulgare* L.) varieties namely BARI Barley-4, BARI Barley-5 and BARI Barley-7 produced by Bangladesh Agricultural Research Institute (BARI). The nutritional compositions and enzymatic activities (α-amylase and protease) in both raw and germinated seeds were changed gradually with germination period. Protein content of germinated seeds for BB-4, BB-5 and BB-7 was recorded as 13.65%, 14.34% and 13.95% respectively. Maximum increase of protein was 20.81% for BB-5 at 48 hours of germination. Gradual decrease was observed at 72 hours of germination and maximum decrease was 7.66% for BB-5 but protein content was higher than non germinated seeds. Enzyme activity of α-amylase and protease showed the results 2.20 U/g BB-5 and 1.16 U/g BB-4. Highest increase of α-amylase and protease activity were found 125.53% for BB-7 and 107.55% for BB-5 at 48 hours of germination. The highest amount of total and reducing sugar was 12.58% for BB-5 and 1.97% for BB-7 respectively at 72 hours of germination. The maximum increase of total sugar was 240.00% and in reducing sugar it was 79.09% for BB-5 and BB-7 respectively. Highest calcium, phosphorus and iron contents were 39.70 (mg/100g), 273.91 (mg/100g) and 8.37 (mg/100g) for BB-4 and BB-7 respectively. The maximum increases of calcium and iron contents were 33.26% and 85.58% for BB-4 and BB-7 respectively at 48 hours of germination. Germinated barley seeds contain more protein with other easily digestible biomolecule than non germinated seeds.

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**Keywords:** Germinated barley; Nutritional compositions and enzyme activities

## Introduction

Barley (*Hordeum vulgare* L.) is of considerable nutritional significance to the human diet contributing a significant portion of the global human diet (Finnie and Svensson, 2009). Barley offers a wealth of potential health benefits (Baik and Ullrich, 2008) containing several vitamins and minerals including niacin (vitamin $B_3$), thiamine (vitamin $B_1$), selenium, calcium, iron, magnesium, zinc, phosphorus and copper. Barley has been proven to help improve certain health conditions. The antioxidants in barley work to slow down the rate of oxidative damage by gathering up free radicals that form when body cells use oxygen (Pins and Kaur, 2006). It is the fourth important cereal crop in the world and third important cereal in Bangladesh (FAOSTAT, 1993 -2002). It occupies about 9.4 % of the total cereal acreage with about 7.8 % of the total cereal production in the world (FAOSTAT, 2007). The total barley production in the world is 135.54 million tons in 2010 - 11 (Agro - stats, 2010). In 2013 -14, the total production of barley in the world was 140.10 million metric tons.

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In 2012 - 13, the production of barley in Bangladesh was 7000 metric tons (USDA, 2013). Seed germination is one of the oldest and most economical methods of producing and preserving food (Billings, 1998). During the germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolitic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development (Rahman *et al*., 2007). In addition, germination provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the seed (Simango, 1997). The initiation of protein synthesis in germinating seed is another important phenomenon. The highest content of protein was found in wheat, spring barley and spring triticale, which exceeded the content of protein found in winter triticale, winter barley and even in rye. Considering the above mentioned facts, the objectives of present investigation are to determine and compare the protein, mineral, starch, total sugar, reducing sugar and enzymatic activity (protease, α-amylase) among different high yielding Barley varieties at different time intervals.

## Materials and methods

### *Barley seed sample collection*

Barley seed samples were collected from Bangladesh Agricultural Research Institute (BARI). All the apparatus were provided by the Food Enzymology Section, IFST, BCSIR, Dhaka-1205.Chemicals and solvents used in the study were of analytical reagents grade.

### *Proximate analysis*

Among proximate analysis, protein content was determined using Macro-Kjeldahl AOAC method (2000). Other proximate analysis including fat, carbohydrate, ash, fibre and moisture contents of raw and germinated maize seeds were 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 H2SO4 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).

### *Enzyme activity assay*

Barley seeds (0.5g) were grinded in a mortar with 0.1M phosphate buffer with respective pH (amylase 6.7 and protease pH 5.5) and finally crushed into paste using a homogenizer. The temperature was maintained at 4$^{o}$C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheese cloth in cold room. The filtrate was then collected and clarified further by centrifuging at 10,000 rpm for 15 min at 4$^{o}$C.

### *Amylase*

Amylase activity was assayed following the method as described by Jayaraman (1981). One percent starch solution was used as substrate (1.0 gm in 100 ml of 0.1M phosphate buffer, pH 6.7). The amylase activity was measured by estimating the release of maltose evaluated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1.0 mg of maltose in 15 min at 37$^{o}$C.

### *Protease*

Protease activity was assayed following the modified method as describe by Mahadehvan and Sridhar (1982). Haemoglobin was used as substrate here. Protease activity was measured by estimating the amount of leucine released from haemoglobin. The amount of leucine released was calculated from the standard curve prepared with leucine. One unit of protease activity was defined as the amount required for liberating 1 mg of leucine in 30 min at 37$^{o}$ C.

## Results and discussion

Barley is a potential cereal and germination is an inexpensive way to develop the food value therefore, it is very important to investigate the biochemical changes among three high varieties of barley. Proximate compositions from different varieties of non germinated barley seeds were analyzed shown in the Table I. The moisture content of BB-4, BB-5 and BB-7 were found 12.9%, 12.3%, 12.8% respectively. The result showed that

ash content of barley seeds BB-4, BB-5, BB-7 were 2.08%, 2.16% and 2.21% respectively (Table I.). Ali *et al*. (1992) reported that ash content of barley seed was 3.90%, which was similar to the result of the present study. The protein content of barley seed BB-4, BB-5 and BB-7 was found to be 11.40%, 11.87% and 11.65% respectively (Table I). The protein content found from this study were similar to the value 12.00% mentioned by Potter and Hotchkiss (1996) for protein content in barley .The fat content of barley seed for BB-4, BB-5, BB-7 was 1.71%, 1.68% and 1.64% respectively (Table I). According to Kamal *et al*. (2013), fat content ranged from 1.5 - 2.9% almost similar to present study. The crude fibre contents of different barley seeds were varied from 5.18 to 5. 96 %.The BB-5 variety was found to have the highest fiber content (5.96%) followed by BB-7 (5.77 %) and the lowest amount was found in BB -4 (5.18%). Ijabadeniyi and Adebolu (2005) reported slightly lower values (2.07 - 2.77%) of the fibre content for the *Zea mays* L. varieties grown in Nigeria. Carbohydrate is the major chemical component of the *H. vulgare* seed. The carbohydrate content of BB-4, BB - 5, BB -7 was found to be 71.91%, 71.99% and 71.77% respectively (Table I). The energy content was varied from 333.617 to 336.236 %. The energy content of BB-4 variety was found to be the highest (336.236 Kcal) followed by BB-5 (335.01 Kcal) and the lowest amount was found in BB-7 (333.617 Kcal). Fairbairn *et al*. (1999) observed the energy content of barley was 336 Kcal.

**Table I. Proximate composition of different varieties of barley seeds**

| Parameters | Name of varieties | | |
|---|---|---|---|
| | BB - 4 * | BB - 5* | BB - 7* |
| Moisture (%) | 12. 9 ± 0.01 | 12.3 ± 0.01 | 12.8 ± 0.01 |
| Ash (%) | 2.08 ± 0.01 | 2.16 ± 0.01 | 2.21 ± 0.01 |
| Protein (%) | 11. 40 ± 0.02 | 11.87 ± 0.02 | 11.65 ± 0.01 |
| Fat (%) | 1. 71 ± 0.01 | 1.68 ± 0.01 | 1.64 ± 0.01 |
| Crude Fibre (%) | 5.18 ± 0.01 | 5. 96 ± 0.01 | 5.77 ± 0.02 |
| Carbohydrate (%) | 71.91 ± 0.01 | 71. 99 ± 0.02 | 71.77 ± 0.01 |
| Energy (Kcal. per100 g) | 336.236 ± 0.01 | 335.01 ± 0.01 | 333.617 ± 0.01 |

*Here, BB-4 = BARI Barley-4, BB-5 = BARI Barley-5, BB-7 = BARI Barley-7
± = Standard deviation based on triplets

Mineral content of germinating barley seeds were shown in the Table II**.** The variety BB-4, BB-5 and BB-7 showed the gradual increase of calcium, phosphorus and iron contents till 48 hours then it was decreased at 72 hours of germination. The result found that maximum calcium content was 39.70 mg/100gm for BB-4 and the increase was 33.26%. The result was in accord with Rupa *et al.* (2018). The variety of BB-7 showed the highest amount of phosphorus 273.91 mg/100 gm at 48 hours of germination and lowest was found 268.11 mg/100gm for BB-5 variety. Phosphorus content of rice, barley and chickpea are almost similar obtained by Ibukun (2008). The phosphorus content in barley grown in Jordan, Morocco by the FAO ranges 179-350 (mg/100g). Maximum increase of phosphorus among the three barley seeds was 12.47% and maximum decrease was 9.51% found in BB-4 and BB-7 respectively.

BB-4, BB-5 and BB-7 variety showed highest iron content 8.37(mg/100 g) for BB-7 after 48 hours of germination. The BB-7 variety showed the maximum increase and decrease of iron 85.59% at 48 hours of

**Table II. Mineral content of different varieties of barley seed during germination**

| Parameters | Variety | Duration of germination | | | |
|---|---|---|---|---|---|
| | | 0 hrs | 24 hrs | 48 hrs | 72 hrs |
| Calcium (mg/100g) | BB-4 | 29.79 ± 0.01 | 35. 04 ± 0.015 | 39. 70 ± 0.02 | 25.08 ± 0.01 |
| | BB-5 | 27.04 ± 0.01 | 30. 52 ± 0.01 | 35.03 ± 0.02 | 24.04 ± 0.01 |
| | BB-7 | 26.05 ± 0.01 | 31.09 ± 0.01 | 33.80 ± 0.01 | 23.05 ± 0.01 |
| Phosphorus (mg/100g) | BB-4 | 240.43 ± 0.01 | 252. 43 ± 0.01 | 270.3 ± 0.01 | 216.33 ± 0.01 |
| | BB-5 | 239.35 ± 0.01 | 249.72 ± 0.01 | 268.11 ± 0.02 | 215.71 ± 0.02 |
| | BB-7 | 242.5 ± 0.01 | 255.30 ± 0.01 | 273. 91 ± 0.01 | 219.42 ± 0.015 |
| Iron (mg/100g) | BB-4 | 4.20 ± 0.01 | 5.81 ± 0.01 | 6.81 ± 0.01 | 5.64 ± 0.03 |
| | BB-5 | 4.38 ± 0.01 | 5. 50 ± 0.01 | 7.19 ± 0.01 | 5. 79 ± 0.01 |
| | BB-7 | 4.51 ± 0.01 | 5.88 ± 0.02 | 8.37 ± 0.01 | 6.39 ± 0.01 |

± = Standard deviation based on triplets

**Table III. Protein and enzyme activity of barley seeds under different germination period**

| Test Parameters | Variety | Duration of germination | | | |
|---|---|---|---|---|---|
| | | 0 hrs | 24 hrs | 48 hrs | 72 hrs |
| Protein (%) | BB-4 | 11.40 ±0.02 | 12.76±0.01 | 13.65 ± 0.01 | 12.25 ± 0.01 |
| | BB-5 | 11.87 ± 0.02 | 13.26 ± 0.01 | 14.34 ± 0.01 | 12.78 ± 0.02 |
| | BB-7 | 11.65 ± 0.01 | 12. 92 ± .01 | 13.95 ± 0.01 | 12.40 ± 0.02 |
| α-Amylase (U/g)* | BB-4 | 0.99 ±0.01 | 1.53 ± 0.02 | 2.16 ± 0.02 | 1.39 ± 0.01 |
| | BB-5 | 1.03 ± 0.01 | 1.66 ± 0.02 | 2.20 ± 0.02 | 1.47 ± 0.02 |
| | BB-7 | 0.94 ± 0.02 | 1.73 ± 0.02 | 2.12 ± 0.015 | 1.41 ± 0.01 |
| Protease (U/g)* | BB-4 | 0.56 ± 0.02 | 0. 94 ± 0.01 | 1.16 ± 0.01 | 0.74 ±0.01 |
| | BB-5 | 0.53 ± 0.01 | 0.89 ± 0.01 | 1.10 ± 0.01 | 0.68 ± 0.01 |
| | BB-7 | 0.57±0.01 | 0.98± 0.01 | 1.13± 0.01 | 0.69± 0.02 |

± = Standard deviation based on triplets.
*Unit/g

**Table IV. Starch, total sugar and reducing sugar content of barley seeds during different germinating period**

| Test Parameters | Variety | Duration of germination | | | |
|---|---|---|---|---|---|
| | | 0 hrs | 24 hrs | 48 hrs | 72 hrs |
| Starch (%) | BB-4 | 59.59 ± 0.01 | 39.18 ± 0.01 | 24.83 ± 0.01 | 16.08 ± 0.02 |
| | BB-5 | 60.54 ± 0.01 | 37.67 ± 0.02 | 23.47 ± 0.02 | 15.81 ± 0.02 |
| | BB-7 | 62.64 ± 0.01 | 38.65 ± 0.01 | 24.67 ± 0.02 | 16.01 ± 0.01 |
| Total sugar (%) | BB -4 | 3. 43 ± 0.01 | 6.09 ± 0.02 | 8.70 ± 0.01 | 11.16 ± 0.01 |
| | BB-5 | 3.70 ± 0.01 | 6.56 ± 0.02 | 10.92 ± 0.01 | 12.58 ± 0.01 |
| | BB-7 | 4.27 ± 0.01 | 7.14 ± 0.02 | 9.29 ± 0.01 | 11.94 ± 0.01 |
| Reducing sugar (%) | BB-4 | 1.19 ± 0.01 | 1.44 ±0.02 | 1.67 ± 0.01 | 1.98 ± 0.02 |
| | BB-5 | 1.14 ± 0.02 | 1.36 ± 0.02 | 1.58 ± 0.01 | 2.00 ± 0.02 |
| | BB -7 | 1.10 ±0.01 | 1.34 ±0.01 | 1. 57 ± 0.02 | 1.97 ± 0.01 |

± = Standard deviation based on triplets

germination and 41.68 in BB-7 at 72 hours of germination. Mubarak A.E. (2004) observed the Iron content of in the range of 8.0 - 9.70 mg/100 gm in mungbean seeds which was closely related to this study.

Protein contents and enzyme activity of Barley seed under different germination conditions were summarized in the Table III. During different germination period, the gradual increase of protein content was observed. The highest protein content was 11.87% for BB-5. Compared to non germinated state (0 hours), the maximum increase of protein was 20.81% in BB-5 followed by BB-4 and BB-7 varieties which was then decreased after 72 hours of germination. Lowest protein content was found to be 12.40% while the decreased was 6.44% after 72 hrs.

Results showed that protein content increased at 24 and 48 hours of germination and then decreased with the progress of germination which was in accord with the results of Rahman *et al.* (2007) and Rupa *et al*. (2018). During different germination period, the highest α-amylase activity was found for BB-5 (2.20 unit/g) and but the tremendous increase was 125.53% in BB-7 followed by BB-4 and BB-5.Then amylase activity was decreased after 72 hours of germination and the maximum decrease was 50.0% for BB-7. The results are in agreement with Liza *et al.* (2010) who reported that the α-amylase activities increased at 24 and 48 hours of germination in chickpea and then decreased drastically from 72 hours.

The highest protease activity 1.16 unit/g was found at 48 hours in BB-4 among three high yielding barley varieties while the protease activity decreased drastically at 72 hours due to germination. In this regard, the maximum increase of protease activity was found 107.55% for BB-5 at 48 hours while the protease activity was decreased maximum 32.14% (0.74 unit/g) after 72 hours germination. According to Rahman *et al*. (2007), the protease activity of mungbean increased tremendously from 131% to 161% at 24 hours of germination and

thereafter decreased, supports the above mentioned results. Rahman *et al*. (2007) showed that α-amylase and protease activity of mungbeans increased at 24 hours of germination and then decreased gradually. Those above observation were similar with the present research.

Results of starch, total sugar and reducing sugar content of barley seed during different germinating period were shown in Table IV.

It was observed that due to germination, starch content of three Barley varieties decreased gradually from 24 to 72 hours. The decrease content of starch was 16.08% whereas the percent was 73.02% found in BB-4 followed by BB-5 and BB-7 at 72 hours of germination. Results are available that starch content decreased with the time progress of germination which is in accord with the result of Kashem *et al.* (1995) and Tuna *et al*. (2017).

The changes of total sugar and free sugar contents of barley seed were shown in Table IV. From the data it was observed that total sugar and free sugar contents were also increased gradually from 24 to 72 hours of germination. The highest total sugar content was 12.58% for BB-5 and lowest content was 11.94% for BB-7 while the maximum increase of was 240.0% in BB-5 followed by BB-4 and BB-7 varieties.

A similar trend in sugar content of maize seeds during germination has been observed. It was observed that during germination, reducing sugar content increased gradually from 24 to 72 hours of germination. The varieties BB-4, BB-5 and BB-7 showed the increases in reducing sugar 1.98%, 2.00% and 1.97% respectively at 72 hours of germination. The maximum increase of reducing sugar content was 79.09% in BB-7 followed by BB-5 and BB-4 varieties. Results are available that reducing sugar content increased with the period of germination which is in accord with the result of Kashem *et al.* (1995) and Tuna *et al*. (2017).

## Conclusion

As one of the first cultivated cereals, barley is fairly suitable for further research because of its short life cycle and physiological characteristics. This study results demonstrated that barley is a cereal products containing higher nutritional values and easy to use to germinate. Barley , a minor crop, could be emerged as a major one because of its nutritional value. Agriculture scientist of Bangladesh could play an important role in enhancing the production of germinated barley together with food items made from it which in turn would contribute to food security as well as earning foreign currency.

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