




Changes in phytase activity, phosphorus and phytate contents during grain germination of barley (*Hordeum vulgare* L.) cultivars

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Abstract Phytase activity, phosphorus and phytate contents in hydroponic fodder (up to 12 days of grain germination) of four Tunisian barley (*Hordeum vulgare* L.) cultivars (Arbi, Ardhaoui, Souihli, and Rihane) were studied. Barley cultivars differed significantly in their phytase activity (0.6–1.6 U/g), phosphorus (3.2–5.0 mg/g DM) and phytate (4.2–4.7 mg/g DM) contents. Phytase increased significantly up to 8 days of germination and decreased steadily thereafter. The phytase activity after 8 days of germination was increased up to tenfold in Arbi and Ardhaoui cultivars, eightfold in Souihli and fivefold in Rihane. The increase in phytase activity during germination was accompanied by a significant decrease in phytate content for all barley cultivars (reduced by 92–97%). The decrease in phytate content

of sprouted barley by germination should improve phosphorus availability of the grain. Based on their phytase activity and phosphorus contents before germination, barley cultivars are ranked as Rihane > Ardhaoui > Souihli > Arbi. However, after germinating for 12 days the differences among varieties in phytase activity, phytate and phosphorus contents became non-significant. Further research is warranted to highlight the effect of germination on other nutritional attributes of hydroponic sprouted barley and its suitability as a feedstuff for farm animals.

Keywords Germination · Hydroponic · Barley · Phytase · Phosphorus · Phytate

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Introduction

Cereals are strategic crops worldwide because of their economic and social relevance as well as the importance of their cultivated areas. Barley is the major cereal crop in many arid areas of the Middle East, North Africa and West Asia characterised by frequent droughts, being vital for the livelihood of many small-scale farms. It has been suggested that barley could replace wheat as the dominant cereal crop, due to its tolerance to drought. Approximately 65% of cultivated barley is used for animal feed and 33% is used for malting, whereas only 2% is used directly for human consumption (Humer and Zebeli 2017). In Tunisia, barley occupies about one-third of the total cereal cultivated area. It is mainly grown for grain production (Stein et al. 2016) and straw for small ruminants during winter. The presence of antinutritional factors in barley grain such as phytate has been a major factor limiting its utilisation (Woyengo and Nyachoti 2013; Nissar et al. 2017). Phytate, *myo*-inositol (1,2,3,4,5,6) hexakisphosphate, is present in high concentrations in many plant feedstuffs (Raboy 2003). It is the major storage form of phosphorus in mature grains and legumes (Kumar et al. 2010). It is well known that phytate alters the solubility, functionality, digestibility and absorption of phosphorus and other minerals, restricting significantly the bioavailability of Zn, Fe, Ca, Mg, Mn and Cu in feed (Wu et al. 2009; Vashishth et al. 2017). Between 60 and 90% of total phosphorus in plant feedstuffs is bound as phytate phosphorus (Wu et al. 2009). Phosphorus in this form is not utilised by non-ruminant mammals and birds due to the lack of sufficient intestinal phytase to catalyse the release of orthophosphate from the phytate molecule in the gastrointestinal tract (Cowieson et al. 2016; Dersjant-Li et al. 2015). Therefore, unabsorbed phytate is excreted and contributes to environmental phosphate pollution (Humer et al. 2015). Phytase (*myo*-inositol hexaphosphate hydrolase) is an enzyme that catalyses the hydrolysis of phytate to *myo*-inositol and inorganic phosphate. It is present in most cereals, but its activity varies widely among species (Bartnik and Szafrńska 1987; Gupta et al. 2015; Vashishth et al. 2017). Wheat, triticale and rye, and even their by-products (bran and pollard), are high in phytase, while barley is somewhat lower and maize has very low phytase activity (Eeckhout and De Paepe 1994). This enzyme is considered environmentally friendly because it

reduces the additional supply of exogenous phosphate and reduces the emissions of phosphate from agricultural animal waste (Nahm 2002; Rao et al. 2009). Since high phytase activity results in enhanced biological availability of phosphorus from plant feeds, it is likely that the use of phytase-rich cereal grains could reduce inorganic phosphorus supplements and the costs of production. In nature, phytase is activated during seed germination. Phytase can be increased by germination of legume seeds and cereal grains (Gupta et al. 2015). To the best of our knowledge, there are few and limited studies reporting on barley phytase activity and its change during germination. Our objective in this present study was to investigate the change of phytase activity, phosphorus and phytate contents during germination of four Tunisian barley cultivars.

Materials and methods

Plant material and hydroponic unit

Grains of four barley (*Hordeum vulgare* L.) cultivars, namely Arbi, Ardhaoui, Rihane and Souihli, were used in this study. Rihane is an improved variety of barley cultivated in north-west Tunisia, whereas Arbi, Souihli and Ardhaoui are local barley cultivars mostly cropped in north-west, centre-east and south-east Tunisia, respectively. Field crops of each barley cultivar were located, and identification of the variety was confirmed by analysis of agromorphological traits. At harvest, samples of grains were collected and preserved to be used as seeds for hydroponic germination. Seedlings of all cultivars were germinated and sprouted at the same time in a steel hydroponic unit (5 × 3 × 3 m) equipped with automatic sprayer irrigation and ventilation devices and with capacity for 90 polyethylene trays sized 70 × 30 cm each. Temperature inside the chamber was set at 21 °C, and the relative humidity was adjusted by regulating air circulation to about 70%. The chamber room was lit for 12–14 h daily by fluorescent tubes equipped with watertight appliances and arranged vertically on the walls, providing 10–15 W/m².

Soaking and germination

Clean seeds of the different barley cultivars were washed and soaked in tap water for 4 h at 23 °C in the dark. Seeds were then treated for 30 min with 0.1%

hypochlorite at 23 °C in the dark in order to reduce fungal contamination without adversely affecting germination percentage. Seeds were sown uniformly in trays at the density rate of 4 kg/m² with restricted chemical input (without pesticides or fertilizers). Three batches (replications) of each cultivar were sown (one tray for each batch) and germinated at 21 °C for 12 days. One representative sample of the sprouted grains (whole sprouts emerging from the seed, also called hydroponic barley fodder) was collected daily from each tray. Immediately after collection, samples were transported in cooling boxes to the laboratory where they were frozen, freeze-dried and stored in a dark dry place. In total, 156 samples were collected during the study (4 cultivars × 13 times (before germination + 12 days of germination) × 3 trays (replicates)).

Chemical analysis

Freeze-dried samples of germinated grains were pulverised into a fine powder in a microcentrifuge tube using a mixer-mill. Phytase activity was determined using the method described by Centeno et al. (2001). Each ground sample (2 g) was weighed in a 50-mL volumetric flask, and homogenised with 25 ml of a sodium phytate solution (1.722 g of sodium phytate (Sigma), 180 mL of H₂O, and 820 mL of 0.25 M acetate buffer, pH 5.5). The mixture was then incubated at 37 °C for 1 h, and the reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid. The phosphorus released was determined spectrophotometrically (700 nm), and the phytase activity was expressed as the amount of inorganic phosphorus released from the sodium phytate solution per minute (1 unit (U) of enzyme activity = 1 μmol P/min) at pH 5.5 and 37 °C.

Total phosphorus concentration was determined by the photometric AOAC 965.17 (Association of Official Analytical Chemists 2005) method, starting with the dry ashing of the sample and solubilisation in dilute HCl followed by the reaction with the molybdovanadate reagent and reading absorbance at 400 nm. Potassium dihydrogen phosphate (KH₂PO₄) was used for the preparation of standard P solutions. Phytate phosphorus was determined according to the indirect method of Haug and Lantsch (1983). Samples (1 g dry weight) were extracted in 10 mL of 0.2 N HCl solution. Thereafter, 0.5 mL of this

extract was taken into glass tubes and mixed with 1 mL of a ferric solution (0.2 g of (NH₄)₂Fe(SO₄)-12H₂O in 100 mL of 2 N HCl). The tubes were heated in a boiling water bath for 30 min and then left at room temperature before adding 2 mL of a 2-2'-bipyridine solution. The decrease in iron determined colorimetrically (519 nm) in the supernatant is a measure of the phytate content, which was expressed in mg per g of dry matter.

Data analysis

Statistical analyses were performed using the general linear models (GLM) procedure of SAS. Analysis of variance (ANOVA), followed by Student–Newman–Keuls range test, was used to examine the differences among the four barley cultivars in phosphorus, phytate and phytase, either before germination or after 12 days of germination, with three replicates per cultivar. The statistical model used was $y_{ij} = \mu + B_i + C_j + \varepsilon_{ij}$, where y_{ij} is each individual observation, μ is the overall mean, B_i is the effect of the i th block ($i = 1, 2, 3$), C_j is the effect of the j th cultivar ($j = \text{Arbi, Ardhaoui, Rihane or Souihli}$) and ε_{ij} is the residual error. Within each cultivar, changes in the same variables (phosphorus, phytate and phytase) during seed germination were assessed by ANOVA, using a design with germination time (day 0–12) as the fixed effect, and replicate (hydroponic tray) as a blocking factor. In this case, the statistical model used was $y_{ij} = \mu + B_i + T_j + \varepsilon_{ij}$, where y_{ij} is each individual observation, μ is the overall mean, B_i is the effect of the i th block ($i = 1, 2, 3$), T_j is the effect of the j th germination time ($j = 0, 1, 2, \dots, 12$), and ε_{ij} is the residual error. The Student–Newman–Keuls range test was used for the multiple comparison of means. Changes in each variable over time were further analysed by orthogonal contrasts, such as polynomial (linear and quadratic) or Helmert to test specific hypotheses regarding the temporal patterns.

Results

Total phosphorus, phytate and phytase activity in grains of the four barley cultivars before germination and at the end of the study are in Table 1. Phosphorus and phytase activity were significantly highest in Rihane grains and lowest in grains of Arbi cultivar,

Table 1 Total phosphorus and phytate contents and phytase activity in grains of four barley cultivars

Barley cultivar	Total phosphorus (mg/g dry matter)		Phytate (mg/g dry matter)		Phytase (units/g dry matter)	
	Before germination	Day 12	Before germination	Day 12	Before germination	Day 12
ARBI	3.21 ^c	4.72	4.32 ^{ab}	0.120	0.60 ^c	2.44
SOUIHLI	4.25 ^b	5.13	4.22 ^b	0.215	0.70 ^{bc}	2.28
ARDHAOUI	3.43 ^c	4.99	4.65 ^a	0.249	0.80 ^b	1.85
RIHANE	4.96 ^a	5.86	4.74 ^a	0.355	1.60 ^a	2.03
SEM ($n = 3$)	0.140	0.599	0.087	0.0565	0.038	0.561
<i>P</i> value	< 0.001	0.601	0.007	0.098	< 0.001	0.881

SEM standard error of the mean (three independent replicates)

^a, ^b, ^cMeans in the same column with different superscripts are significantly different ($P < 0.05$)

Table 2 Changes in total phosphorus (mg/g dry matter) during germination in grains of four barley cultivars grown in a hydroponic system

	Days of germination	Barley cultivar			
		ARBI	SOUIHLI	ARDHAOUI	RIHANE
	Day 0	3.21 ^b	4.25	3.43 ^b	4.96
	Day 1	3.14 ^b	4.08	3.43 ^b	5.03
	Day 2	3.61 ^{ab}	4.11	3.80 ^{ab}	5.35
	Day 3	3.64 ^{ab}	4.17	3.88 ^{ab}	5.44
	Day 4	3.69 ^{ab}	4.35	3.96 ^{ab}	5.56
	Day 5	3.96 ^{ab}	4.46	4.17 ^{ab}	5.77
	Day 6	4.18 ^{ab}	4.58	4.22 ^{ab}	5.72
	Day 7	4.23 ^{ab}	4.40	4.62 ^{ab}	5.53
	Day 8	4.70 ^a	4.55	4.63 ^{ab}	5.67
	Day 9	4.89 ^a	4.86	4.82 ^{ab}	5.69
	Day 10	4.80 ^a	4.89	4.96 ^a	5.74
	Day 11	4.78 ^a	4.98	4.67 ^{ab}	5.88
	Day 12	4.72 ^a	5.13	4.99 ^a	5.86
SEM ($n = 3$)		0.389	0.324	0.356	0.361
<i>P</i> value		0.023	0.370	0.036	0.781
Linear		< 0.001	0.002	< 0.001	0.023
Quadratic		0.459	0.473	0.496	0.377

SEM standard error of the mean (three independent replicates)

^a, ^bMeans in the same column with different superscripts are significantly different ($P < 0.05$)

whereas phytate content was lowest in Souihli and highest in Ardhaoui and Rihane cultivars. These effects were only significant before germination ($P < 0.05$). However, no differences among cultivars were detected after 12 days of germination.

Germination had no effect ($P > 0.05$) on phosphorus content in grains of Souihli and Rihane cultivars (Table 2). Phosphorus increased ($P < 0.05$) in grains of Arbi cultivar during germination to reach a maximum after 9 days of germination. A similar increase ($P < 0.05$) was observed for Ardhaoui

cultivar, so that phosphorus after 10 or 12 days of germination was greater than that on days 0 and 1.

In the four barley cultivars, phytate content decreased linearly ($P < 0.05$) throughout germination (Table 3). This decrease was statistically significant ($P < 0.05$) compared with the value before germination from day 5 (Souihli), day 6 (Arbi) or day 7 (Ardhaoui and Rihane) of germination for all cultivars. On day 12 of germination, phytate content represented only between 3 and 8% of the initial values in non-germinated grains (day 0).

Table 3 Changes in phytate content (mg/g dry matter) during germination in grains of four barley cultivars grown in a hydroponic system

Days of germination	Barley cultivar			
	ARBI	SOUIHLI	ARDHAOUI	RIHANE
Day 0	4.32 ^a	4.22 ^a	4.65 ^a	4.74 ^a
Day 1	3.32 ^{ab}	3.82 ^{ab}	4.12 ^{ab}	3.71 ^{abc}
Day 2	3.03 ^{abc}	3.93 ^a	3.58 ^{abc}	3.77 ^{ab}
Day 3	3.33 ^{ab}	3.66 ^{ab}	3.48 ^{abcd}	3.70 ^{abc}
Day 4	3.20 ^{ab}	3.57 ^{ab}	3.19 ^{abcde}	3.42 ^{abc}
Day 5	2.46 ^{abcd}	2.52 ^{bc}	2.31 ^{abcdef}	2.49 ^{abcde}
Day 6	2.22 ^{bcde}	2.19 ^{cd}	2.29 ^{abcdef}	2.56 ^{abcd}
Day 7	1.61 ^{bcdef}	1.37 ^{cde}	1.54 ^{bcdef}	1.71 ^{bcde}
Day 8	1.49 ^{bcdef}	1.26 ^{cde}	1.63 ^{bcdef}	1.57 ^{bcde}
Day 9	1.05 ^{cdef}	1.05 ^{de}	1.31 ^{cdef}	1.49 ^{cde}
Day 10	0.89 ^{def}	0.95 ^{de}	0.90 ^{def}	0.75 ^{de}
Day 11	0.26 ^{ef}	0.45 ^e	0.74 ^{ef}	0.28 ^e
Day 12	0.12 ^f	0.22 ^e	0.25 ^f	0.36 ^{de}
SEM (<i>n</i> = 3)	0.285	0.185	0.358	0.313
<i>P</i> value	< 0.001	< 0.001	< 0.001	< 0.001
Linear	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	0.543	0.876	0.325	0.934

SEM standard error of the mean (three independent replicates)
 a, b, c, d, e, f Means in the same column with different superscripts are significantly different (*P* < 0.05)

Germination of barley grains was associated with a significant linear and quadratic increase (*P* < 0.05) in phytase activity of all studied cultivars (Table 4), with a peak value observed after day 8 of germination. In

comparison with values before germination, phytase activity became significantly increased (*P* < 0.05) from day 6 (Arbi, Souihli, Rihane) or day 7 (Ardhaoui.). This increase was more pronounced in Arbi

Table 4 Changes in phytase activity during germination in grains of four barley cultivars grown in a hydroponic system (units/g dry matter)

Days of germination	Barley cultivar			
	ARBI	SOUIHLI	ARDHAOUI	RIHANE
Day 0	0.60 ^d	0.70 ^e	0.80 ^d	1.60 ^{ef}
Day 1	1.12 ^d	1.18 ^e	1.04 ^d	1.02 ^f
Day 2	1.28 ^d	1.55 ^{de}	1.75 ^{cd}	1.99 ^{def}
Day 3	2.05 ^{cd}	2.20 ^{cde}	2.27 ^{cd}	2.32 ^{cdef}
Day 4	2.23 ^{cd}	2.22 ^{cde}	2.84 ^{bcd}	3.01 ^{cdef}
Day 5	3.12 ^{abcd}	3.08 ^{bcde}	3.05 ^{bcd}	2.55 ^{cdef}
Day 6	5.37 ^{abc}	4.13 ^{abcd}	4.25 ^{abcd}	4.41 ^{bcd}
Day 7	5.80 ^{ab}	5.12 ^{ab}	6.85 ^{ab}	6.62 ^{ab}
Day 8	6.05 ^a	5.83 ^a	7.68 ^a	7.72 ^a
Day 9	5.18 ^{abc}	4.60 ^{abc}	6.23 ^{abc}	4.59 ^{bc}
Day 10	3.31 ^{abcd}	3.87 ^{abcd}	4.31 ^{abcd}	3.94 ^{cde}
Day 11	2.64 ^{bcd}	2.85 ^{bcde}	2.79 ^{bcd}	2.70 ^{cdef}
Day 12	2.44 ^{bcd}	2.28 ^{cde}	1.85 ^{cd}	2.03 ^{def}
SEM (<i>n</i> = 3)	0.569	0.356	0.321	0.350
<i>P</i> value	< 0.001	< 0.001	< 0.001	< 0.001
Linear	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	< 0.001	< 0.001	< 0.001	< 0.001

SEM standard error of the mean (three independent replicates)
 a, b, c, d, e, f Means in the same column with different superscripts are significantly different (*P* < 0.05)

and Ardhaoui cultivars (tenfold), followed by Souihli (eightfold) and Rihane (fivefold). Thereafter, from day 8 to day 12 of germination, phytase activity of all cultivars followed a steady and significant decrease ($P < 0.05$).

Discussion

Differences among barley cultivars in total phosphorus, phytate and phytase activity in the grains

Phytase, the enzyme responsible for the dephosphorylation of phytic acid, has been detected in a number of ungerminated cereals (Eeckhout and De Paep 1994; Bergman et al. 2000; Viveros et al. 2000). The endogenous phytase activity in cereal grains has the potential to contribute to the gastrointestinal hydrolysis of phytate in non-ruminant animals (Azeke et al. 2011). In our study, significant differences ($P < 0.05$) among barley cultivars were observed in the phytase activity (0.6–1.6 U/g). Dai et al. (2010) reported a range of phytase activity (0.22–0.99 U/g) in different barley cultivars. However, higher phytase activity values (2.3 U/g) for barley grains were observed by Steiner et al. (2007). In our study, phytase activity varied within a narrow range. Similarly, Azeke et al. (2011) pointed out a low phytase activity ranging between 0.41 and 0.67 U/g for rice, maize, millet, sorghum and wheat grains. In contrast, Steiner et al. (2007) reported large differences (0.4–6.0 U/g) among oats, barley, triticale, rye and wheat. The discrepancies among studies observed may be due to the cereal species, cultivar and analytical procedures used for the determination of phytase activity. Phytase activities in cereals based on extraction methods are considerably lower than those obtained by direct incubation methods (Steiner et al. 2007). The incomplete extraction of plant phytase might be attributed to the proteolytic degradation and a partial association of the enzymes with membrane structures (Greiner and Egli 2003). Additional factors such as differences in the composition of the cell walls, interaction of phytase with morphological grain fractions and differences in the location of the enzymes within the grains could be responsible for the incomplete and different extraction rates of plant phytases (Steiner et al. 2007).

Phytic acid (phytate) is the phosphorus storage compound in plants, where it accounts for 60%–90% of the seed phosphorus (Lott et al. 2000). Cereals contain lower phytate contents than legumes (Azeke et al. 2011). In our study, phytate content in ungerminated barley grain varied from 4.2 (Souihli) to 4.7 mg/g (Rihane). These values are within the range reported by Dai et al. (2010) (3.4–9.2 mg phytate/g in grains of wild and cultivated Tibetan barleys). However, other studies (Steiner et al. 2007) have reported a lower value (2.6–2.8 mg/g) for barley. Such differences are expected since phytate content is formed during maturation of plant seeds and in dormant seeds (Loewus 2001). Therefore, differences in phytate contents of barley grains could be associated to different factors of variation such as differences in stage of maturity, harvest year, genetics, cultivar, climatic conditions, location, soil type and fertiliser application (Steiner et al. 2007; Wu et al. 2009). Phytic phosphorus in cereal grains is unavailable to non-ruminant animals due their lack of endogenous (digestive) phytases. During seed development, phytate is deposited as mixed phytate salts of potassium, magnesium, calcium, zinc and iron. Hence, phytic acid may also reduce the bioavailability of other essential dietary nutrients such as minerals, proteins and amino acids (Wu et al. 2009; Vashishth et al. 2017).

The total phosphorus contents differed among cultivars ranging between 3.2 (Arbi) and 5.0 mg/g DM (Rihane). This variation is consistent with values reported in the literature for different cereal grains. Azeke et al. (2011) observed a range between 3.3 mg/g for wheat and 4.3 mg/g for rice. Nour et al. (2010) reported a narrow range of phosphorus contents (4.4–4.6 mg/g DM) in three sorghum cultivars. Differences in phosphorus content of grains may be influenced by the phosphorus status of soil, fertilizer, and the root uptake of phosphorus. Considering total phosphorus and phytate content in seeds, it was confirmed that up to 90% of total phosphorus is bound as phytate phosphorus in cereals and legumes (Wu et al. 2009).

Effects of germination on total phosphorus, phytate and phytase activity in barley grains

Germination is a process commonly used in legumes and cereals to increase their palatability and nutritional value (Peer and Leeson 1985). Most usually,

hydroponic barley fodder is harvested after 8 days of germination (Peer and Leeson 1985). In our study, germination was extended up to 12 days to investigate if further valuable changes could occur in phosphorus and phytate contents in sprouted barley grain. Phosphorus content increased steadily during germination in grains of all the four barley cultivars, although differences among days were not always significant. A similar trend of phosphorus contents was observed by Azeke et al. (2011) who reported that after germinating for 10 days, phosphorus contents of many cereal grains (rice, maize, millet, sorghum and wheat) increased by 8–20%. These authors suggested that the increase in phosphorus would result initially from changes in dry matter due to the sprouting process (Azeke et al. 2011) and afterwards from phosphate translocation, which plays a significant role in metabolism during grain germination. Nour et al. (2010) reported a phosphorus increase of 19–36% for three varieties of sorghum after 3 days of germination. This increase may be related to the release of P bound to phytate during germination due to the increased phytase activity. The different values found in the literature on the content of phosphorus of cereals are related to cultivars, germination process, time of harvest and processing conditions (pH, temperature).

During grain germination some antinutritional compounds can be inactivated or broken down, such as phytate through the activation of endogenous grain phytase (Gupta et al. 2015). In our study, germination resulted in increased phytase activity of all samples screened. This could be expected since phytase in dry seeds is inactive until water is absorbed. During germination, phytase is progressively activated hydrolysing gradually more phytate and releasing phosphorus to supply the mineral to the sprouting plant. This process has been described in many other cereals (Bartnik and Szafrńska 1987; Sung et al. 2005; Azeke et al. 2011). Azeke et al. (2011) reported a significant increase (3–16-fold) of phytase activity in different cereal-germinating grains. In our study, the maximum peak value for phytase activity was reached the 8th day of germination, and the increase over the basal value before germination was more pronounced for Arbi and Ardhaoui (tenfold), followed by Souihli (eightfold) and Rihane (fivefold). The magnitude of this increase will depend on the cereal grain (species, variety and cultivar), and drying and storage conditions. Azeke et al. (2011) reported a maximum 16-fold increase in

phytase activity for rice grains after 10 days of germination. Grains used in our study were collected from different farms located in different regions of Tunisia (north-west; centre and south). In addition, the conditions of germination, such as temperature, affect the intrinsic phytase activity (Sung et al. 2005), so that germinating barley at 25 °C resulted in higher phytase activity than at 20 °C or 15 °C (Sung et al. 2005). Germination in our study was carried out in a hydroponic unit production where average room temperature was 21 °C.

Some lag phase (5–6 days) was observed in all the samples before any significant increase in phytase activity was recorded. Other authors have reported faster activation of phytase (increased after 24 h) when germinating barley (Sung et al. 2005), maize, sorghum or wheat (Azeke et al. 2011). It is likely that during germination the expression of phytase is delayed. After 8 days, phytase activity declined slightly for all the cultivars. A similar quadratic trend was reported for other cereals (Sung et al. 2005; Azeke et al. 2011). This could be due to the degradation of the enzyme by proteases or to a feedback inhibition caused by the phosphate accumulated (Sung et al. 2005).

Phytase activity was increased during germination causing the hydrolysis of phytate that was therefore consistently reduced in the grains of all the screened barley cultivars, up to a minimum phytate content observed after 9–12 days. During the initial lag phase for phytase activity (first 4–5 days of germination), non-significant changes in phytate were observed. In general, the kinetics of phytate degradation were similar to those described by Blaabjerg et al. (2012). During germination endogenous phytase breaks down phytate (Kumar et al. 2010; Gupta et al. 2015) releasing inorganic phosphate that can be used by the growing plant. High moisture storage of barley also increases P solubility (Ton Nu et al. 2015). The reduction of phytate during germination will enhance the availability of phosphorus and thus increase the palatability and nutritional value of the grains (Torres-Pitarch et al. 2017). Azeke et al. (2011) reported decreases of 81–88% in phytate contents of rice, maize, millet, sorghum and wheat grains during 10 days of germination with the minimum value being recorded after 8–10 days of germination. Such differences could be attributed to the inter- and intra-species variability in their intrinsic phytate-degrading activity

and capacity to dephosphorylate phytate, and the properties of the enzymes (protein stability) and optimal conditions (pH and temperature) for phytate degradation (Gupta et al. 2015). Likewise, the light during germination has been found to be an important factor in the reduction of phytate, and in our study, germination was under a photoperiod of 16 h.

By releasing bound phosphorus in feed ingredients of vegetable origin, phytase makes more phosphorus available to the animal and contributes to improve phosphorus utilisation and to reduce phosphorus excretion and its impact on environmental pollution.

Conclusions

Barley cultivars differ in the phytase activity, phytate and phosphorus content in grain. Based on their phytase activity and phosphorus contents before germination, barley cultivars are ranked as follows: Rihane > Ardhaoui > Souihli > Arbi. Intrinsic phytase activity in the grain is increased during germination, reaching a maximum peak after 8 days of germination. The increase in phytase activity is accompanied by a significant decrease (of up to 97%) in the phytate content, releasing inorganic phosphorus. Thus, germination of barley grains can contribute to improve phosphorus availability and utilisation by the animal. Differences among cultivars are diminished after germination, so that no differences in phosphorus, phytate or phytase are observed after 12 days of sprouting. Further research is warranted to highlight the effect of germination on other nutritional attributes of hydroponic sprouted barley and its suitability as a feedstuff for farm animals.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

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