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Research Article

Isolation and Study of the Nutritional Variability of Peripheral Layers of Barley Grains During Development

Lahouaria Labga¹, Samira Meziani^{1*}, Haoud Khadidja¹, Saidani Souad¹, Tasleem Tahir Aysha²

¹Department of Biology, Faculty of Life and Nature Sciences, Djillali Liabes University, Laboratory of Biotoxicology, 12 Rue Oulhaci Mokhtar, Sidi Bel Abbes, Algeria

²Department of Biosciences, Comsats University, Chak Shahzad Campus, Parkistan Road, Islamabad 45550

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Abstract

Barley (*Hordeum vulgare* L.) is one of the grain cereals widely cultivated for human and animal consumption. The objective of this study was to determine the components of the peripheral layer (PL) of 4 stages of development of barley grain (*Hordeum vulgare* L.) (Saida 183) by manual isolation, as follows, Mg, Mn, and Ca content shows a progressive decrease through the development cycle from (1.888; 0.0645; 8.345) mg/l DM in ripening stage respectively, while Fe and Zn are increased in maturation stage (0.663; 0.360) mg/l of DM. The K content presented an important increase from 7.7 mg/ml in the maturity with a value of 30 mg/ml. The average values of crude fiber content varied from 8.4 to 16.6. These values increase significantly with the development of the barley in different stages. The phytic acid content in the fourth stage (maturation) was 8.96 ± 0.56 mg/g DM, and the proline content was very low in the first stage with a value of (2.474 ± 0.069) mg/ml DM and in the maturation stage the value was high with a value of (7.148 ± 0.248) mg/ml DM. The optical observation of the different microstructures and progressive changes that were undergone from the first filling stage of the mid-milk stage, soft dough stage, physiological stage, and ripening stage were studied. By optical observation, there is the formation of the first protective envelope of the grain, so all the tissues of the external pericarp that is grouped with the testa. The aleurone layer was structured in three rows with an important condensation of the cells of the albumen sub-structures that are involved in the storage.

Keywords: *Hordeum vulgare* L.; peripheral layer; histological; grain development.

Introduction

In the region called the Fertile Crescent (now Iraq and Iran), the geographical origin of barley (*Hordeum vulgare* L.) can be traced back to several millennia before J-C. The world production of maize, wheat, rice, and barley reached more than 1148, 765, 755, and 158 MT of cereal respectively [1]. Foods that have a cereal base are the main source of energy, protein, B vitamins, minerals and food for the current world population [2]. The use of grains has evolved considerably, and it is divided among human feed,

animal feed and non-food applications [3]. It is through structure and composition that the hardness of grains can be determined [4] and therefore, gives a capacity to the processing of grains in industrial food processes such as food manufacturing. There are two main types of barley, two-row and six-row of ears [5]. The main use of barley is for animal feed, in the form of barley meal or fodder; the barley is also used as a malting and brewing grain in the manufacture of beer and whisky [6]. In Algeria, the most important cereal is barley (*H. vulgare* L.). According to a survey of Polish households the cereal consumption provides 20-30 % of the average intake of protein, thiamine, phosphorus and zinc, and 10-20 % of many nutrients such as sodium, calcium, potassium, unsaturated fatty acids, niacin, riboflavin and vitamin B6 [7]. Barley grain

* Corresponding author: Meziani Samira, Email:

meziani_samira@yahoo.fr

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has a complex material contains embryo which is responsible for vital activities of the grain. Dead cells filled with starch granules have a reserve of nutrient source (endosperm), testa and pericarp is semi-permeable membrane consisting of tubular cells, aleurone layer is 'a peripheral layer of cells contains proteins, lipids, vitamins, phytic acid and cellulose [8].

The most peripheral tissues (pericarp, testa) are little or not hydrolyzed by hydrolytic enzymes such as xylanases, unlike the aleurone layer [9]. The localization of substances is often performed by light microscopy to characterize the structure of cereal grains; on the other hand, the localization of other substances is most often performed using selective dyes, which reveal the distribution, for example: starch and polysaccharides [10]. For cereal grains there are no specified dyes for its staining, its localization requires immunolabeling or staining by Xylene Probe, which are more advanced and effective methods than direct staining [11]. Barley grains are mainly composed of carbohydrates (60 to 65% of the dry weight of barley) which are located mainly at the endosperm and 25% of amylose of linear structure, with bonds of type α (1-4) [12]. The protein content of barley grains is in the order of 10 to 17% [13, 14] but there are also lipids in small amounts (2-3%) [8,3]. Barley is characterized by the abundance of glutamic acid and proline, major characteristics of prolamins. It is a good source of minerals (such as Potassium, Phosphorus, and Magnesium). These minerals from grains can contribute significantly to the intake in the feed ratio [3]. The main objective of this study illustrated the structural distribution of the components in the cells of the endosperm and aleurone of the developing barley, based on the optical microscopic observation; analyze the gross chemical composition, and mineral composition.

Material and Methods

Biological material

The plant material used in this experiment is a line of barley (*Hordeum vulgare* L.) of the variety with Saida 183 in full development and the grains were sown on 07/11/2019. Saida 183 is local barley with 6 rows, semi-late, with medium and hollow straw, medium tillering, good productivity [15, 16]. The experiment was carried out at the level of the national institute of large-scale crops (ITGC) of Sidi Bel Abbes, Algeria. The peripheral layers are obtained after manually separating the bran from the grain.

Table 1. Agronomic, morphological, cultural and Qualitative characteristics of the tested varieties [16]

Saïda 183	Characteristics
Origin	Algeria (local)
Morphological characteristic	Morphological characteristic
- Characteristic of the ear	Very loose
- Color of the spike	White
Height of the plant at maturity	90cm
Cultural characteristic	Cultural characteristic
Alternative	Fall
- Vegetative cycle	Semi-early
- Tallage	Medium
- Cultural characteristic	Cultural characteristic
To cold	Resistant
To lodging	Sensitive
To drought	Resistant
Qualitative characteristic	High 1,4%
- Thousand kernel weight (TKW)	

Plant sample preparation

Grain detection was done with magnifying binocular magnifier (ZEISS, Germany) according to each stage of development, the endosperm powder obtained is not considered in this study (they will be the subject of further work). The peripheral layers of each phase as well as the endosperm powder were stored at (- 80 °C) until analysis.

The samples were prepared and observed by the light microscopy method according to Nougarede (1969) [17], we used the grains of each phase of development of barley in two extractions, then stored in 70% ethanol at 4 °C until the day of use, having followed the method that requires several steps to allow good observation by light microscopy. The fixation was made by formaldehyde to 36.99% that was used in different water baths after using melted kerosene to 56 °C until solidification, after passing in microtome for a very fine section of 4 to 5 μ m, passing in dewaxing with toluene and dehydration by alcohol baths of decreasing degree (ethanol 100, 95, 70), finalized by a distilled water bath. The staining with toluidine blue requires important steps to realize the mounting and the observation by optical microscopy.

Extraction and determination of phytate

The phytic acid content was determined by the colorimetric method with a slight modification, using sulfosalicylic acid with a colored complex formation [18, 19]. The extraction was performed by using 150 mg of each sample which was mixed with 5 ml of 2.4% HCL in each tube, left in the dark for 2 h under orbital shaking every 10 min for

15 sec using a vortex, after the solution was centrifuged 30 min at 6000 rpm at 20 ° C. At the end of 2 h a part of supernatant of 200 µl was diluted 25 times in the test tubes with 4,8 ml of distilled water by stirring the tubes during 5 seconds. By taking 750 µl of dilution 1/25 (the standard range) were mixed with 250 µl of Wade's rose solution (Wade's rose, HCL 2.4%) mixed vigorously in a vortex mixer and centrifuged at 10000 rpm at 20 °C during 20 min, the absorbance was read at 500 nm on a spectrophotometer and the results were expressed in mg/g for the quantity of phytic acid, all the tests were carried out in triplicate.

Determination of the minerals contents

The Na, K, Mg, Ca, Zn, Mn, and Fe content was assayed according to Rodier (2005) [20]. Mineralization that prepared from a stock solution of 100 mg/l for each phase of development of successive barley grain peripheral layer with 37% hydrochloric acid, nitric acid 69% and completed with distilled water. In 'to weigh 0,5 mg of each sample prepared for testing after drying and grinding into a very fine powder in a 50 ml beaker, to avoid any contamination the glass equipment was cleaned with distilled water and dried in an oven after each use. A standar 0, 45 µm filter paper and an adjustable 1 ml micropipette were used; aqueous solutions of reagents and standards were prepared using ultrapure water. The concentrations of heavy metals in the peripheral layers of barley were determined by atomic absorption spectrophotometry (AAS) with flame (AA-7000 SHIMADZU), the quantification of trace metals was performed with flame spectrometry (Jenway).

Determination of crude fibers

The determination of crude fiber was done according to Ween AOAC (1993) [21] in samples dried in an oven at 105-110°C for a few hours. A 1 g portion of the sample was ground with a Moulinex to obtain a fine powder that was collected by sieving to 1mm, then was placed in a glass crucible with glass frit bottom (ref; 11:1140, VELP SCIENTIFICA s.r.l, Via Stazione, 16USMATE (MI) Italy) 1,25 % sulfuric acid to the 150ml mark and 3-5 drops of N- octanol as an anti-foaming agent were added. The samples were boiled for 30 minutes, the sulfuric acid was drained off, and the solid samples were washed three times with 30 mL of hot distilled water. After the last wash, 150 ml of preheated 1.25% potassium hydroxide (KOH) and 3-5 drops of the antifoaming agent were added. The filtrates were boiled for 30 minutes. The contents of the crucible were washed three times with 25 ml of acetone.

Dry weight was determined after drying in an oven at 105°C for one hour and cooling in a desiccator. The ash content was determined after combustion for 4 h at 550 °C in a high temperature furnace after cooling for 1 h in a desiccator and the residue was weighed, the gross content is calculated as follows:

$$\% \text{ in fiber} = [(F1-F2)\backslash F0]] \times 100$$

where:

F0: weight of the dry sample

F1: weight of the crucible with residue

F2: weight of crucible with residue near ash

Determination of proline

Among the twenty main amino acids that enter the constitution of proteins is the pyrrolydine 2-carboxylic acid or proline. The proline extraction was carried out according to the method described by [22] simplified and developed by [23]. 0,1 g of dried and grounded plant material was taken and placed in test tubes, to which 2 ml of 40% methanol was added. The samples were heated in a water bath at 85 °C, for 1 hour and after cooling, 1ml of each extract was taken. To each sample tube 1 ml of acetic acid and 1 ml of reagent prepared from 120 ml distilled water, 300 ml acetic acid, 80 ml orthophosphoric acid and 25 mg ninhydrin were added. The whole was boiled in a water bath at 100 °C for 30 minutes, after cooling; 5 ml of toluene was added. After vigorous stirring, two phases were observed, the upper phase containing proline was removed and the lower aqueous phase without proline was eliminated, the phase containing proline was dehydrated by adding sodium sulfate (Na₂SO₄). The final, colored solution was determined was determined spectrofotometrically at 538 nm; the values obtained were converted into proline content, the standard curve was established from a series of solutions of known proline concentration.

Statistical analysis

To compare the relationship between micronutrients in developing barley grains, all the results were obtained in three replicates, and they are expressed as mean ± standard deviation (means plus or minus standard deviation as well as representations) and all the parameters were submitted to an analysis of variance (one factor) with the statistical software EXCEL 2010.

Results and Discussion

The microstructure of the developing barley grains was revealed by optical microscopy (Figure 1) using toluidine blue as a specific dye. Observing the cross-section of these four barley grain stages

(Saida 183), the major part of the barley grain was constituted from the outside to the inside. Different tissues were distinguished in the whole of the external layers, first the envelope or testa surrounded by the internal and external pericarp, it constitutes the first protective envelope of the

grain then the layer of aleurone with two to three rows of cells. In barley grains, the outer layers were separated into two composite layers, the first layer consisted of the whole pericarp and testa. The residues of the unicellular epidermis and aleurone were isolated as the second layer.

Figure 1. Cross section of barley grain at each stage of development observed under an optical microscope (magnification x). (The soft barley grain allowed obtaining a composite layer of pericarp, testa, aleurone and endosperm: the sections are stained with toluidine blue). Stage 1: semi milky; stage 2: Soft dough; Stage 3: Physiological maturation; Stage 4: Maturation. Structural composition: PL: Peripheral Layer; AL: Aleurone Layer; ALC: Aleurone Layer Cells; SE: Starchy Endosperm

At the first stage (mid-milk), it was observed the absence of the envelope of the fruit (pericarp), and the presence of testa which is a little separated from the hyaline band, entered in the interior there is the formation of the layer aleurone and of some cells of albumen. For the second stage (soft paste) there is the beginning of the formation of the external envelope (pericarp) that is attached to the testa with the formation of a layer of aleurone and cells of albumen. Arriving at the third stage (physiological maturation) where there is the appearance of the external envelope, the testa, the layer of aleurone and the cells of the albumen are well condensed. In the fourth stage (maturation) we observed the formation of all the tissues of the seed, the external pericarp which is

the first protective envelope of the grain, which is grouped with the testa. The aleurone layer was presented in three rows and an important condensation of the cells of the albumen.

Slight differences were observed in barley grain samples according to [24] barley tissues did not separate in the same way as wheat, even though the dissection procedure was similar. Although the exact location of the outer pericarp, which was crushed during grain development [25], was not easy to detect in the dissected layers in barley cultivars, its presence or absence could not explain this low amount. It could rather be related to the type of barley (a2rangs), since all these characteristics tend to reduce the proportion in the barley grain [26]. The relative proportion of the

aleurone layer in the whole outer layer was higher in barley grain than wheat grain where the value was about 45% [24, 26]. This could be related to the relative thinness of the other tissues of the maternal barley grain, the amount of aleurone layer in the barley grain being comparable to that of wheat grain [24].

Phytic acid

The results of phytate content show that all stages of development of barley grains, which contain phytate have varying amounts from one stage to

another in increasing order ranging from 5.22 to 8.96 mg/g. The phytate content of the peripheral layers of the four stages of developing barley grain was determined. Stage one (mid-milk) has the lowest phytate content with 5.22 ± 0.267 mg/g DM, this value starts to increase in the second phase (soft dough) with 6.22 ± 0.166 mg/g DM until stage three (physiological ripening) with 7.57 ± 0.120 mg/g DM, stage four (ripening) the phytate content has 8.96 ± 0.56 mg/g DM. Figure 2 shows the phytate content of barley grains Saida183 in development.

Figure 2. Phytic acid content of barley grain (Saida 183) in full development. Stage 1: semi milky; stage 2: Soft dough; Stage 3: Physiological maturation; Stage 4: Maturation.

Phytic acid is a molecule of plant origin that plays the main role in storing phosphorus in plant tissues. Phytic acid synthesis is procured in developing grains [27,28], it plays a role in the vegetative cycle to prevent very early germination and is a natural defense against grain predators, releasing large amounts of phosphorus for energy production [29]. Phytic acid is mainly stored in the aleurone layer (about 80%) or germ [30,31]. According to Schlemmer et al. (2009) [31] the phytic acid content between (3.8 and 11.6) mg/g DM is comparable to that obtained by our variety, these values are of the same order of magnitude as those observed for barley [32] or wheat [33]. The study from [34, 35] contacted the values generally ranging from 1.42 to 20.70 mg/g. The analysis of variance shows that the phases in the development of barley grain from milky phase to maturity have significant effects ($p < 0.05$) on phytic acid content. These values have deprived to the half, we explain these results by the endogenous phytase activity synthesized during

the germination period which continues the bioavailability during the whole vegetative cycle, but hydrolysis develops slowly and the difference of reduction differs from one species to another of cereal rye>wheat>barley>corn [36, 37]. Some researchers stated that the phytase activity of cereals in the native state is influenced by cultivar, climatic conditions like heat and humidity. This phytase activity has recorded a high content for rye grains and the lowest is proven for corn grain [38, 39]

Mineral composition

The measured mineral content of the peripheral layers of the different developmental stages of barley grains (Saida183) and the differences in the concentrations of the metallic trace elements was calculated relative to the dry weight of the extract of the peripheral layer and are expressed in mg/ml per 100 g of DM for the Na and K and in mg/l per 1000 g (ppm) for Mn, Fe, Zn, Ca and Mg are shown in Table 2.

Table2. Mineral content of barley grain (Saida 183) in full development in mg/ml (Na and K) and mg/l (Mn, Fe, Zn, Ca and Mg)

Minerals	Stage 1	Stage 2	Stage 3	Stage 4
K	7.7 ± 0.424	10.5 ± 0.707	13.9 ± 0.141	30 ± 1.414
Na	18.75 ± 0.353	22 ± 0.141	24.5 ± 0.707	10.6 ± 1.272
Mn	0.0645 ± 0.0006	0.023 ± 0.0019	0.022 ± 0.005	0.021 ± 0.001
Fe	0.550 ± 0.028	0.342 ± 0.003	0.458 ± 0.004	0.663 ± 0.002
Zn	0.691 ± 0.003	0.037 ± 0.001	0.042 ± 0.005	0.360 ± 0.004
Ca	8.345 ± 0.002	2.763 ± 0.006	2.717 ± 0.002	1.355 ± 0.007
Mg	1.888 ± 0.001	1.737 ± 0.016	1.728 ± 0.006	1.626 ± 0.017

The analysis of variance revealed that the contents of (Na), (Mn, Fe, and Mg), (0.00066) mg/ml, (0.0014; 0.0001; 0.00022) mg/l of DM are respectively significant, while the contents of (K),

(Ca, and Zn), (3.7912) mg/ml, (7.287; 2.2099) mg/l of DM, there is not significant difference at the 5 % risk of error across the different phases of development (Figure 3).

Figure3. The mineral content of developing barley grain (Saida 183) in different stages. Stage 1: semi milky; stage 2: Soft dough; Stage 3: Physiological maturation; Stage 4: Maturation. The results for the different metals are significantly different

The content of Mg, Mn and Ca presents a progressive decrease through the development cycle from (1.888; 0.0645; 8.345) mg/l DM in mid-milk stage to (1.626; 0.021; 1.355) mg/l DM at the maturation stage respectively, while Fe and Zn in the midmilk stage presents a value of (0.550; 0.691) mg/l DM after it starts to decrease until the maturation stage which increased to (0.663; 0.360) mg/l DM. For Na in phase 1 presents a value of (18.75) mg/ml or it begins to increase in phase 2 and phase 3 with values of (22 and 24.5) mg/ml respectively, arrived at phase 4 it decreased until (10.6) mg/ml. The potassium (K) presents an important increase from (7.7) mg/ml at phase 1 until phase 4 with a value of 30 mg/ml. Natural metal elements are essential substances for our organism which are indispensable for the

proper functioning of the organism. They are present in very small quantities in the form of traces in non-excessive concentrations. Minerals are present in small quantities in barley grain and the main ones are potassium, manganese and copper often associated with salts (phosphates, chlorides or sulphate) [40]. The peripheral layers include a living cell tissue rich in minerals providing about 40% of the minerals of the grain, phosphorus (phytate), potassium, magnesium, zinc and iron [41- 44]. A decrease in certain metals such as the results found in this study more particularly at the level of Mg, Mn and Ca and also on the value of Zinc for different stages, indicates that there is a probability that phytic acid which one of the primary causes of mineral decrease according to a study by [45,46] have

also shown that the minerals in barley grain are found in sufficient concentration to meet the nutrient requirement of the seedling. the most abundant in barley grains [47], metals like (Fe, Zn, Ca, Na, Mn, Mg) plays an important role in the biological system [48]. A great variability in mineral content from one sample of the peripheral layer to another has been highlighted in the literature, these differences are under the influence of environmental factors that characterize the grain culture and the varietal effect [49,50]. Magnesium is also localized in the bran and a aleurone layer which is required for photosynthesis in the peripheral layers during the first 30 days of grain formation and accumulation and a mineral deficiency of Mg in cereals reduces photosynthesis of several metabolic functions which leads to lower grain weight and weaker defenses against stress [51]. In barley grains, the peripheral layer is the primary storage site for phosphate, magnesium, potassium, sodium, and calcium, accumulating more than 70% (97% for magnesium) of the endosperm reserves of these minerals [52]. Iron plays an important role in the production of hemoglobin and red globin, insufficient iron intake results in anemia [53]. Zinc promotes normal growth and development during pregnancy, childhood and adolescence, affects the immune system, wound healing, and the senses of taste and smell [54]. Cereals are used as a staple food in developing countries, macro-micronutrients are a good source for human nutrition or the human body is unable to produce them.

The content of crude fiber and proline

The crude fiber and proline contents of the different peripheral layers of developing barley grains (Saida 183) are presented in Table 3 and Figures 4a and 4b. The average values of crude fiber content varied from 8.4 to 16.6, these values increase significantly with the development of barley of different stages, which are presented by Figures 4.

According to our results has the phase of maturity is expressed at 16.6% this content is inferior to that [55] is estimated at 27.33% of DM, but it is close to the results reported by [56] which range from 18% to 24%. Comparing the fiber content in other cereal species (millet, wheat, barley) [57] note that barley is the richest in fiber; its content is about 26% of DM. According to [58] that the peripheral tissues contain the major part of fiber and minerals, in fact, these tissues represent

approximately 14% of the grain in mass contain 75% of the fibers present in the grain according to the histological origin, the structure and properties of the fibers vary. The grain is much richer in raw cellulose for barley, if the rate is more than 5%, these elements form cell walls, and their energy inputs are relatively low because of their low digestibility. The wheat bran is richer in insoluble fiber than barley, on the other hand, it has been found that people who follow cereal-based diets such as wheat or barley have a beneficial physiological effect on the postprandial insulin response in obese humans who are at risk of insulin resistance [59, 60]. Products with new functional and nutritional properties are a prerequisite for better acceptance of barley, e.g. as high dietary fiber products [61, 62]. According to Biel et al. (2015) [63], barley grain dietary fiber (dietary fiber, TDF) contains 265 g in Kg DM, classified into soluble and insoluble fibers, based on their ability to dissolve in water, their ease of digestion by floral bacteria in the gut, and other chemical properties such as water retention.

Proline is a multifunctional amino acid; it is implicated in some processes of plant development such as germination and reproduction. According to the results obtained in the four stages, the accumulation of proline revealed a significantly difference at the level of ($p < 1\%$) which is presented in Figure 5. The proline content was very low in the first stage with a value of (2.474 ± 0.069) mg/ml of DM, through the two stages of development the proline content was (4.624 ± 0.307) mg/ml of DM for stage two, was (4.8 ± 0.19) mg/ml of DM for the third stage and in the maturation stage the value of proline was important with a value of (7.148 ± 0.248) mg/ml of DM. Our results indicate that proline content increases significantly and highly significantly with temperature and plays a role in achieving ionic and osmotic equilibrium in the different developmental stages of barley grain (Saida 183). Proline accumulation is an important process for developing crop tolerance under adverse environmental conditions [67] and can be considered as an indicator of crop defense mechanism in response to multiple stresses or [68] concluded that proline accumulation increases under stress and optimizes grain yield, and [69] indicated that heat and water stress stimulated the concentration of proline and Osmo protector significantly. The study for [67] reported an unattainable concentration of proline under stress conditions in resistance genotypes.

Table 3. Dietary fiber content expressed as percentage of dry matter (DM) and proline in mg/ml

Stages	Stage1	Stage 2	Stage 3	Stage 4
Crude fiber %	8.4	10.4	12.2	16.6
The level of proline (mg/ml)	2.474 ± 0.069	4.624 ± 0.307	4.8 ± 0.19	7.148 ± 0.248

Stage 1: semi milky; stage 2: Soft dough; Stage 3: Physiological maturation; Stage 4: Maturation.

Figure 4. The Fiber content and the proline content of the four of developing barley grain (Saida 183). Stage 1: semi milky; stage 2: Soft dough; Stage 3: Physiological maturation; Stage 4: Maturation.

Conclusions

For thousands of years, mankind has been feeding on cereal grains, which are considered a good source of food. The microstructure of the developing barley grain presents that it is a good source of micronutrients especially fiber, proline, and minerals with its constitution in three rows of aleurone layer. According to the present results, the peripheral layers of the developing barley grain is an important source of minerals, fiber, phytate, and proline at different stages of development, this can be related to the environmental conditions, the richness of the soil in heavy metals, the variety of barley used, and the extraction solvent. As a consequence, barley bran could be a good option to be introduced in daily food.

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