

The nutritional quality of North African barley genotypes

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ABSTRACT: Barley has interesting characteristics for human health such as fiber, antioxidants, vitamins, minerals, proteins and especially β -glucans, which make it one of the best choices of whole grain. Because of its importance for human nutrition, β -glucan and protein contents were determining factors in the grains quality. The aim of this study was to analyze the nutritional grain quality of North African barley collection. Grain color, protein and β -glucan contents, starch and mineral elements contents were determined in sixteen barley genotypes. Obtained results showed that the average of protein content, varied from 10,76 to 14,13%, the β -glucan content varied from 2,18 to 4,75%, starch content varied from 52 to 60% and mineral elements varied from 1.82 to 2.52. The richest genotypes in terms of protein and β -glucan contents were the naked barley genotypes (V10 and V23) from Tunisia and Egypt respectively. In opposite, these two genotypes were the poorest in mineral elements comparatively to the remainder ones. Although some hulled barley genotypes (V7, V8) showed high levels of protein, the naked barley seeds have, in addition, an appreciable color similar to durum wheat allowing them to be easily mixed with wheat in the process of making pasta.

KEYWORDS: Barley, β -glucans, nutritional quality, proteins, starch.

1 INTRODUCTION

Barley may be classified into two-row, four-row or six-row varieties. There is either malting barley or barley for human and animal consumption. It may be hulled or naked grain [1]. Despite its number of row or its status (naked or hulled), it was demonstrated that barley endosperm contributes to grain hardness, to protein content and to starch and β -glucan contents [2]. According to these authors, barley contains high levels of β -glucans, which constitute approximately 75% of the endosperm cell walls, with 20% arabinoxylans and proteins.

It has been also shown by several authors that barley grain usually contains 2 to 10% β -glucan ([3], [4], [1]). However, further research conducted on barley mutants or on naked and waxy barley lines, have shown higher β -glucan values exceeding the 10% ([5], [4]).

A growing interest for β -glucan component was investigated in other species (oat). Redaelli et al. (2013)[6] have demonstrated that the total β -glucan content in oat is ranged from 2.85 to 6.77% (DM) and their solubility were significantly influenced by both genotype and growing environment.

Due to barley β -glucans content, significant interest in health has been reported by several researchers ([7], [8], [9]). We can list the cholesterol and glucose reduction, the obesity reduction, as well as the heart disease and the type 2 diabetes.

The grain color was shown to be linked to nutritional quality of barley seeds [10]. Indeed, this color can vary from light yellow to purple, blue and black, which is mainly caused by the anthocyanins level. Colored types are also receiving more attention, these days, because of their high antioxidant content. Colored barley varieties (purple or black), have properties that can make them an effective treatment for a number of skin problems [11]. These varieties are rich in anti-acne compounds that could offer benefits for people with damaged skin or acne, hyper pigmentation or age spots [11]. The high levels of anthocyanins found in purple or black barley can also help slow skin aging and prevent wrinkles.

Grain color is also an important indicator of food industrial quality. In fact, some naked barley was sometimes incorporated into durum wheat flour to make pasta associating its therapeutic effect and its appreciable color. However, the majority of currently available barley gives dark products, non appreciable by the consumer. On the other hand, naked barley can be mixed until 20% of durum wheat without any darkening or crumbling of the formed pasta [12].

The studies conducted by some authors ([13], [14]) on barley showed that protein levels vary widely depending on genotypes and climatic conditions, mainly rainfall, during the growing season. These authors have reported that barley grain protein content varies between 12 and 16%, but some exceptional genotypes exceed the 16% content. They have also indicated that protein grain content is an important indicator of barley quality, regardless of its use. Besides the varietal effect and climatic conditions during the growing season, altitude also plays an important role in the genotype protein content. Similarly, [15] showed a negative correlation between grain yield and protein content; which means that a rainy year produces high grain yield but with low protein contents and vice versa. They explained this negative correlation by the dilution of nitrogen content into the grain carbon-rich molecules. So what would happen if barley grown in an environment similar to North Africa, experiencing repeated periods of drought?

The objective of this study is to evaluate the nutritional quality of North African barley collection grown in rain fed conditions and to identify the genotype (s) that could be used as brood stock in a breeding program for nutritional quality improvement.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

In the frame of previous research project (New Partnership for African Development=NEPAD), thirty one (31) North African barley genotypes were characterized on the molecular level and showed high genetic diversity allowing to cluster them according to their eco-geographical origin or according to the caryopsis character (hulled or naked caryopsis) ([16]). Among these genotypes, sixteen ones (16) were selected on the base of greater genetic distance or on the base of contrasting characters (early/late; hulled/hulless; erected/prostrate; productive/less productive) and used for multiple evaluation.

Five genotypes from Tunisia [Kairouan (V4), Rihane (V7), Sidi-Bouزيد (V8), Sabra (V9), Tombari (V10)], five genotypes from Algeria [Techedrette (V15), Saïda (V17), Sidi-Mehdi (V18), Ras-El-mouche (V19), Naïlia (V20)] and six genotypes from Egypt [Giza 130 (V23), El Arich (V24), Ksar (V25), Giza 2000 (V26), Giza 125 (V29) and Giza 131 (V30)] were used in this study.

2.2 FIELD EXPERIMENTAL DESIGN

The trial was conducted at the National Agronomic Research Institute of Tunisia for two consecutive growing seasons (2014 and 2015). Sixteen barley genotypes previously selected as part of a research project, were assessed in the field using a split plot experimental design with three replications. The soil on which the tests were carried out showed the following characteristics: clay 22.5%, silt 31.5%, very fine sand 12.0%, fine sand 20%, coarse sand 12% and organic matter 2%. The fertilization consisted of 80 kg P₂O₅/ha, just before sowing date and 20kg of (Ammonitrate)/ha, at full tilling stage, was applied each year. The seeding density was calculated on the basis of 250 grains/m².

Each barley genotype was represented by an elementary plot of 4m² (two meters wide and two meters long), replicated three times and conducted under rainfall water regime.

The rainfall recorded during the two years of experimentation (September to June) was virtually similar (424 mm in 2013-2014 and 401 mm in 2014-2015).

At harvest time, grain samples were taken from each plot, for different analysis.

2.3 MEASUREMENT OF QUALITY PARAMETERS

2.3.1 ASSESSMENT OF GRAIN COLOR

The carotenoid pigmentation content in cereal grain is considered as a criterion for the raw materials quality used in agri-foodstuffs, and it is one of the first factors appreciated by the consumer when purchasing cereal products. For these reasons we considered this factor and assigned a scale from 0 to 3 to each barley flour color, according to what [17] have suggested. (0 = yellow, 1 = yellowish, 2 = dirty-white, 3 = gray).

2.3.2 ANALYSIS OF THE B-GLUCAN CONTENT

According to several authors ([18]; [6]; [19]), barley contains many natural health-beneficial compounds, including soluble and insoluble fiber called β -glucan. This substance is present in a proportion of 2 to 10% in barley.

The analysis of the β -glucan content was carried out using the assay kit provided by Megazyme International, Ltd., Wicklow, (Ireland) and according to the protocol described by Havrlentova and Kraic (2006)[20]. To determine the total β -glucan amount (mixed-linkage [(1-3) (1-4)] - β -D-glucan), 0.5 g of flour of each barley genotype was put in 50 ml Falcón tubes in which we added 1 ml of ethanol solution (50% v/v) and 5 ml of sodium phosphate buffer (20 mM, pH 6.5).

Then all tubes were well mixed (by vortex) and incubated in a boiling water bath for 2 min, followed by further shaking for 3 min, then cooled to 40°C. When the tube temperature was stabilized at 40°C, 0.2 ml of the lichenase enzyme (10U) was added and then the tubes were incubated for 1 h at 40°C with an intermediate stirring of the mixture. Subsequently, the volume of each tube was adjusted to 30 ml with distilled water and the content of each tube was thoroughly mixed and centrifuged at 1000 rpm for 10 min.

Aliquots of 0.1 ml of each supernatant were transferred into three 15 ml Falcon tubes. One of them received 0.1 ml of sodium acetate buffer (50 mM, pH 4.0) (blank) and the two others received 0.1 ml of β -glucosidase and then incubated at 40°C for 15 min. After that, 3 ml glucose oxidase peroxidases were added and all tubes were further incubated at 40°C for 20 min.

At the end of this incubation, the absorbance was measured at 510 nm using a spectrophotometer (Bio rad, Smart Spec 3000). The standard glucose solution was used as a standard and the calculation was performed according to the formula described below [21].

$$\beta\text{-glucane (\% w/w)} = \frac{\Delta A \times F \times 300 \times 100 \times 162}{1000 \times W \times 180}$$

$$\beta\text{-glucane (\% w/w)} = \frac{\Delta A \times F \times 27}{W}$$

ΔA : The difference in absorbance between the sample and the blank

F: conversion factor of the absorbance value in μg of glucose

W: Dry weight of the barley flour sample used

300 = corrective volume (0.1 ml taken from 30.0 ml).

1/1000 = Conversion of μg to mg

100 / W = Factor expressing the β -glucan amount in percent of the weight of barley flour

162/180 = Conversion factor of free glucose to anhydrous glucose, produced in β -glucan

2.3.3 ANALYSIS OF GRAIN PROTEIN CONTENT

The protein grain content was determined by Kjeldahl method. Three replications for each genotype were performed. The Kjeldahl method involves the three-stage analytical procedure: mineralization, distillation and titration.

The mineralization of flour sample is performed in digestion tubes that were inserted into an electrically heated block. The block allows the mineralization of 6 flour samples at once. The blank sample (parallel) is necessary to compensate the effect of used chemicals and water. To carry out this analysis, 1 g of barley flour of each genotype was put in weighting bottle and set to the digestion tubes. Carefully 14.5 ml of concentrated sulphuric acid (H₂SO₄) and 2 catalyst tablet (Se+K₂SO₄) was added to the sample in tubes. The mixture was then heated gradually up to 440°C. After 1.5 h the mineralization was finished. The tubes put out block on ceramic plate and let 30 min to cool. The sample was correctly mineralized to be transparent and colorless.

Distillation of ammonia was performed in the distillation unit which is steam generator. The ammonia released was driven by water vapor. The dosage of reagents (sodium hydroxide solution, storage solution, 40%, v/v) and the distillation process was controlled automatically. The cooled mineralized sample was dilute by 75 ml of distil water and insert to the distil unit (left side). On the right side of unit, we placed the Erlenmeyer flasks with 25 ml of boric acid solution with indicator (4%, w/v). Appropriate amount of sodium hydroxide was added automatically to the tube and the generator of steam was turned out. The complete distillation of ammonia was finished in 4,5 minutes. Water vapor condensed in the cooler and the distillate flowed into the Erlenmayer flask.

After distillation, we titrated the "green" color of solution with 0.1 mol l⁻¹ hydrochloric acid (HCl) to "grey" color of solution.

The nitrogen content of the sample was calculated as a function of the ammonia amount produced, proportional to the volume of acid used.

$$[N\%] = [(V-V_0) \times 0.1 \times C_{(HCl)} \times M_{(N)}] / m_{(V)}$$

Where:

V= consumption (volume) of HCl (mL),

V₀ = consumption (volume) of HCl for blank sample (mL),

C_(HCl) = molar concentration of HCl (mol l⁻¹),

M_(N) = molar mass of nitrogen (14 g mol⁻¹),

m_(V) = the sample weight (g).

According to Dumas et al. (2007)[22], the protein content of barley was:

$$N\% \times 5.83$$

2.3.4 ANALYSIS OF PROTEIN AND STARCH CONTENT BY INFRATEC

The Infratec has been specially developed to analyze the whole grain, flour, semolina and other cereal products. The use of this technology makes it possible to analyze a wide range of parameters (moisture, protein, specific weight, oil, starch, etc.) on multitude crops. The use of the "infratec", to estimate the grain composition has become widespread in recent years in grain storage silos. Today, almost all barley and wheat harvesting is controlled by this method at the silo reception.

We used this technology to measure the protein content but also the starch and the water content of the barley grain, firstly because this apparatus became available at our Laboratory and then for its simplicity of use, its speed response and non-destructive process.

2.4 STATISTICAL ANALYSIS

Data collected over two years of experimentation were subjected to variance analysis using SAS statistical software (SAS Inst, 1990). A comparison of the means was performed using the Fisher's Smallest Significant Difference (SMDP) test at the 5% probability level for the parameters studied, when the effect of one of the factors studied was significant.

3 RESULTS AND DISCUSSION

3.1 GRAINS COLOR

The composition and the organoleptic characteristics of barley grains have an important influence on its quality byproducts. The scale previously established has attributed 0 (yellow) to V10 and 1 (yellowish) to V23. The reminder genotypes share the other scales (2: dirty-white or 3: gray).

Among the barley genotypes used in this study, the naked ones V10 (Tombari) and V23 (Giza 130) were identified as the most appreciable, on the basis of their color (Figure 1). This result is confirmed by [12] and [23] who proved that cross-cutting of durum wheat by several barley genotypes including V10 to produce pasta (Tagliatelle), showed the superiority of this genotype over the other hulled ones and recognized that color and appearance are the most reliable indicators of food quality.



Fig. 1. Color of the two barley genotypes V10 (left) and V23 (right)

Similarly, [24] proposed several factors including the grain color as reliable indicator of malting quality of barley. These authors have shown also that the color index obtained by image analysis has a significant positive correlation with the quality of the malt obtained. For these reasons we considered the seeds color as a factor of the quality appreciation.

3.2 β-GLUCAN CONTENT

The analysis of barley genotypes showed a significant variability in β-glucan content, ranging from 2.18 (V20) to 4.75% (V10) (Figure 2). These values are in agreement with that of [3], suggesting that barley seeds usually contains 2-10% of β-glucan. However, [5] reported that some naked and waxy barley varieties contain more than this level.

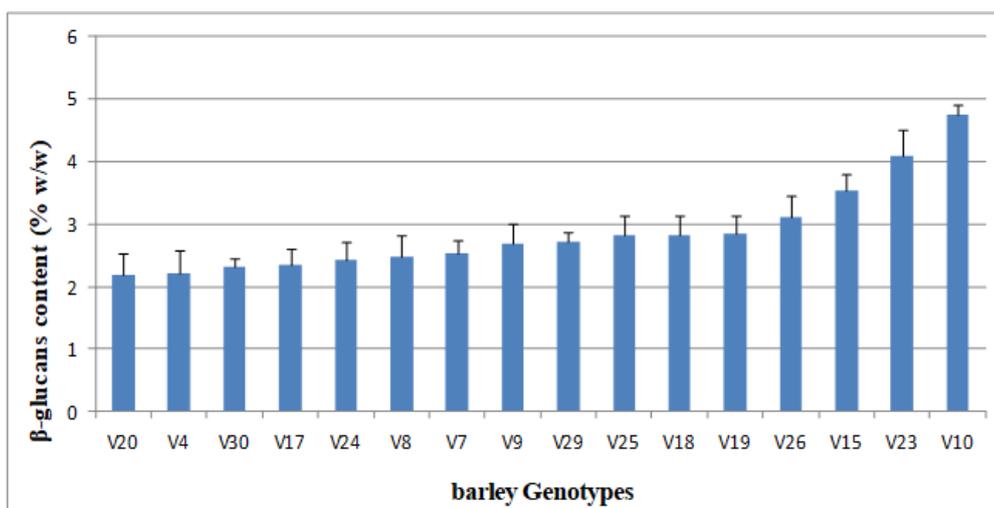


Fig. 2. Variation of β-glucan content in barley genotypes

The variance analysis of the β -glucan content showed significant difference between the different genotypes (Table 1). The Newman-Keuls test, classified the genotypes into 5 distinct groups (Table 2) where V20 (Neïlia), V4 (Kairouan), V30 (Giza 131) and V17 (Saïda) have the lowest values while V10 (Tombari) has the highest value. It should be noted that V10, V23 and V15 which have the highest values (4.75, 4.10 and 3.53 respectively), constitute, each one, a separate group. These three genotypes, particularly, V10 and V23 could be recommended for human diet because of their richness in β -glucan in addition to their therapeutic effect recognized in all barley genotypes. A recent study conducted by [25] have confirmed that hull-less barley β -glucan reduces the concentration of plasma cholesterol in hypercholesterolemic hamsters. Likewise, [26] have also confirmed the positive effect of β -glucan in enhancing some cell-mediated immune responses in broilers.

Table 1. Variance analysis of the β -glucan content

Source	Degree of Freedom	Sum of Squares of Deviations	Mean Square	F Value	Probability (Pr>F)
Model	15	45.43528590	3.02901906	34.24	<.0001
Error	80	7.07814813	0.08847685		
Corrected Total	95	52.51343403			

Table 2. Distribution of barley genotypes in homogeneous classes based on β -glucan content

Barley Genotypes	β -glucan (en %)	homogenous Class
V20 (Neïlia)	2,18	E
V4 (Kairouan)	2,20	E
V30 (Giza 131)	2,31	E
V17 (Saïda)	2,34	E
V24 (El-Arich)	2,41	DE
V8 (Sidi Bouzid)	2,49	DE
V7 (Rihane)	2,53	DE
V29 (Giza 125)	2,70	DE
V9 (Sabra)	2,68	DE
V25 (Ksar)	2,82	DE
V18 (Sidi Mehdi)	2,83	DE
V19 (Ras El-Mouche)	2,85	DE
V26 (Giza 2000)	3,10	CD
V15 (Tichedrett)	3,53	C
V23 (Giza 130)	4,10	B
V10 (Tombari)	4,75	A

It should be noted that V10 and V23 are two hullless genotypes and their high content in β -glucan was in agreement with what many authors have reported. For instance, [27] confirmed that naked and waxy barley grains contain higher β -glucan content than normal grains. This significant difference between the genotypes tested is, mainly, due to the varietal effect rather than the environmental factor, according to what [28] have reported. The results obtained are also in agreement with those of [29] and [30] who showed a positive correlation between β -glucan richness and barley nudity (nud locus).

3.3 PROTEIN CONTENT (KHJELDAHL METHOD)

The analysis of protein content is shown in Figure 3. This values varied from 10.67% in V20 (Naïlia) to 14.13% in V10 (Tombari).

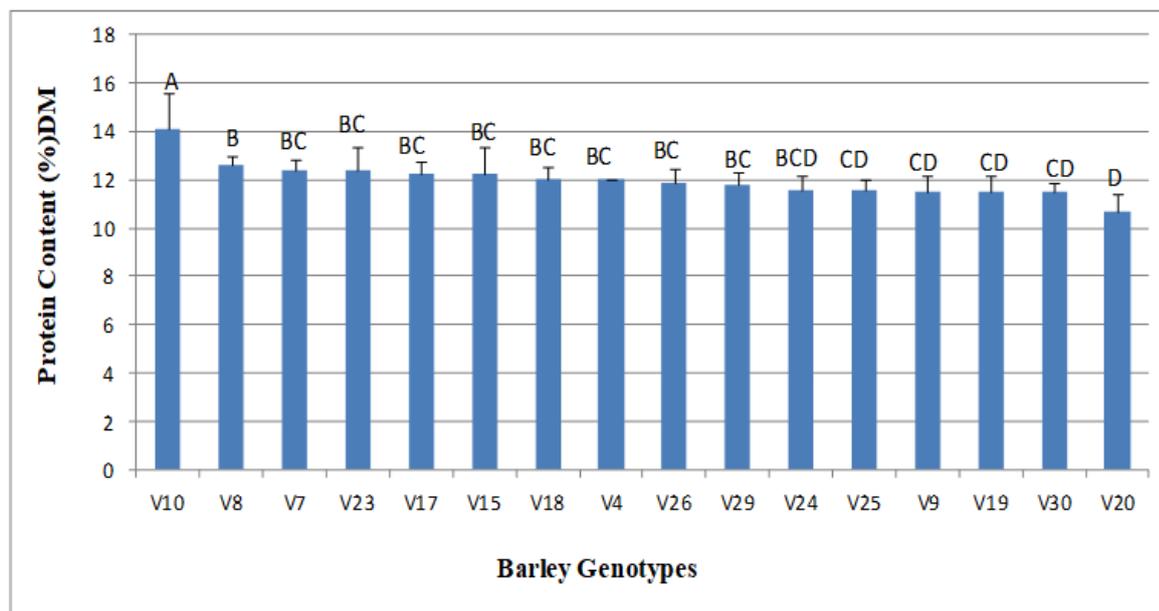


Fig. 3. Protein content in different barley genotypes using the Kjeldahl method

Variance analysis of this parameter showed a significant difference among genotypes (Table 3). The Newman-Keuls classification showed four significantly different groups.

Table 3. Variance analysis of the protein content

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Probability (Pr>F)
Model	15	24.72236458	1.64815764	4.59	0.0001
Error	32	11.47886667	0.35871458		
Corrected Total	47	36.20123125			

V10 genotype had the highest value (14.13%), while V20 had the lowest one (10.67%). The other genotypes are intermediate. Except for V10, the obtained values are, for the most part, consistent with what [31] and [32] have obtained during two growing seasons. However, the genotypes studied in this work were generally richer than those studied by [32], may be because the difference in varietal genetics or the difference in growing conditions (moisture, fertilization, ...) and/or the negative correlation between grain yield and protein content reported by several authors ([33], [15]). These authors stated that high grain yield is negatively correlated to the protein content. They explained this negative correlation by the dilution effect of grain nitrogen into the carbon-rich molecules.

The low protein content observed in some barley genotype can be increased by nitrogen fertilization or by introgression of some genes conferring high protein content, according to what [34] has identified in wild barley and proposed to improve the protein content of domesticated varieties.

3.4 PROTEIN CONTENT (INFRATEC METHOD)

Barley grain analysis by "infrared" (Figure 4), still shows the superiority of V10 on all studied barley genotypes. The value obtained for V10 (13.76%) is similar to what we found by the Kjeldahl method (14.13%).

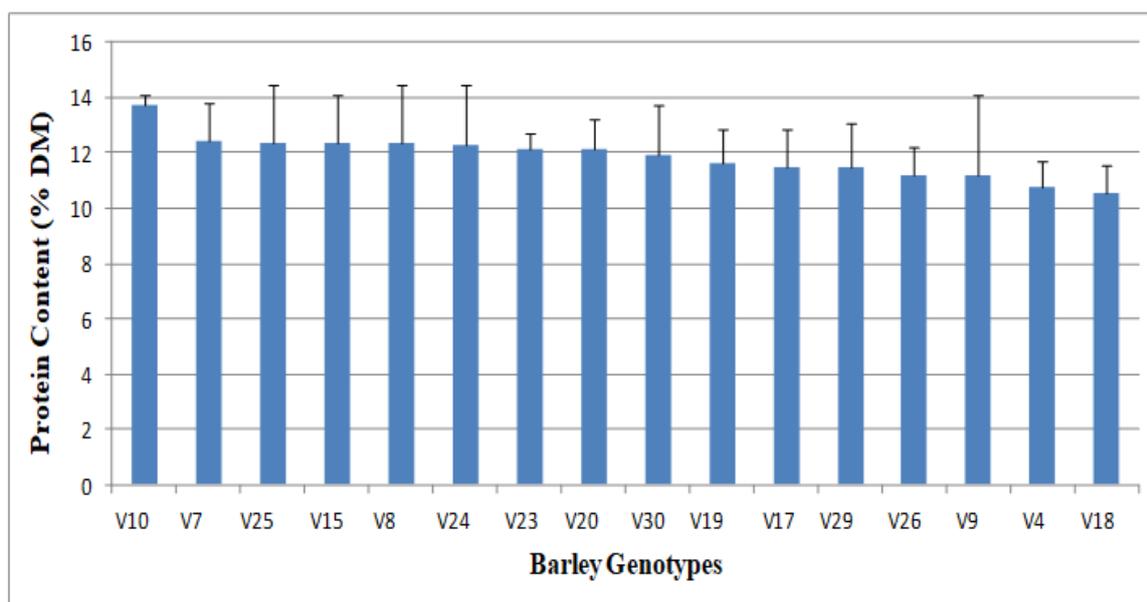


Fig. 4. Protein content, in the different barley genotypes, using "Infratec" method

On the other hand, the values obtained for the other genotypes are also similar to what we obtained by the kjeldahl method, however the position of the genotypes changes a little bit according to the method used.

The Kjeldahl method is the most universal technique for nitrogen analysis. In fact, this is the only method that can be used to measure the nitrogen content of very diverse samples. However, its slowness and its high cost (chemicals consumed) forces users to look for a simpler method (Infratec), reliable and allowing not only protein analysis but also grain moisture and starch content, with reproducibility higher than the reference methods used

3.5 STARCH AND ASH CONTENT

Table 4 showed that the naked genotypes (V10 and V23) are the richest in protein and β -glucans but also the poorest in mineral ash, compared to the others. Furthermore, V9 and V24 have the highest starch content against V18 and V19 who have the lowest content. Because of their high starch content, the first two genotypes are an essential energy component of human food [35]. Likewise for animal feed, starch is used to formulate high energy rations needed by highly productive dairy cows.

Table 4. Biochemical components of the studied barley grains

Génotypes	Protéines	β -glucanes	Amidon	Cendre
V4	11.99 \pm 0.06	2.21 \pm 0.38	53.8 \pm 5.0	2.3 \pm 0.17
V7	12.41 \pm 0.44	2.53 \pm 0.20	57,1 \pm 1.19	2.23 \pm 0.009
V8	12.58 \pm 0.42	2.49 \pm 0.34	57,4 \pm 0.93	2.13 \pm 0.027
V9	11.52 \pm 0.65	2.68 \pm 0.33	60.0 \pm 1.23	2.10 \pm 0.024
V10	14.13 \pm 1.5	4,75 \pm 0.17	56,6 \pm 1.85	1.82 \pm 0.07
V15	12.24 \pm 1.09	3.52 \pm 0.27	54.0 \pm 0.85	2.52 \pm 0.007
V17	12.26 \pm 0.48	2.34 \pm 0.28	53.8 \pm 1.66	2.42 \pm 0.017
V18	12.03 \pm 0.52	2.83 \pm 0.30	52.6 \pm 2.6	2.14 \pm 0.14
V19	11.52 \pm 0.68	2.85 \pm 0.27	52.0 \pm 1.50	2.10 \pm 0.03
V20	10.67 \pm 0.76	2.18 \pm 0.35	56.00 \pm 1.43	2.22 \pm 0.009
V23	12.41 \pm 0.98	4.09 \pm 0.41	56,7 \pm 1.404	1.94 \pm 0.008
V24	11.6 \pm 0.54	2.41 \pm 0.29	59 \pm 1.22	2.25 \pm 0.18
V25	11.56 \pm 0.49	2.82 \pm 0.31	57,5 \pm 0.95	2.26 \pm 0.16
V26	11.85 \pm 0.63	3.10 \pm 0.36	57,1 \pm 1.92	2.23 \pm 0.18
V29	11.82 \pm 0.52	2.71 \pm 0.16	58 \pm 2.04	2.24 \pm 0.024
V30	11.49 \pm 0.36	2.31 \pm 0.15	56,5 \pm 1.25	2.18 \pm 0.019

These results are comparable to those reported by [36]; [37] and [38]. A negative correlation between organic compounds (proteins and β -glucans) and ash content was established. This finding was also reported by [39] who showed also a negative correlation between micronutrients and organic barley grain compounds.

On the other hand, this table showed that the highest constituents are starch, which varied from 52.0 to 60.0% DM and crude protein, which varied from 10.67 to 14.13% DM. Ashes are minor constituents and was formed mainly of potassium and phosphorus as it was demonstrated by [38]. The differences in chemical composition observed in this study are attributed to genetic differences since all these genotypes were grown under the same environmental conditions.

4 CONCLUSION

This work aimed to study the nutritional quality of sixteen (16) North African barley genotypes. Grain quality analysis included grain color, protein content, β -glucan, starch and ash content. The results obtained pointed out the superiority of two naked genotypes originating from Tunisia (V10) and Egypt (V23).

The V10 genotype has showed the highest protein and β -glucan contents but also the lowest ash content. On the starch level, the V9 and V24 genotypes were the richest while V18 and V19 were the poorest. Thus, the Tunisian naked genotype (V10) and, to a lesser degree, the Egyptian naked genotype (V23) have proved to be the most nutritional, since they combined three quality criteria making them as the most interesting barley genotypes for human health and for a possible mix with wheat to make bread and pasta.

Undeniably, the addition of small quantity of this genotype's flour to wheat could improve the appearance, the nutritional quality of the bread and/or pasta in addition to its therapeutic effect due to its high β -glucan content.

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