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Comparative Characterization of Grain Protein Content and Composition by Chromatography-Based Separation Methods (SE-HPLC and RP-HPLC) of Ten Wheat Varieties Grown in Different Agro-Ecological Zones of Algeria

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Abstract: To characterize and compare the protein quality of ten durum wheat genotypes grown under three cropping modalities in Algeria (subhumid in Algiers, subhumid-semiarid in Constantine, and semiarid in Sétif), the protein profile of their kernels was performed by High-Performance Liquid Chromatography (SE-HPLC and RP-HPLC). The "variety" factor has a major impact, mainly on the insoluble fraction (Fi), on the gliadin/glutenin ratio, on the large and small glutenin aggregates (F1 and F2, respectively), and on ω-gliadins and high molecular weight albumins (F3). Conversely, the total protein content and the albumin-globulin fraction (F5) depend mainly on the environment. The α- β- and γ-gliadins (F4) are equally dependent on variety and environment. The subhumid-semiarid agroecological conditions of Constantine (SH-SA) favored an important accumulation of proteins (14.1%), particularly by an increased synthesis of omega gliadins and high-molecular-weight glutenin subunits (HMW-GS), compared to those of Algiers (SH) and Sétif (SA). For these latter environments, metabolic-type proteins are predominant, reflected in a higher F5 fraction ($p < 0.05$) (albumin and globulin), and significantly more alpha-beta and gamma gliadins. The use of chromatographic analyses to characterize wheat genotypes remains a reliable tool for breeding and variety promotion programs and can provide a better understanding of the ecophysiology of cereal crops.

Keywords: gluten; gliadins; glutenins; proteins; RP-HPLC; SE-HPLC; wheat

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1. Introduction

In Algeria, the need for durum wheat and its by-products for public consumption are not yet covered by the national production, even though the cultivation of this cereal occupies the largest national area of agricultural land exploited, with 1,579,080 ha in 2019 [1]. The technological quality of wheat grains is strongly influenced by growing conditions (climate, soil, and cultivation practices) and by genetics [2]. Genetic factors mainly affect the protein composition while cropping conditions are essential for the quantity and proportion of the different classes of proteins [3–5]. Based on their solubility in different solvents, wheat proteins have been classified into albumins, globulins, gliadins, and glutenins. Albumins and globulins belong to the category of soluble proteins (in water and saline solutions), which are necessary for cell metabolism but are of little technological interest. In contrast, gliadins and glutenins, which form the gluten, are the grain storage proteins belonging to the category of water-insoluble proteins. However, they are soluble in diluted alcohols,

acids, and bases, as well as detergents, to varying degrees. For the study of the structure of gluten proteins (gliadins and glutenins), spectroscopic methods, such as infrared and Raman, have been applied [6]. Gliadins are divided into four families: omega, alpha, beta, and gamma; whereas glutenins are grouped into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenins (LMW-GS). Wheat proteins can also be classified according to their quaternary structure to mono and polymeric proteins. Monomeric proteins such as gliadins are responsible for the viscosity and extensibility properties of pasta, while polymeric proteins such as glutenin confer elasticity and tenacity properties. Glutenins are present in the mature grain in the form of aggregates of varying sizes that are extremely important in defining the technological properties of wheat. The amount and size distribution of polymeric and monomeric proteins can be measured by Size Exclusion High-Performance Liquid Chromatography (SE-HPLC). In common wheat, they have been correlated with baking quality [7]. The quantity of polymeric proteins in durum wheat has also been related to the rheological properties of dough [8] and artisanal pasta [9]. The amount of gliadin is strongly influenced by agronomic conditions and increases with nitrogen fertilization [10]. Variations in the omega-gliadin content and the ratio HMW-GS/LMW-GS were considered as indicators of sulfur fertilization [11]. Furthermore, Reversed-Phased High-Performance Liquid chromatography (RP-HPLC) methods have been optimized for the separation of HMW-GS as a valuable tool in wheat breeding programs targeting end-product quality [12].

For commercial quality traits, protein content is a commonly required criterion. The latter is essential in several aspects since the occurrence of yellow berry, which affects the texture of the albumen in durum wheat, and the protein content are inversely related parameters [13–15]. Different factors seem to be at the origin of yellow berry, on the one hand, environmental factors (nitrogen supply, climate, and soil), and on the other hand, genetic factors.

In Algeria, very few studies have applied HPLC methods to study the protein composition of durum wheat. Two published works are reported, one on the analysis of a set of wheat genotypes in a subhumid-semiarid area of Constantine [16], and the other concerned only the evaluation of gluten in a powder of commercialized vital wheat gluten in the context of the study of its toxicity for celiac patients [17].

This work, as a first comparative study in multi-conditions of cropping, aims to characterize using chromatographic methods (RP-HPLC and SE-HPLC) the protein content and composition of ten durum wheat genotypes, and to analyze the variation of the protein profiles of these varieties, according to their agro-ecological growing regions in Algeria.

2. Materials and Methods

2.1. Study Area and Growing Condition of the Studied Wheat Varieties

Ten durum wheat (*Triticum durum* Desf.) varieties (var 1 to var 10) were selected (Table 1) and cultivated during the same year on fallow land located at three experimental research stations of the Institute Technique des Grandes Cultures (ITGC), located in three agro-ecological zones, El Khroub (Constantine), Sétif, and Oued Smar (Algiers), which represent the diversity of Algerian agro-climate, soils, and their irrigation (Figure 1).

The ITGC Algiers site (36°43′ N, 30°84′ E), according to Emberger's classification [18], belongs to the subhumid bioclimatic stage (SH), with mild winters. The average annual precipitation is 727 mm and the yearly average temperature is 19.4 °C. The coldest month of the year is February with an average temperature of 13.2 °C while August is the coldest with an average temperature of 25.9 °C. The soil at the site is clay-loam. The climate of the El Khroub ITGC (36°26′ N, 06°66′ E) varies from subhumid to semi-arid (SH-SA), with cool winters. The average temperature of the year is 15.9 °C with a lower average value in February (6.7 °C) and a higher mean in July (26.2 °C). The average annual rainfall is about 519 mm. In this area, the soil is carbonated with a silty-clay texture. In the Setif ITGC (36°12′ N, 05°24′ E), climatic conditions are semi-arid (SA), with cold winters and a cumulative rainfall of 377.7 mm. The average temperature during the year is 14.4 °C;

February wis the coldest month of the year with an average temperature of 4.0 °C and July is the warmest (26.1 °C). The soil at this site is clayey to silty-clayey in texture.

Table 1. Characteristics of the different wheat varieties involved in the study.

Varieties	Characteristics
var 1: Waha	High productivity—semi-dwarf
var 2: GTA/Dur	High productivity—good quality
var 3: Stk/Haul/Heca-1	Mexican Cross—good quality
var 4: Ammar-8	Intensive advanced lineage
var 5: Msbi-1/Quarmal	Semi-dwarf—productive
var 6: Azeghar-1/6/Zna-1/5/Awl 1/4/Ruff//jo/Cr/3/F9.3	Crossover ICARDA—good adaptation
var 7: Ville mur/3/Lahn//Gs/Stk/4/Dra2/Bcr/5/Bcr/Lks4/4/	Advanced lineage—Cross. ICARDA-CIMMYT
var 8: Gsb/1/4/D68-1-93A1A//RuFF/Fg/3/Mtl5/5/Wdz6/Gi/4	Var. CIMMYT—Adapted.
var 9: Lahaucan	Adapted to water stress
var 10: Da-6Black awns/3/Bcr//Memo/God	Good production—good adaptation

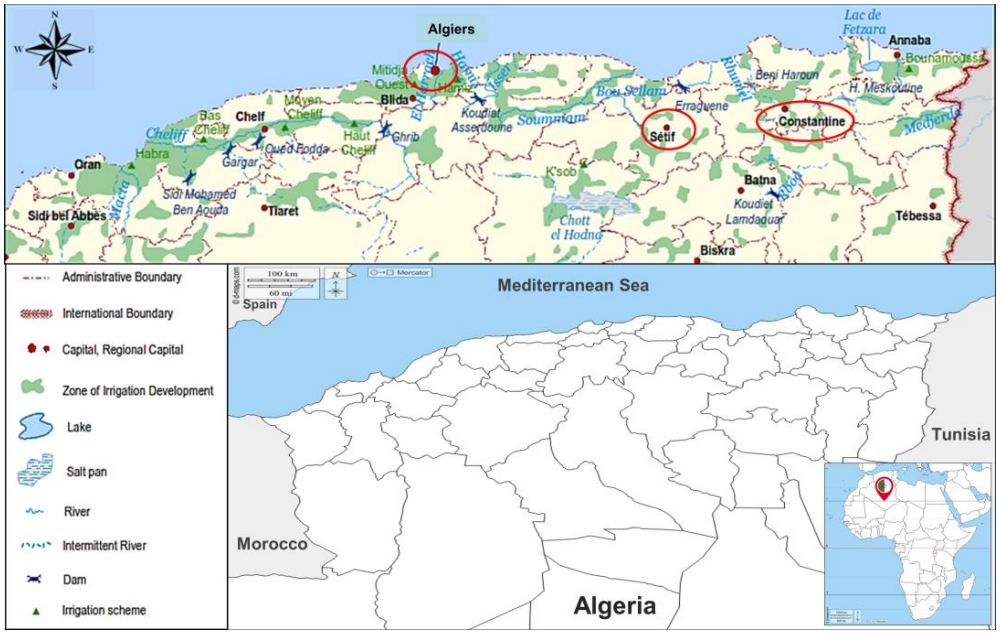

Figure 1. Irrigation characteristics in the studied agroecological zones (red circles) modified from FAO—AQUASTAT [19].

The experimental design was a Randomized Complete-Block Design with 4 replications; the elementary plot comprises six lines of five meters in length, spaced 0.20 m apart. They received basic fertilization of 46 units of phosphorus (46 kg of P2O5/ha) and 46 units of nitrogen in cover (46 kg/ha of nitrogen), at tillering. Sowing was carried out during the period from late November to mid-December at a dose of 100 kg/ha and harvesting took place from mid-June to early July. This experimental protocol was applied identically at the three stations.

2.2. Chromatographic Quantitative Analyses of Wheat Grain Proteins

Chromatographic techniques, SE-HPLC and RP-HPLC, were used to separate and quantify the different classes of durum wheat storage proteins. RP-HPLC was carried out to separate and quantify the proteins on the basis of their hydrophobicity and to distinguish between the different classes of glutenins (HMW-GS and LMW-GS) and gliadins (ω-, α/β-, and γ-gliadins). SE-HPLC was used to separate and quantify glutenin polymers and monomeric proteins (albumins/globulins and gliadins) (Figure 2).

2.2.1. Analysis of the Protein Composition of Wheat Grains by SE-HPLC

SE-HPLC was used to determine the proportion of polymeric and monomeric durum wheat proteins, the gliadin-to-glutenin ratio (Glia/Glu) and the amount of unextractable polymeric protein (UPP).

To do that, wheat total proteins were extracted from the ground kernels or flour, according to Dachkevitch and Autran [7], with some modifications concerning duration, sodium dodecyl sulfate (SDS) concentration, two steps extractions, and sonication power setting for durum wheat. Thus, 160 mg of ground kernels/flour were suspended in 20 mL of 0.1 M sodium phosphate buffer pH 6.9 containing 1% (*w*/*v*) of detergent (SDS) and stirred for 80 min at 60 °C. After centrifugation, (39,000× *g*; 30 min; 20 °C), the supernatant containing SDS soluble proteins was kept and stored (−20 °C) until analysis. The extraction pellet containing the protein fraction, insoluble in the detergent (Fi), was re-extracted with the same buffer (5 mL), and the residual proteins were solubilized using ultrasound according to the procedure described by Morel et al. [20], using a sonicator (Vibracell 72434, Thermo Fisher Scientific, Illkirch-Graffenstaden, France) equipped with a 3 mm probe for 3 min at 7.5 watts. After centrifugation (39,000× *g*; 30 min; 20 °C), the new supernatant was stored until analysis. The proteins recovered after the different extraction steps were separated by SE-HPLC using an Alliance 2695 System (Waters, Saint Quentin en Yvelines, France) on a TSKgel G4000 SWXL column (7.8 mm i.d. × 30 cm, TOSO BIOSCIENCE GmbH, Griesheim, Germany), protected by a TSK gel SWXL guard column (6 mm i.d. × 4 cm, TOSO BIOSCIENCE GmbH, Griesheim, Germany), according to Dachkevitch and Autran [7]. Twenty microliters were injected onto the column and the proteins were eluted at ambient temperature with 0.1 M sodium phosphate buffer (pH 6.9) containing 0.1% (*w*/*v*) SDS, at a flow rate of 0.7 mL·min^{-1}, and absorbance was measured at 214 nm with a 2998 Photodiode array detector (Waters, Saint Quentin en Yvelines, France). Chromatographic system was controlled by the Empower 3 software (Waters, Saint Quentin en Yvelines, France). For the same wheat sample, two chromatograms were obtained. The first chromatogram ("1st supernatant") was divided into 5 fractions: F1 to F5. The fraction marked F1 corresponds to large protein or aggregates of very high molecular weights (>630 kDa). These aggregates are composed of glutenin subunits (LMW-GS and HMW-GS). The F2 fraction (630–116 kDa) corresponds to smaller glutenin aggregates. The F3 fraction, comprised between 116 and 65 kDa, includes omega-gliadins and albumins of high molecular weight. The F4 fraction (65–21 kDa) is essentially composed of gliadins, while the F5 fraction (<21 kDa) contains albumins and globulins (Figure 2). The bigger aggregates are released using ultrasound and constitute the area of the second chromatogram ("Fi"). "Fi" was considered for the calculation of the UPP% (unextractable polymeric protein) which corresponds to the quantity of polymers not solubilized during the first extraction, expressed in % of the total polymers obtained during the two extractions (Fi + F1 + F2). Finally, the sum of the total areas of the two chromatograms ("1st supernatant" + "Fi divided by 4") can be related to the amount of total protein in the grain. The total protein content was deduced from the SE-HPLC data and expressed as a percentage, in g/100 g wet matter (WM), in relation to the sample as it is, with no correction to the dry matter.

2.2.2. Analysis of the Composition of Gliadins and Glutenins of Wheat Grains by RP-HPLC

The extraction of gliadins and glutenins was carried out according to Wieser et al. [21] from 50 mg of ground grains. The albumins/globulins were first removed by two successive

washes (10 min with stirring at room temperature) with a 0.067 M $HKNaPO_4$ buffer and a pH 7.6 buffer containing 0.4 M NaCl (500 µL). After each wash, the suspension was centrifuged at 6000× *g* for 15 min and the supernatant was discarded. After the second wash, the gliadins were extracted by resuspending the pellet with 500 µL of 60% (*v*/*v*) ethanol. The extraction was carried out while stirring for 10 min at room temperature, and the suspension was then centrifuged for 20 min at 6000× *g*. The supernatant was kept, and the pellet was re-extracted 2 more times under the same conditions. The supernatants were pooled (=1500 µL) and stored. The residual pellet was again taken up for the extraction of glutenins with a solution (500 µL) containing 50% (*v*/*v*) aqueous 1-propanol, 2 M Urea, 1% (*w*/*v*) dithioerythritol, and 0.5 M Tris HCl (pH 7.5). The suspension was purged with an inert gas (argon or helium) and incubated at 60 °C, for 30 min with intermediate stirring after 15 min, before being centrifuged as above. The supernatant was kept, and the pellet was taken up again under the same conditions. The supernatant was added to the previous one (=1 mL). The gliadins and glutenins contained in the two extracts were separated on a C18 (2.1 mm i.d. × 25, 5 µm, 300 Å) ACE column (AIT, Houilles, France) using an Alliance 2695 System (Waters, Saint Quentin en Yvelines, France), under the conditions described by Samson et al. [15], with modifications. Ten microliters were injected into the column and proteins were eluted at 50 °C with the following elution system: (A) 0.1% (*v*/*v*) trifluoroacetic acid (TFA) and (B) 0.1% TFA (*v*/*v*) in acetonitrile. The gradient was as follows: 0 min 28% B, 30 min 56% B, 33 min 38% B, 38 min 90% B, 41 min 28% B, and 51 min 28% B. Elution flow rate was maintained at 0.2 mL·min^{-1} and a 2998 Photodiode array detector (Waters, Saint Quentin en Yvelines, France) was used, and absorbance recorded at 210 nm (Figure 2). Empower 3 software (Waters, Saint Quentin en Yvelines, France) was used to control the system, acquire data, and make integrations. Gliadins were divided into 3 classes: omega-gliadins, alpha/beta-gliadins, and gamma-gliadins; whereas, glutenins were separated into LMW-GS and HMW-GS.

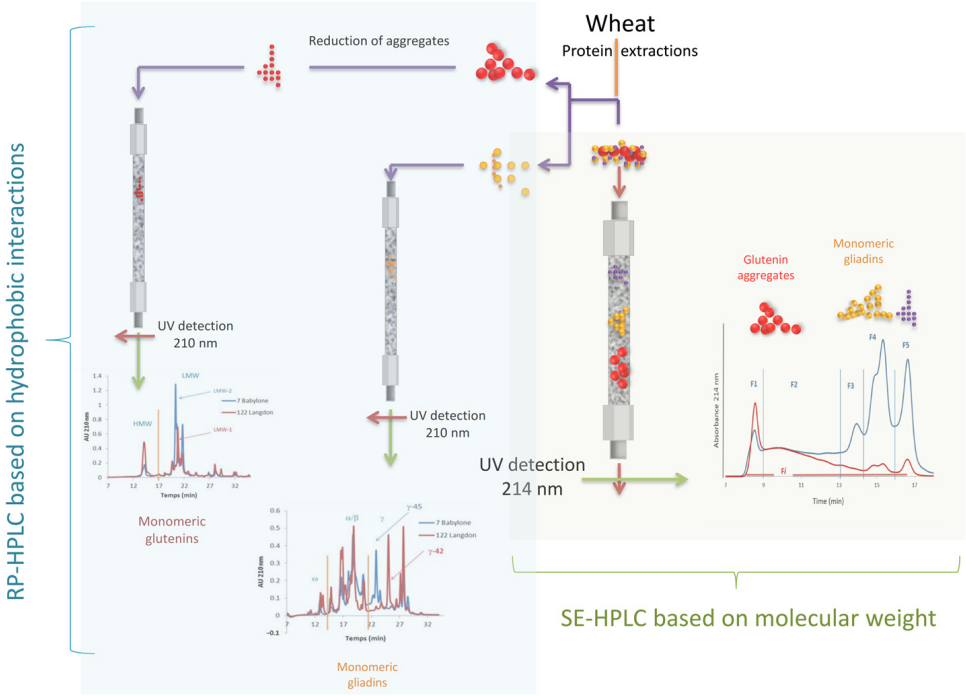

Figure 2. Methodology of chromatographic analysis of wheat proteins.

2.3. Statistical Analysis

Analyses of wheat protein content and composition data were performed using the SAS statistical system, version 9.3M2 software package (SAS Institute Inc.: Cary, NC, USA, 2012). The variation in the reserve protein composition of the studied wheat varieties was

analyzed by generalized linear model (GLM), using ANOVA for each factor, variety, and environment. Student–Newman–Keuls (SNK) post hoc test was applied to compare the mean values of protein fraction, protein ratio, and glutenin and gliadin compositions as a function of "variety" and "environment" variables. In addition, the sum of squares was considered to estimate the overall variance. A significance level of 0.05 was assumed for all tests.

3. Results and Discussion

In the Mediterranean area, the variability of precipitation during the grain-filling period may be related to large fluctuations in the yield of durum wheat grains and influence the technological quality aspects including the dynamics of grain dry mass and protein accumulations as well as glutenin polymer assembly. This relationship has been reported by different authors in the Mediterranean regions who showed that this effect is mainly due to climatic conditions during grain-filling, and assimilate accumulation in the grain [22].

The protein composition of gluten is one of the main factors affecting the technological quality of durum wheat. Gluten is mainly composed of two large families of proteins: gliadins, soluble in alcoholic solutions and glutenins, polymeric proteins, extractable under more drastic conditions (use of detergents, ultrasound, etc.). The first studies on the relationship protein composition/technological quality of durum wheat have demonstrated a link between gamma gliadins type 42 and weak gluten and gamma gliadins type 45 and strong gluten [23,24]. Subsequently, it was shown that the low molecular weight glutenin subunits (LMW-GS) encoded by genes close to those encoding gamma gliadins were in fact responsible for the difference in quality between glutens [25]. Two types of LMW-GS profiles: LMW-1 (associated with gamma gliadins 42) and LMW-2 (associated with gamma gliadins 45) largely explain the quality of durum wheat gluten [26–30]. If we consider high molecular weight glutenin subunits (HMW-GS), it has been shown that in common wheat, there is a relationship between the type of HMW-GS encoded at locus *Glu-B1* and the quality of the pasta, in particular the superiority of HMW-GS types 6 + 8 or 7 + 8 over type 20 x + 20 y.

3.1. Composition in Gliadins and Glutenins of the Studied Varieties

RP-HPLC makes it possible to study, on the one hand, the differences between varieties linked to genetics (presence and absence of peaks) and, on the other hand, those linked to agro-environmental conditions (height of peaks and relative proportion of different protein classes). The RP-HPLC profiles of gliadins (Figure 3) and glutenins (Figure 4) show both genetic differences between varieties but also some similarities.

Thus, for gamma gliadins (Figure 3), all varieties except variety 1 (gamma 42) are of type gamma 45.

In association with the gamma gliadin type, the low molecular weight glutenins are LMW-2 (LMW-$2^{(-)}$ or LMW-2) for all varieties except variety 1, where they are LMW-1 (Figure 4). Regarding the composition of high molecular weight glutenins, 4 varieties were identified as type 20 x + 20 y (varieties 2, 5, 7 and 10) and 5 as type 7 + 8 (varieties 1, 4, 6, 8 and 9), while variety 3 could not be identified with certainty, only electrophoresis would have allowed to do so. The identification of HMW-GS was done by comparing the studied varieties with French varieties whose HMW-GS type was known with certainty. For variety 3, it was not possible to link it with a known type. The most frequent types are 7 + 8, 6 + 8, 20 x + 20 y, and to a lesser extent 13 + 16. There are other types whose frequencies are very low. Moreover, the frequencies also vary with the countries [31,32]. According to Rekowski [33], an emphasis on protein composition may help to develop more efficient wheat varieties with optimal baking quality under water shortage. However, the results also highlight the need to evaluate the drought tolerance of wheat genotypes under their respective environmental conditions.

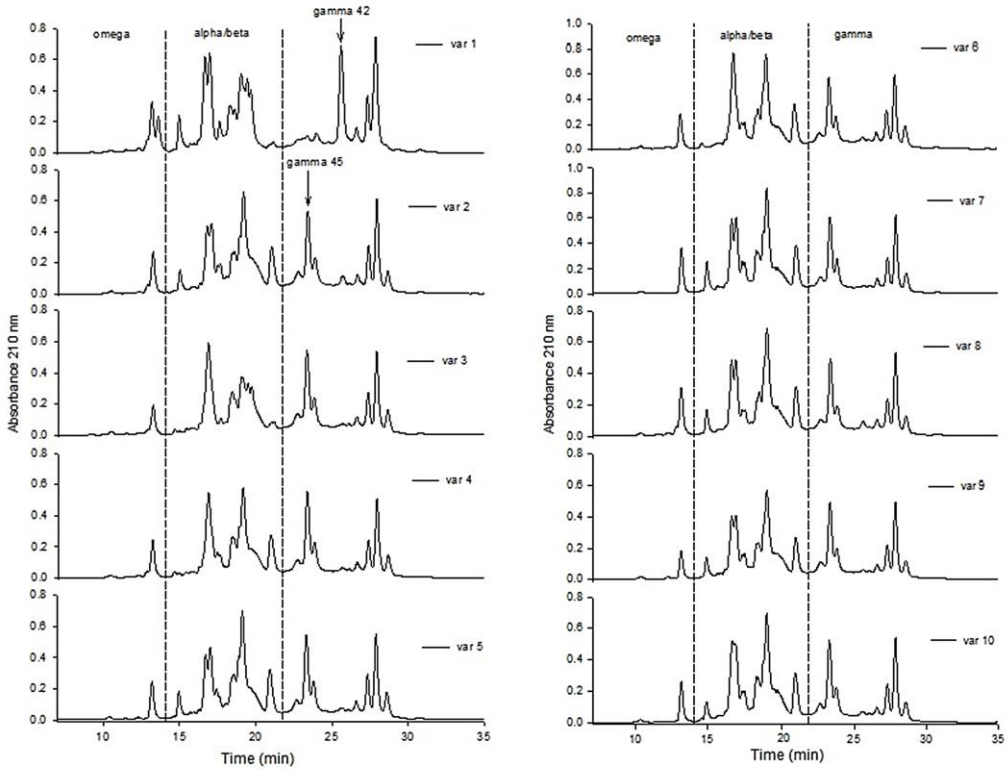

Figure 3. RP-HPLC profiles of gliadins (ethanol extracts) for the studied wheat varieties.

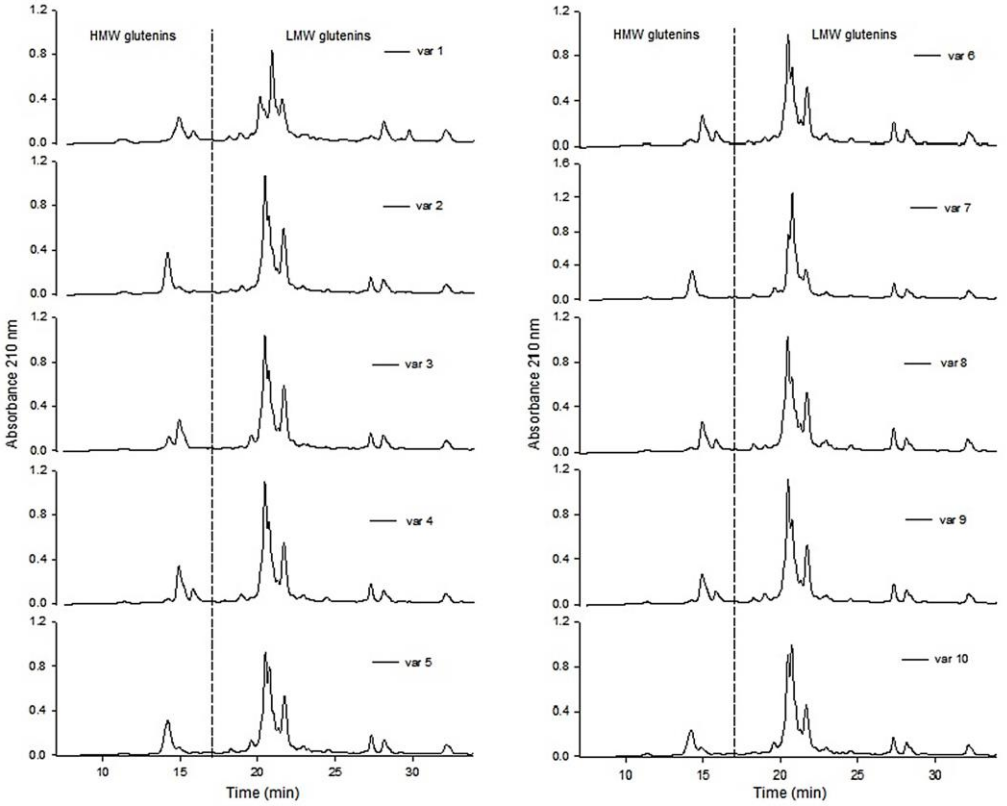

Figure 4. RP-HPLC profiles of glutenins (propanol/urea/DTE extracts) for the studied wheat varieties.

3.2. Effect of Varieties and of Cultivation Modalities in the Three Environments (SH-SA, SA, and SH) on the Content and Composition of Protein Reserves of Algerian Wheat

To evaluate the variety and environmental effect on the content and composition of storage proteins of the studied varieties grown under three conditions (stations of Constantine (SH-SA), Sétif (SA), and Algiers (SH)), the total proteins of the grain were extracted and analyzed by SE- HPLC, of which the results are presented in Table 2. On the other hand, the gliadins and glutenins were separated and analyzed by RP-HPLC, the relative proportion of each of the classes is presented in Table 3.

The total grain proteins were separated according to their size by SE-HPLC. Glutenins that have a strong capacity to complex in aggregates of large size constitute the F1 and F2 fractions (soluble aggregates), while the insoluble aggregates appear in the Fi fraction.

The average repartition of the different protein fractions is represented in Figure 5. As expected, the major part (38% on average) of the proteins present in the grains are α-, β-, and γ-gliadins (F4 fraction), followed by an almost equal proportion (between 15 and 19%) of small glutenin aggregate proteins (F2), albumins, and globulins (F5) (Figure 5). The percentage of Fi (insoluble proteins) is of the same order as that of F3 (ω-gliadins and high molecular weight albumins).

The protein level varied on average from 11.5% (var 10) to 13.3% (var 1) but the differences are not significant (Table 2); whereas it varied significantly according to the environment: from 10.6% in Sétif (SA) to 14.1% in Constantine (SH-SA).

Depending on the variety and on the cropping location, these percentages varied. The highest coefficient of variation is noted for Fi (CV = 13.7%) and F1 (CV = 12.1%). For the other fractions, the CV ranges from 2.7% (F4) to 5.5 (F5) (Table 2).

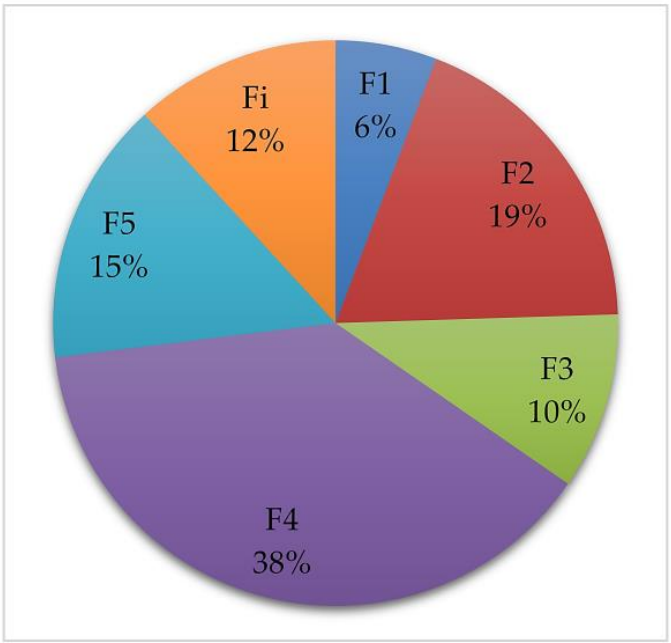

Figure 5. Average repartition of the grain durum wheat proteins in the 5 fractions and Fi fraction (means of all varieties and all locations). F1 = large glutenin aggregates; F2 = small glutenin aggregates; F3 = ω-gliadins and high molecular weight albumins such as β-amylase; F4 = α-, β-, and γ-gliadins; F5 = albumins-globulins, Fi = insoluble fraction.

Regarding the F1 and F5 fractions, no significant difference is noted between the varieties ($p < 0.05$). For F1, there is a huge difference among the varieties concerning the coefficient of variation: var 4 and var 6 have significant differences ($p < 0.05$) for this fraction according to the environment. Similarly, concerning Fi and UPP, the highest coefficients of variation are obtained for varieties 6 and 7.

The highest value of UPP was measured for variety 8 and the lowest one for var 7 (variety of type HMW 20 x + 20 y). When considering Glia/Glu, the higher ratio was found for var 1 (1.31) and the lowest for var4 (0.94). This could be explained by the fact that breeding programs have promoted varieties with the gamma 45 type, or the LMW-2 type, which express more LMW-GS at the expense of gliadins [34].

Interactions between variety and environment are illustrated in Figure 6 for UPP criteria. Some varieties have a higher UPP in location A (var1, 3, and 5), some others in location SH (var 2 and 7), and the others in location SH-SA (var4, 6, 8, and10). Such types of interaction were also found for other criteria.

According to the study of Gagliardi et al. [22], variation in the proportion of non-extractable polymeric protein (UPP%) revealed an overall significant effect of year (Y), genotype (G), nitrogen level (N), and their interactions. Moreover, Mefleh et al. [35] found that the variation in UPP% between wheat genotypes was independent of their allelic glutenin composition. This parameter and the ratios between protein groups and subunits do not appear to be affected by grain nitrogen content.

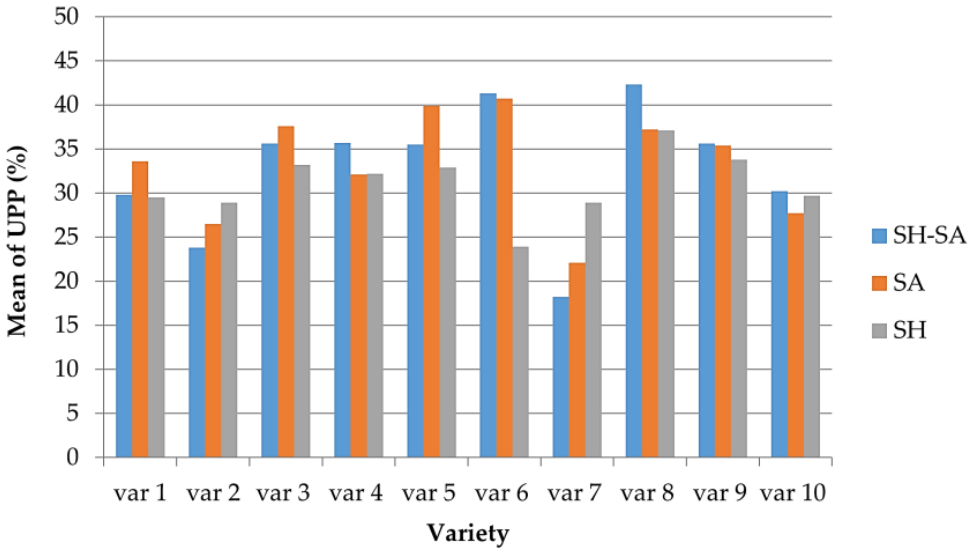

Figure 6. Mean of UPP (%) by variety and environment. SH-SA: suhumid-semiarid, SA: semiarid, and SH: subhumid.

The SH-SA modality is characterized by a higher average total protein content (14.1%), which seems to be due to a higher proportion of gliadins (F4), while the F5 fraction is reduced. The Glia/Glu ratio is higher in this location (Table 2).

The two modalities SA and SH generate lower total protein contents with a significantly higher proportion of soluble proteins (F5).

In the semiarid location (SA), the genetic variability was reduced compared to the other two locations. Indeed, whatever the fraction, the coefficient of variation (CV) is significantly lower (Table 2). This seems to be due to the stressed conditions of this location, that did not allow the varieties to well express their potential. Recently, in a Mediterranean semiarid area of Italy (Sicily), a negative impact of maximum temperatures on grain yield, and a positive relationship between minimum temperatures and protein content, during grain–filling periods, were confirmed [36].

Table 2. Mean and Coefficient of Variation of each parameter according to the variety and the environments.

Variables			SE-HPLC Protein Fractions (% of the Total Protein Content)						UPP (%)	Glia/Glu	Protein (%)
			F1	F2	F3	F4	F5	Fi			
Variety	var 1	mean	4.9 ^{a}	16.9 ^{b}	11.9 ^{a}	41.1 ^{a}	15.5 ^{a}	9.7 ^{bc}	31.0 ^{ab}	1.31 ^{a}	13.3 ^{a}
		CV	3.5	8.9	8.6	6.1	10.9	8.8	7.4	16.6	12.2
	var 2	mean	6.9 ^{a}	20.1 ^{a}	10.0 ^{b}	38.5 ^{bc}	14.9 ^{a}	9.7 ^{bc}	26.4 ^{bc}	1.05 ^{b}	12.4 ^{a}
		CV	10.2	2.8	3.2	3.5	11.4	9.4	9.7	11.1	2.4
	var 3	mean	5.6 ^{a}	18.8 ^{ab}	9.5 ^{b}	37.8 ^{bc}	14.9 ^{a}	13.4 ^{ab}	35.5 ^{ab}	1.00 ^{c}	12.2 ^{a}
		CV	3.7	1.9	7.9	2.1	8.1	8.8	6.2	9.9	1.7
	var 4	mean	6.5 ^{a}	19.6 ^{a}	9.4 ^{b}	36.6 ^{c}	14.9 ^{a}	13.0 ^{ab}	33.3 ^{abc}	0.94 ^{d}	11.7 ^{a}
		CV	20.5	5.9	9.1	7.4	11.1	7.4	6.2	14.9	13.4
	var 5	mean	5.3 ^{a}	18.9 ^{ab}	9.9 ^{b}	37.6 ^{bc}	14.5 ^{a}	13.7 ^{ab}	36.1 ^{ab}	0.99 ^{d}	12.6 ^{a}
		CV	3.3	4.3	6.1	4.3	10.0	12.1	9.8	12.4	5.3
	var 6	mean	5.2 ^{a}	18.0 ^{ab}	10.0 ^{b}	39.1 ^{abc}	15.0 ^{a}	12.7 ^{ab}	35.3 ^{ab}	1.09 ^{c}	13.2 ^{a}
		CV	25.0	12.8	2.6	2.3	7.6	27.9	28.0	11.8	2.4
	var 7	mean	6.7 ^{a}	19.8 ^{a}	9.9 ^{b}	40.3 ^{ab}	15.2 ^{a}	8.0 ^{c}	23.1 ^{c}	1.17 ^{b}	12.5 ^{a}
		CV	9.5	2.1	6.1	5.8	11.8	27.1	23.5	21.6	9.2
	var 8	mean	5.1 ^{a}	17.8 ^{ab}	10.1 ^{b}	37.6 ^{bc}	14.7 ^{a}	14.6 ^{a}	38.8 ^{a}	1.00 ^{d}	12.8 ^{a}
		CV	3.4	4.8	3.0	5.8	16.0	8.7	7.7	17.5	4.6
	var 9	mean	5.6 ^{a}	18.6 ^{ab}	10.2 ^{b}	37.2 ^{c}	15.6 ^{a}	13.0 ^{ab}	34.9 ^{ab}	1.00 ^{d}	11.6 ^{a}
		CV	5.4	1.2	3.5	6.2	13.0	4.9	2.8	17.8	7.3
	var 10	mean	6.1 ^{a}	18.7 ^{ab}	10.2 ^{b}	38.6 ^{bc}	16.1 ^{a}	10.2 ^{bc}	29.2 ^{abc}	1.10 ^{c}	11.5 ^{a}
		CV	2.5	1.1	1.7	5.5	13.8	5.0	4.5	18.9	4.7
Environment	SH-SA	mean	5.7 ^{a}	18.3 ^{a}	10.3 ^{a}	40.4 ^{a}	13.3 ^{b}	11.9 ^{a}	32.8 ^{a}	1.13 ^{a}	14.1 ^{a}
		CV	19.0	7.8	9.2	5.5	7.6	21.9	18.5	8.9	13.3
	SA	mean	5.9 ^{a}	18.5 ^{a}	9.7 ^{a}	37.5 ^{b}	16.0 ^{a}	12.3 ^{a}	33.3 ^{a}	1.03 ^{b}	10.6 ^{c}
		CV	9.5	3.7	3.7	2.6	3.7	13.7	11.7	7.8	5.9
	SH	mean	5.7 ^{a}	19.3 ^{a}	10.2 ^{a}	37.4 ^{b}	16.0 ^{a}	11.2 ^{a}	31.0 ^{a}	1.03 ^{b}	12.5 ^{b}
		CV	16.7	8.1	10.0	4.2	3.6	26.6	22.8	5.9	12.9
General means			5.8	18.7	10.1	38.4	15.1	11.8	32.4	12.4	1.07
General CV			12.1	5.2	5.2	2.7	5.5	13.7	12.9	6.7	5.9

$^{a–d}$ Mean values labeled with the same letter(s) within a column are not significantly different using Student-Newman-Keuls (SNK) test ($p > 0.05$). F1 = large glutenin aggregates; F2 = small glutenin aggregates; F3 = ω-gliadins and high molecular weight albumins such as β-amylase; F4 = α-, β-, and γ-gliadins; F5 = albumins-globulins; Fi = insoluble fraction; Glia = Gliadins; Glu = Glutenins; SH-SA subhumid-semiarid; SA semiarid; SH subhumid.

Table 3 contains the information generated from the ANOVA concerning the contribution of each factor (variety and environment) to the variability of the protein fractions. The factor "Variety" mainly explains most of the fractions, except fraction F5. Notably, the insoluble fraction (Fi) and the F1 and the gliadin/glutenin ratio (Glia/Glu) are mostly explained by the variety, and did not depend on the location.

On the contrary, the protein content and the albumins-globulins fraction (F5) are mainly dependent on the environment. The α-, β-, and γ-gliadins (F4) are both equally dependent on variety and environment. The residuals (error) of the ANOVA model (Y = var + environment + error) are relatively high, showing that other undefined factors play a role in the variation of the parameters (Table 3).

Table 3. Contribution (expressed in sum of squares values) of factors "variety" and "environment" to the variability of the studied parameters.

Sum of Squares Factor/Total Sum of Squares (%)	Protein Fraction							Protein	Glia/Glu
	F1	F2	F3	F4	F5	Fi	UPP		
Variety	60	54	65	40	9	71	65	12	70
Environment	2	11	10	45	73	3	3	73	14
Residuals	38	35	25	15	18	26	32	15	16

F1 = large glutenin aggregates; F2 = small glutenin aggregates; F3 = ω-gliadins and high molecular weight albumins such as β-amylase; F4 = α-, β-, and γ-gliadins; F5 = albumins-globulins, Fi = insoluble fraction.

The results obtained by RP-HPLC on gliadin and glutenin composition of the 10 varieties, according to the SH-SA, SA, and SH culture sites are indicated in the Table 4. Concerning the varieties, significant differences are obtained between varieties for each studied parameter. The main CV (9.4%) is obtained for HMW-GS and therefore for the ratio HMW/LMW. The HMW-GS percentage is the lowest for variety 5 (12.9%) and the highest for variety 1 (16.9%). Regarding the detail of the gliadin composition, the modality SH-SA promotes a higher synthesis of omega gliadins (Table 4), which probably explains the increase in the F4 fraction (Table 2).

The SH-SA environment is also distinguished by a significantly higher HMW/LMW glutenin ratio (Table 4).

The important variation of the parameter HMW/LMW seems essentially due to the SH-SA environment. This limiting environment, that, on average, minimized the genetic variability for the protein fractions, maximized the synthesis of omega gliadins and HMW-GS (Table 4). In a controlled environment study, flour protein composition of hard red spring wheat was similar for grain produced at 24/17 °C or 37/28 °C, when supplied with post-anthesis N (NPK) [37]. Since SA and SH do not differ statistically from each other, but do differ with SA-SH, it appears that the agroecological classification alone is not so robust in explaining the differences in the data. More detailed environmental factors are needed to explain them.

The higher proportion of omega-gliadin in "var 1" (25.5%) also seems to be an indicator of the "variety" effect since its sum of squares value shows an SS of 47% (Table 5). It may be related to a lower technological quality of this variety [38].

As suggested by Gagliardi et al. [22] who observed a variation in gluten protein assembly in the four genotypes depending on the season, the characterization of wheat grain proteins could help breeding programs select genotypes adapted to cope with ongoing climate changes in the Mediterranean growing region.

Table 4. Gliadin and glutenin composition of the ten varieties in the three environments.

		Gliadin Classes (% of Total Gliadin)			Glutenin Classes (% of Total Glutenins)		
		Omega	**Alpha/Beta**	**Gamma**	**HMW-GS**	**LMW-GS**	**HMW/LMW**
Variety							
var 1	Mean	25.5 ^{a}	33.9 ^{b}	40.7 ^{ab}	16.9 ^{a}	83.1 ^{b}	0.21 ^{a}
	CV	12.8	10.3	3.7	12.1	2.4	15.5
var 2	mean	20.6 ^{b}	39.2 ^{a}	40.3 ^{ab}	13.7 ^{ab}	86.3 ^{ab}	0.16 ^{ab}
	CV	3.4	2.2	1.1	19.9	3.1	24.1
var 3	mean	22.0 ^{b}	35.5 ^{b}	42.6 ^{a}	14.3 ^{ab}	85.7 ^{ab}	0.17 ^{ab}
	CV	3.5	5.8	3.1	11.7	1.9	12.5
var 4	mean	21.0 ^{b}	38.5 ^{a}	40.5 ^{ab}	13.7 ^{ab}	86.3 ^{ab}	0.16 ^{ab}
	CV	4.5	4.3	3.1	27.0	4.3	31.2
var 5	mean	20.1 ^{b}	39.4 ^{a}	40.5 ^{ab}	12.9 ^{b}	87.1 ^{a}	0.15 ^{ab}
	CV	8.1	3.5	0.6	23.6	3.5	29.0
var 6	mean	21.2 ^{b}	38.7 ^{a}	40.1 ^{ab}	13.7 ^{ab}	86.3 ^{ab}	0.16 ^{ab}
	CV	6.4	4.1	1.3	14.8	2.4	16.5
var 7	mean	22.2 ^{b}	40.3 ^{a}	37.5 ^{c}	14.2 ^{ab}	85.8 ^{ab}	0.17 ^{ab}
	CV	8.4	3.2	2.9	13.8	2.3	15.1
var 8	mean	21.3 ^{b}	40.2 ^{a}	38.5 ^{bc}	13.4 ^{ab}	86.6 ^{ab}	0.16 ^{ab}
	CV	14.8	5.6	3.7	11.1	1.7	13.3
var 9	Mean	20.4 ^{b}	38.7 ^{a}	41.0 ^{ab}	14.6 ^{ab}	85.5 ^{ab}	0.17 ^{ab}
	CV	7.5	2.8	1.3	9.9	1.7	11.8
var 10	mean	21.6 ^{b}	40.0 ^{a}	38.4 ^{bc}	13.3 ^{ab}	86.7 ^{ab}	0.16 ^{ab}
	CV	8.0	3.9	1.4	10.5	1.6	13.3

Table 4. *Cont.*

		Gliadin Classes (% of Total Gliadin)			Glutenin Classes (% of Total Glutenins)		
		Omega	**Alpha/Beta**	**Gamma**	**HMW-GS**	**LMW-GS**	**HMW/LMW**
Environment							
SH-SA	Mean	23.4 [a]	36.9 [b]	39.7 [a]	16.2 [a]	83.8 [b]	0.19 [a]
	CV	9.8	6.8	5.2	5.7	1.10	6.5
SA	Mean	20.9 [b]	38.9 [a]	40.2 [a]	12.8 [b]	87.3 [a]	0.15 [b]
	CV	8.3	7.5	4.3	17.5	2.5	21.9
SH	Mean	20.4 [b]	39.5 [a]	40.1 [a]	13.2 [b]	86.8 [a]	0.15 [b]
	CV	5.4	3.7	2.9	8.9	1.4	9.8
General means		21.6	38.4	40.0	14.1	85.9	0.16
General CV		5.0	3.4	2.5	9.4	1.5	11.2

[a–c] Mean values labeled with the same letter(s) within a column are not significantly different using the Student–Newman–Keuls (SNK) test ($p > 0.05$). SH-SA = subhumid-semiarid; SA = semiarid; SH = subhumid; HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenins.

Table 5. Contribution (expressed in sum of squares values) of factors "variety" and "environment" to the variability of the studied parameters.

Sum of Squares Factor/Total Sum of Squares (%)	Gliadin Classes			Glutenin Classes		
	Omega	**Alpha/Beta**	**Gamma**	**HMW-GS**	**LMW-GS**	**HMW/LMW**
Variety	47	64	75	25	25	27
Environment	37	20	1	52	53	54
Residuals	15	16	24	23	22	19

HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.

4. Conclusions

The use of the chromatography-based separation methods (SE-HPLC and RP-HPLC) to analyze the protein composition in several durum wheat genotypes appears essential to understand the link between their diversity and the technological properties of wheat-based food. The results show that, in the considered year, the agro-climatic conditions in Constantine (subhumid-semiarid) favored protein accumulation compared to that obtained in Algiers (subhumid) and Setif (semiarid). According to these results, these conditions have an impact on the quantity of protein but also, to a lesser extent, on the quality of these proteins.

The genetic factor seems to have a preponderant role in the determination of the studied quality parameters of durum wheat, and interactions may be observed with the environment. Assessment of agroecology-dependent changes in protein parameters using advanced analytical chemistry methods could be a reliable approach in a durum wheat breeding program.

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