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Original Article

Proteome consistency of the aleurone layer in grain of major wheat species grown over different years

Samira Meziani [1] *, **Isabelle Nadaud** [2]

[1] Laboratory of Biotoxicology, Djillali Liabes University, Faculty of Life and Natural Sciences, Department of Biology, Algeria
[2] UMR. INRA GDEC-UCA 1095, 5 Chemin de Beaulieu, 63000 Clermont-Ferrand, France

ABSTRACT

Background: Aleurone layer (AL), being a living cell layer among the peripheral layers of the grain structure obtained after milling wheat, is rich in, vitamins, minerals, and antioxidants potentially nutritional value of the flour. **Objectives:** To isolate AL in the mature grain of the three major species; Common wheat (CW), Durum wheat (DW), and Einkorn wheat (EW) that were grown at two different years as well as to analyze and compare their proteomes revealed through two-dimensional electrophoresis (2DE) and image analysis. **Methods:** The AL was hand dissected and unicellular purity verified using scanning electron microscopy. AL proteins were separated using IEF pH3-10 X SDS-PAGE then Coomassie-stained. The gels were scanned and the images were compared using Samespot (Nonlinear Dynamics) and were proteins identified using mass spectrometry and database interrogation. **Results**: For CW and DW samples, no significant quantitative or qualitative differences were observed between the AL proteome in the two years. However, a few quantitative differences were revealed between EW for AL of 2006 and 2007. The identified proteins were classified in the carbohydrate pathway and stress defense response. **Conclusion:** This remarkable stability over environmental growing conditions strengthens the need to pay greater attention to this unicellular living cell of the wheat grain.

Keywords: Wheat, durum, einkorn, aleurone layer, metabolic pathways

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1 Introduction

Wheat represents one of the main cereals consumed by humans and animals. Knowledge of wheat grain structure and the composition of storage proteins, starch, lipids, vitamins, and micronutrients is essential for producing high-quality grain [1,2]. Wheat species, such as durum and einkorn wheat are high nutritive value of their grain [3]. Durum wheat is an extremely important species in Mediterranean regions, where is used in the production of semolina and pasta [4]. Einkorn wheat is one of the ancient wheats and is often grown in environmentally friendly organic farming. This wheat is also known for its high protein and carotenoid contents [5,6]. Einkorn wheat is a potential source of food with high nutritional properties [7]. The peripheral layers (PL) of wheat, including the aleurone layer (AL), are a considerable source of micronutrients. The AL is used as a food ingredient for animal feed, the presence of its nutritional and phytochemical elements increases the quality of food [8,9]. For several years, studies on AL have mainly focused on analyzing its nutritional potential for health. AL contains vitamins more particularly vitamin B and E, and minerals [10] and phenolic antioxidants, mainly ferulic acid compounds, which exhibit a potential nutritional value. However, few studies have been carried out to study the potential health effects of isolated aleurone on biomarkers for cancer [11].

Numerous studies focusing on the proteome variations of the wheat grain were reported since the last decade. Based on the two-dimensional electrophoresis of the proteins extracted either from grain peripheral layers [12] or from the hand isolated AL using binocular [13], a large set of enzymes and proteins, specific of this unicellular layer, were revealed. In the aim to compare AL of the major wheat species, three representatives of common wheat (*T. aestivum*) and three varieties of *T. durum* were analyzed [14]. The same proteomic approach was also developed in the comparison of AL from the mature grain of three representative cultivars of *T. aestivum* and *T. monococcum* [15]. The influence of different growing environmental conditions on the AL proteome was also partly reported in a previous study [14,15]. Our study was focused on possible proteomic variations of the AL isolated in mature grain from one variety of common (*T. aestivum*), durum (*T. durum*) and Einkorn (*T. monococcum*) wheat grown at two consecutive years in different conditions. Each of these cultivars has been produced in nurseries, under conventional growing conditions, with sufficient mineral fertilization to achieve high grain yields. The production of these cultivars was carried out with complete phytosanitary protection on the plant and the ear.

* **Corresponding author:** Samira Meziani Laboratory of Biotoxicology, Djillali Liabes University, Faculty of Life and Natural Sciences, Department of Biology, Algeria. Telephone No: 00213 560 337 210. E-mail: meziani_samira@yahoo.fr

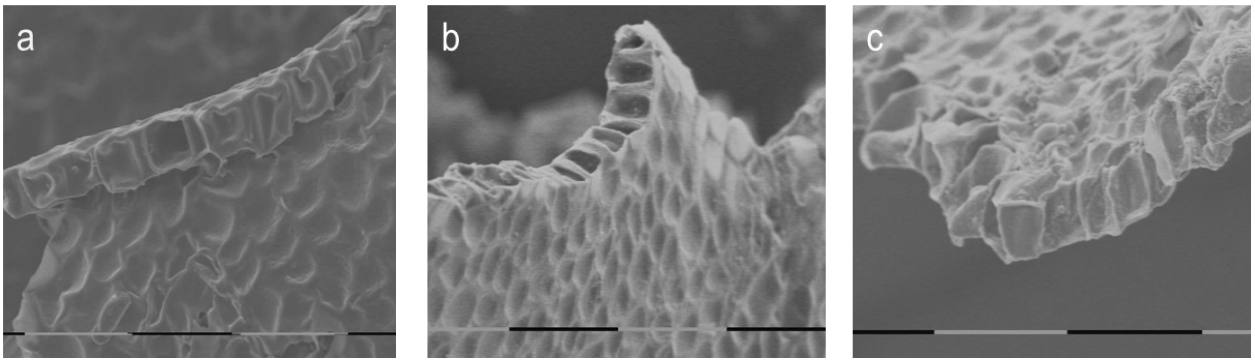

Figure 1: Transversal scanning electro-microscopy of aleurone layer fully separated from the other layers. Scale 100 µm. (a) AL CW, (b) Al DW, (c) AL EW

AL CW: Aleurone layer in common wheat ALDW: Aleurone layer in durum wheat AL EW: Aleurone layer in Einkinon wheat

2 Material and Methods

2.1 Plant material

One cultivar representative of each specie was used to characterize the AL. The three cultivars such as the common wheat Chinese Spring (CS); durum wheat Bidi 17 (Bidi17) and the Einkorn wheat DV92 (DV92), were collected from different experimental CS. Bidi 17 was grown in 2004 and 2007, not at two consecutive years like the CS and DV92. The dissection of the ALs was performed under the binocular microscope [13,14]. The isolated AL was kept at -80 °C before protein extraction and characterization.

2.2 Protein extraction and quantification

Total protein contents were quantified using the method reported in previous studies [12,15,16]. For each AL sample, from a given genotype, the samples were ground in liquid nitrogen introduced into Eppendorf tubes and separated into two biological extracts, allowing two repetitions per extract. These two extracts were then stored at - 80 °C for analysis [17].

2.3 Proteins identification

In this part, the spots, which are qualitatively and quantitatively different, have been selected and which the p-value is greater than or equal 0.05 to cut and hydrolyze by an enzyme and then identified by MALDI-TOF mass spectrometry. According to some authors [14,18] proteins are identified if they match a single reference in the databases: NCBI Viridiplantae, Poacea, and Wheat EST database.

3 Results

As reported in previous publications the protein maps of the AL showed a wide distribution of spots in the pI range from 3 to 10 and a mass range between 10 and 110 kDa for CW and DW, respectively [14]. Each 2DE revealed more than one thousand AL spots for each variety, while others were found common (identical position for pI and apparent MW) between CW and DW as well as for CW and EW (Figure 2). Thanks to the linear relation of the Coomassie staining, between protein contents and optical density, the proteome comparison was carried out for DW grown in 2004 and 2007. Neither qualitative nor quantitative differences between the grains harvested during the two different years were observed.

Proteins grouped in specific zones

Most of AL grown in the two years, had spots localized in three main zones (Figure 2). In addition, the results of the image analysis of AL proteome comparison did not reveal any qualitative differences between samples of EW grown in 2006 and 2007. Among eleven hundred AL spots, only 15 spots have significantly different in abundance between the two consecutive growing years of EW.

Zone I was composed of two proteins. Aldose reductase (spot 1302) involved in metabolism pathway; involved in environmental functions, metabolism and defense, and including polysaccharide, glycolysis, 1peroxyredoxin (spot 1717) both involved was involved in protection against oxidative stress (Table 1: supplementary data) This protein was more abundant in EW grown in 2006. Zone II and III out of these 15 spots were more abundant in EW grown in 2007. Ten spots were identified as Glo-3 involved in storage proteins; this globulin is light molecular weight. Among the nine spots, seven were globulins (spot number 2184, 2369, 2370, 3387, 2313, 3390 and 2249) of low molecular weight (19 kDa to 37 kDa). Those spots were obtained in the acid and basic region of the gel, when, one was a glyoxalase family protein (spot 2605) that was more abundant in EW grown in 2006.

4 Discussion

The proteomic approach used in our study was based on manual dissected AL under binocular. At least 30 grains per biological replicate were needed to isolate AL with sufficient protein amount to perform 2DEs for a given genotype.

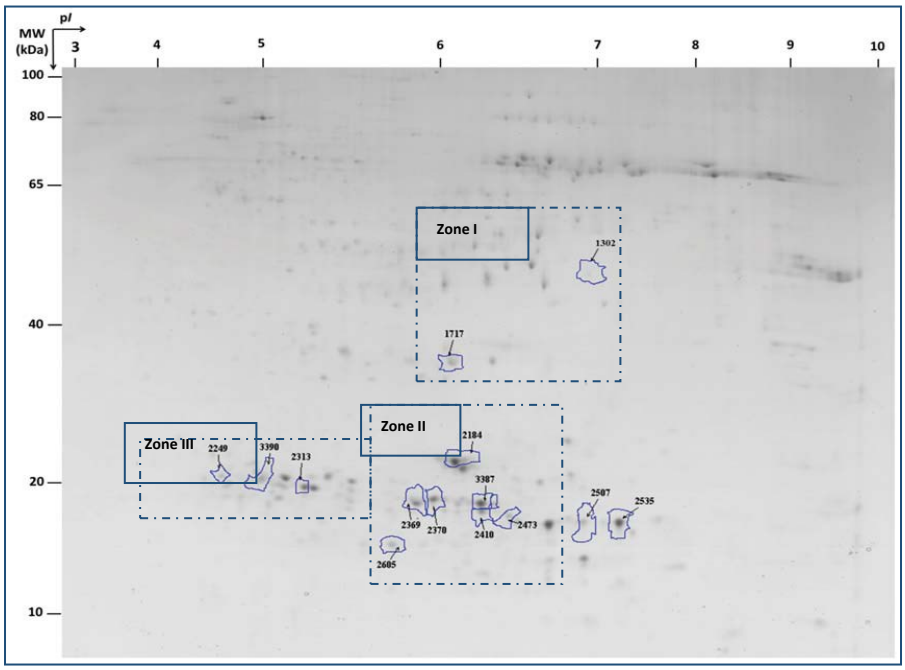

Figure 2: 2DE gel immobiline pH 3-10 x SDS-PAGE of the proteins extracted from AL of EW. The normalized volume of the surrounded and numbered spots differed significantly between the two growing years

The laborious hand isolation of AL was verified for each species using scanning electron microscopy (SEM) to guarantee the purity of the AL isolated (Figure 1). The proteome analysis revealed that the number of AL protein spots was not additive like the cumulated genomes in wheat grain: *i.e.*: common and durum wheat's genome ABD and AB did not show three times and two times the number of spots of Einkorn cultivar (genome A). Instead, most of the spots were revealed as common in interspecies proteomic comparisons [14]. For each of these species, a comparison of AL proteome grown in different environmental and weather conditions did not reveal any differences in terms of presence or absence between the AL proteome of the grains harvested the two years. In addition, no quantitative differences, in terms of spot abundance, were detected between AL proteome for both common wheat CW and DW. Only 15 spots (1 % of the stained AL spots) displayed differences between the two growing years for the EW. Why did we obtain such a consistency in the proteomic composition of the AL of wheat grain? - First, we may recognize that all wheat was grown under complete fungicide protection. Spike diseases, may attack grains and consequently induce different protein expressions, such as protease inhibitors, in the AL. - Secondly, field growing conditions, which differed mainly in temperature and rainfall amount, had an impact on the grain size and grain yield. The proteome of the AL in hexaploid and tetraploid wheat cultivars was however not quantitatively affected, whereas only EW had few proteins whose abundance was affected. This finding showed that the AL proteome is highly genetically controlled. The fact that most of the AL protein spots were common between the three species, and revealed through high-resolution 2DE gels, suggests that many genes encoding specific AL proteins in the Einkorn (genome A) are also present and probably also duplicated in the genome B and D of durum wheat and common wheat. Together with the gene duplication, the passage from the genome (A) to the durum wheat (genome AB) and common wheat (genome ABD) has undoubtedly led to a specialization of genes making it possible to respond better to climatic and environmental variations. The polyploid wheat possessing a larger area of production has progressively acquired more genes related to environmental response as compared to Einkorn with a narrower area of production. Thirdly, the possible major reason of this remarkable AL proteome consistency was due to the fact that AL is a living cell layer. The grain peripheral layers, composed of emptied cells together with endosperm, are all dead tissues. The AL and embryo are the living tissues of mature grain. Hence, to protect the endosperm sac against fungi attacks and possible consequences of microflora from hydrolytic influence, the AL has undoubtedly acquired during wheat evolution the needed numerous genes associated with proteome consistency and better response to environmental variation. Nadaud *et al.*, [18] analyzed the AL proteome evolution at fifteen stages during grain development.

Previous proteomic analyses showed that globulins were the major AL proteins (representing 65% of CW spots) of mature seed [19,20]. In our study, seven globulins were significantly more abundant in EW grown in 2007 than in 2006. These globulins correspond to 7S-globulins, which were identified in wheat species [19,20]. Globulins are involved in response to biotic stresses

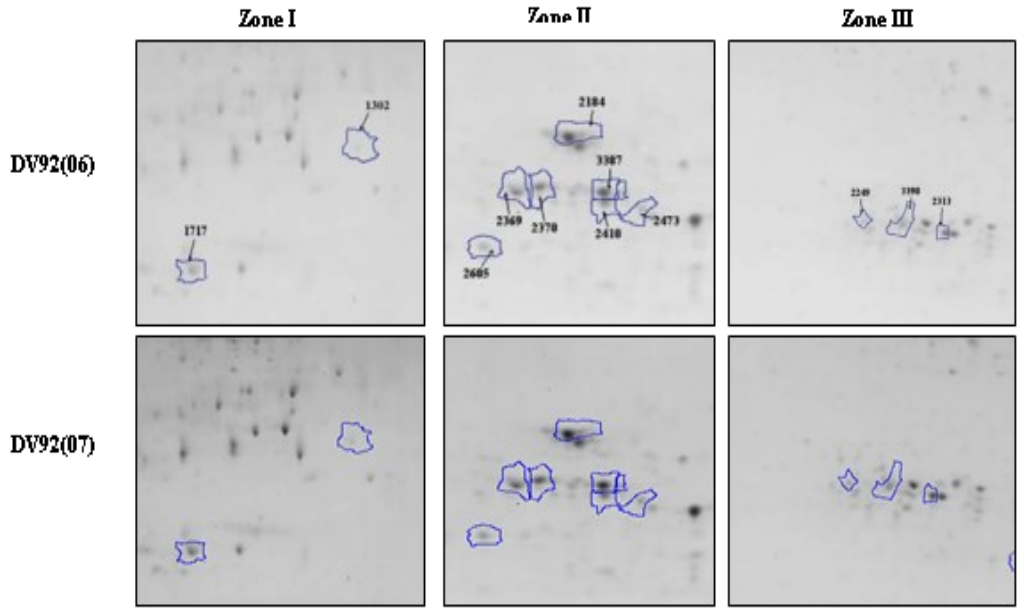

Figure 3: The three major zones where qualitative and quantitative differences for the AL proteins of the EW in mature wheat significantly between the two growing years

(synthesis of defense enzymes and inhibitors to face fungi attack, and bacteria) during grain formation, in response to abiotic stresses (mainly thermal and water) during the grain storage period [21]. Globulins are also involved in the synthesis of enzymes such as proteases and amylases in response to the stimulation of gibberellic acid causing germination.

Other proteins differently expressed in EW were identified: one belonging to stress/defense family proteins: the 1-Cys peroxiredoxin (1-Cys Prx), this protein possesses thioredoxin peroxidase activity, and plays a role in the reduction of hydroperoxides, being induced during oxidative stress. This protein present in cereals, which identified in the wheat endosperm (2), and AL (14), the 1-Cys peroxiredoxin genes are present in seeds, i.e. the embryo and the AL. The protein is expressed during the maturation phase of the grain, a sensitive phase in grain development but also during germination, where this protein is involved in protection against oxidative stress [19]. One aldose reductase and one glyoxalase were differently expressed in 2007 and 2006 in DV92. These two enzymes participate in the conversion of glucose to fructose, the detoxification of methylglyoxal, and the aldehydes of cellular metabolism (Table 1: supplementary data).

5 Conclusion

The aleurone layer constitutes an important source of micronutrients and its role in the mobilization of kernel reserves for germination has been widely studied. Our results clearly showed the proteome consistency (or proteome stability) of the AL from mature grains collected in the three major wheat species, when grown at different years with fungicide protection. This finding needs to be confirmed with wheat harvested in different controlled environments. Consequently, the genetic improvement of the nutritional value of wheat should make significant advances particularly through the knowledge acquired on the AL for human health benefit.

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References

1. Evers, T., & Millar, S. (2002). Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science*, *36*(3), 261-284. https://doi.org/10.1006/jcrs.2002.0435
2. Skylas, D., Mackintosh, J., Cordwell, S., Basseal, D., Walsh, B., Harry, J., Blumenthal, C., Copeland, L., Wrigley, C., & Rathmell, W. (2000). Proteome approach to the characterisation of protein composition in the developing and mature wheat-grain endosperm. *Journal of Cereal Science*, *32*(2), 169-188. https://doi.org/10.1006/jcrs.2000.0321
3. Abdel-Aal, E. M., Young, J. C., Wood, P. J., Rabalski, I., Hucl, P., Falk, D., & Frégeau-Reid, J. (2002). Einkorn: A potential candidate for developing high lutein wheat. *Cereal*

Chemistry Journal, *79*(3), 455-457. https://doi.org/10.1094/cchem.2002.79.3.455

4. Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*, *45*(1), 88-96. https://doi.org/10.1016/j.jcs.2006.07.004
5. Hidalgo, A., Brandolini, A., Pompei, C., & Piscozzi, R. (2006). Carotenoids and tocols of einkorn wheat (Triticum monococcum SSP. monococcum L.). *Journal of Cereal Science*, *44*(2), 182-193. https://doi.org/10.1016/j.jcs.2006.06.002
6. Hejtmánková, K., Lachman, J., Hejtmánková, A., Pivec, V., & Janovská, D. (2010). Tocols of selected spring wheat (Triticum aestivum L.), einkorn wheat (Triticum monococcum L.) and wild emmer (Triticum dicoccum Schuebl [Schrank]) varieties. *Food Chemistry*, *123*(4), 1267-1274. https://doi.org/10.1016/j.foodchem.2010.05.064
7. Lavelli, V., Hidalgo, A., Pompei, C., & Brandolini, A. (2009). Radical scavenging activity of einkorn (Triticum monococcum L. subsp. monococcum) wholemeal flour and its relationship to soluble phenolic and lipophilic antioxidant content. *Journal of Cereal Science*, *49*(2), 319-321. https://doi.org/10.1016/j.jcs.2008.12.004
8. Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science*, *46*(3), 327-347. https://doi.org/10.1016/j.jcs.2007.09.008
9. Hemery, Y., Lullien-Pellerin, V., Rouau, X., Abecassis, J., Samson, M., Åman, P., Von Reding, W., Spoerndli, C., & Barron, C. (2009). Biochemical markers: Efficient tools for the assessment of wheat grain tissue proportions in Milling fractions. *Journal of Cereal Science*, *49*(1), 55-64. https://doi.org/10.1016/j.jcs.2008.07.006
10. Carole, A., Lullien-Pellerin, V., Abecassis, J., & Rouau, X. (2002). Intérêt nutritionnel de la couche a aleurone du grain de blé. *Sciences des Aliments*, *22*(5), 545-556. https://doi.org/10.3166/sda.22.545-556
11. Brouns, F., Hemery, Y., Price, R., & Anson, N. M. (2012). Wheat aleurone: Separation, composition, health aspects, and potential food use. *Critical Reviews in Food Science and Nutrition*, *52*(6), 553-568. https://doi.org/10.1080/10408398.2011.589540
12. Tasleem-Tahir, A., Nadaud, I., Girousse, C., Martre, P., Marion, D., & Branlard, G. (2011). Proteomic analysis of peripheral layers during wheat (Triticum aestivum L.) grain development. *PROTEOMICS*, *11*(3), 371-379. https://doi.org/10.1002/pmic.201000333
13. Laubin, B., Lullien-Pellerin, V., Nadaud, I., Gaillard-Martinie, B., Chambon, C., & Branlard, G. (2008). Isolation of the wheat aleurone layer for 2D electrophoresis and proteomics analysis. *Journal of Cereal Science*, *48*(3), 709-714. https://doi.org/10.1016/j.jcs.2008.03.004
14. Meziani, S., Nadaud, I., Gaillard-Martinie, B., Chambon, C., Benali, M., & Branlard, G. (2012). Proteomic analysis of the mature kernel aleurone layer in common and durum wheat. *Journal of Cereal Science*, *55*(3), 323-330. https://doi.org/10.1016/j.jcs.2012.01.010
15. Meziani, S., Nadaud, I., Gaillard-Martinie, B., Chambon, C., Benali, M., & Branlard, G. (2014). Proteomic comparison of the aleurone layer in triticum Aestivum and triticum Monococcum wheat varieties. *Current Proteomics*, *11*(1), 71-77. https://doi.org/10.2174/15701646116661404152243 48
16. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
17. Rabilloud T., Charmont S. (2000). Detection of Proteins on Two-Dimensional Electrophoresis Gels. In: Rabilloud T. (eds) Proteome Research: Two-Dimensional Gel Electrophoresis and Identification Methods. Principles and Practice. Springer, Berlin, Heidelberg. 107-126. https://doi.org/10.1007/978-3-642-57105-3_5
18. Nadaud, I., Girousse, C., Debiton, C., Chambon, C., Bouzidi, M. F., Martre, P., & Branlard, G. (2010). Proteomic and morphological analysis of early stages of wheat grain development. *PROTEOMICS*, *10*(16), 2901-2910. https://doi.org/10.1002/pmic.200900792
19. Loit, E., Melnyk, C. W., MacFarlane, A. J., Scott, F. W., & Altosaar, I. (2009). Identification of three wheat globulin genes by screening a triticum aestivum BAC genomic library with cDNA from a diabetes-associated globulin. *BMC Plant Biology*, *9*(1), 93. https://doi.org/10.1186/1471-2229-9-93
20. Jerkovic, A., Kriegel, A. M., Bradner, J. R., Atwell, B. J., Roberts, T. H., & Willows, R. D. (2010). Strategic distribution of protective proteins within bran layers of wheat protects the nutrient-rich endosperm. *Plant Physiology*, *152*(3), 1459-1470. https://doi.org/10.1104/pp.109.149864
21. Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: Structures, properties and role in grain utilization. *Journal of Experimental Botany*, *53*(370), 947-958. https://doi.org/10.1093/jexbot/53.370.947

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