

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Short Communication

ISSN 2320-480X
JPHYTO 2019; 8(6): 303-305
November - December
Received: 18-10-2019
Accepted: 07-12-2019
© 2019, All rights reserved
doi: 10.31254/phyto.2019.8607

Meziani Samira

Biotoxicology Laboratory, Faculty of
Naturel Science and life Djillali Liabes,
University Sidi Bel Abbès, Algeria

Benali Mohammed

Biotoxicology Laboratory, Faculty of
Naturel Science and life Djillali Liabes,
University Sidi Bel Abbès, Algeria

Correspondence:

Meziani Samira
Biotoxicology Laboratory, Faculty of
Naturel Science and life Djillali Liabes,
University Sidi Bel Abbès, Algeria
Email: meziani_samira@yahoo.fr

Identification of globulins in aleurone layers of wheat species in mature grain

Meziani Samira*, Benali Mohammed

ABSTRACT

Subject description: The aleurone layer is a living tissue that contains many bioactive compounds. This layer is rich up mostly of proteins, minerals and vitamins. **Objective:** this work makes it possible to search for sites carrying post-traditional modifications on the sequences of three types of globulins identified that allow us to predict virtual reality. **Results:** The proteomic approach allowed us to identify numerous reserve proteins including three types of globulins (Glo3, Glo-3C, Glo3B) belonging to carbohydrate metabolism., 78% homology between Glo-3 and Glo-3 3B was found and 93% homology between Glo-3 and Glo-3C. Type 3B globulins are potentially three times more methylated, hydroxylated and much more ribosylated ADP than type 3 globulins. Type 3C globulins could be completely phosphorylated and five times more than type 3 and 3B globulins. They will then agree to confirm by chemical analysis and mass spectrometry this work.

Keywords: Wheat, aleurone layer, globulins, sequences, post-traditional modifications.

INTRODUCTION

Wheat is one of the most widely grown cereals in the world. Today, several studies have focused mainly on the extraction and separation of starch located in the endosperm to produce white flour. The germ and the inner and outer peripheral layers (Son) are excluded and used mainly for feeding cattle [5]. Among the tissues of bran, the layer aleurone (AL) which is the objective of this study, this layer is a living tissue of the mature grain or is located all the nutrients necessary for its development during germination. These nutrients are rich in proteins, particularly lysine, vitamins (B1, B2, B3, B6, B9, and E) and minerals (P, k, Mg, Mn, and Fe) are also found. In terms of health, it is a tissue that has a high antioxidant potential including bioactive compounds that are responsible for the health benefits of whole grains, as well as foods rich in these foods. The aleurone layer of wheat grains, because of its richness in micronutrients, is an important target for the enrichment and improvement of flours and other cereal products. In addition to its richness in vitamins and minerals, it seems to be also involved in the health benefits observed in the experimentation of AL fortified meal diet, semi complete (or complete) by the reduction of cardiovascular diseases, diabetes or colon cancer.

The methods of protein isolation, the separation and the characterization of the proteins of different layers of wheat, especially the aleurone layer, have been developed in recent years [12, 5, 7] and on peripheral layers and aleurone layers of two soft wheat cultivars by [2] and [7]. The analysis of this AL would therefore be to provide information on the origins of the different protein on 2DE gels from the major wheat genomes by analyzing the hexaploids. The objective of this study is to see the presence of post-translational modification sites of globulins identified in aleurone layer for wheat grain by a proteomic approach. The proteome of the aleurone layer of the cultivated wheat species therefore opens up possibilities for future research.

MATERIELS AND METHODS

Two wheat species studied in this work, soft wheat (AABBDD genome) and durum wheat (AABB genome). The isolation and extraction of the AL was carried out on 40 grains per species, selected according to their shapes in order to easily separate the layers with aleurone and to constitute homogeneous samples for the characterization.

Proteins extraction in aleurone layer

The AL proteins were extracted in 400-500 µL in buffer extraction (7 M urea, 4% chaps, 2 m thiourea, 1.2% destruct reagent (Amersham Biosciences) and 1% IPG 3-10 buffers (Amersham Biosciences), as previously described [7].

2D-electrophoresis and images analysis et identification

The gels were create from two independent extractions to allow comparison with all the extracts of AL and to have statistically reliable results. This approach is preceded by [7]. The preparative gels were stained with Coomassie Brilliant Colloidal Blue (CCB) G-250 according to [8] and improved by [9]. The selected protein spots were excised from preparative gels according to [4]. Subsequent identification of the peptides was performed using a MALDI-TOF Voyager Pro-DE mass spectrometer (Applied Biosystems, Framingham, MA, USA) as described [4].

Possible identification of post-translational modifications for the type of globulins

Using the sequences of globulins it is possible to investigate whether these proteins carry putative sites of post-translational modifications such as phosphorylations. The sequence analysis of types in 3 globulins using the NetPhos software (www.cbs.dtu.dk/services/NetPhos/) allows us to examine whether serines, threonines and tyrosines are potentially phosphorylated.

RESULTS AND DISCUSSION

In this work, the application of proteomics gives information in the study of the origins of the proteomes specific to the aleurone layers of the major wheat genomes has been achieved, by analyzing and determining on the genomes of wheat species, the distinction and the homology of proteins between species. This work offers the additional benefits that should be useful to better understand the functional properties of proteins of different species related to specific roles of synthesis, metabolism, whose nutritional and health benefits are an important point for future research. Since the proteins in the aleurone layer are essentially of the albumin-globulin type, they were extracted with the CHAPS buffer, Urea, Thiourea classically used for this type of protein. The technique of two-dimensional electrophoresis (E2D) implemented (iso-electrofocusing in a pH gradient 3-10 on 'strip' of 24cm) offered an optimal possibility of separating these proteins on the same gel.

The results in proteomics obtained by Meziani *et al.*, 2012 [7], allowed us to detect the similarity and the difference in protein between the different comparisons, the proteins of aleurone tissue have a role in the synthesis and assembly of proteins and which are the majority and similar in the wheat species studied, however, the profile, number and expression of proteins differ from one species to another. For these reasons, more than 95% of the revealed spots could be statistically comparable (quantitatively). Proteins varied quantitatively and qualitatively within each of the species belong are the majority in all species of wheat. 50% of them proved to be globulin-like reserve proteins. In the two comparisons between soft wheat and durum, it is found that globulins which are quantitatively different are more than 90% of them more abundant in wheat than in durum wheat. This indicates that there are genomes B and D. These globulins are mainly present in the vacuoles, or globular structures, which contain with the globulins an abundant amount of phytic acid (on average estimated at 152 mg g⁻¹ MS CA, [3] and, in addition to phosphorus, also elements such as Zn, Fe, Na, Mg and Al [11]). The proteomic approach has made it possible to identify and characterize three types of globulins. Glo-3,

Glo-3B and Glo-3C dominate in all species; they represent the majority of the identified proteins. These globulins correspond to 7S-globulins, whose genes have already been identified in hexaploid, tetraploid and diploid wheat species [6]. The three types of globulins observed in the AL analyzes fall into two classes, the highest category being between 45 kDa and 75 kDa and the second lower PM 20-30 kDa (Figure 1). This last class does not seem to correspond to the identified genes and could either result from the expression of pseudo-genes (partially truncated genes).

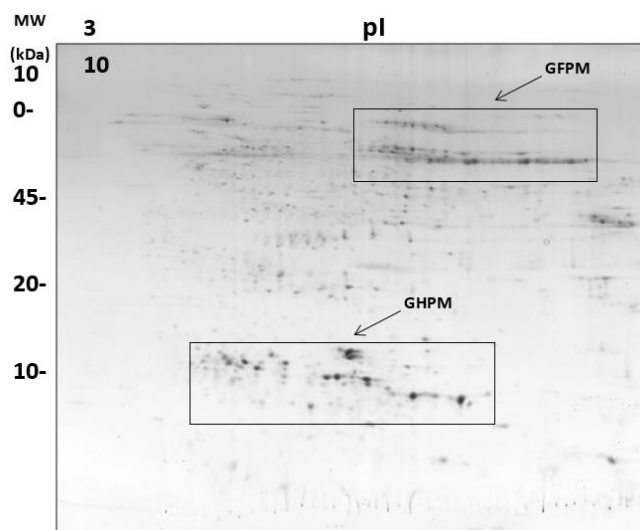


Figure 1: Images of a 2D IPG gel pH (3-10) 24 cm X SDS-PAGE of wheat aleurone layer (AABBDD). The spots are important major proteins identified. GFPM: Low molecular weight globulins. GHPM: High molecular weight globulins. [7]

It is very interesting to identify the presence of these three globulins in AL that are not studied previously. Glob-3 largely dominates its presence in the AL, all the same, some differences were evident, we have also evidences by the importance of Glo-3 and Glo-3B at the level of localization compared to Glob-3C, which were interspecific occurring in the genomes of wheat. Another point that was found that storage proteins (globulin3 and 3C) had higher expression levels in genomic in aestivum wheat (AABBDD) than in genomic durum wheat (AABB). The appearance of proteins in the genome (AABBDD) probably explains that the genes that control these proteins are located in the genome (AABB), but their expression is regulated by a suppressor located in the DD genome. Globulins are very different because they result, as we have seen, from the expression of several genes present on the genome A but presumably also duplicated on the genomes B and D. Moreover these reserve proteins do not seem to have enzymatic function clean, at least known to date. They thus have, like the albumen reserve proteins (gliadins and glutenins) accumulated mutations, revealed by their very high intra-specific diversity, which do not alter their structure and their reserve function of amino acids necessary for the synthesis of many enzymes that intervene in defense stress reactions and protection of parts of the grain before germination. It is also in the AL that the synthesis and accumulation of secondary metabolites occur, especially the B vitamins predominantly present in this tissue. We thus note that our study is a first approach opening real avenues of investigation to address the genetic and physiological aspects of the nutritional value of wheat. At the level of the globulin sequences of CAs themselves, 78% of homology between Glo-3 and Glo-3B was found and 93% of homology between Glo-3 and Glo-3C (Figure 2).

```

Glo-3
2HII  GIGlo-34005 COGIC
MATRARVITPLLFLGLTSLFAAAVSASHDEEEDRRGGRSLQQCVQR
NQRDRPRYSHARGVRESDDDQQQLHHEQERGRGRHRIHTE
REEEQGRGRGRHGQGEREEEQGRGRGRGGERDEEHGDGRRPVV
FGPFRSFRRIIRSDHGFVKALRPFDEVSRLLRGIRM4RVAIMEVNPRAF
VVPGLTDADGVGYVAQGEGLTVIENGEKRSYTVRQGDVIVAPAGS
IMHLANTDGRRLKLVIAKILHTISVPGKFQYFSAKPLLASLSKRVLTAAL
KTSDFERLGSLLGSRQGKFEFFKSSISIVRASEFQI BELRRQASEGDQGH
HWPLPPFRGDSRDFTNLLQRPKIANRHGRLYEADARSFHALAQHD
VRVAVANITPGSMTAPYLNTQSFKLAVVLEGEVEIVCPHLGRDSE
RRQEHEGKGRWRSEEEEDDRRQRRRRSGSGSEEEEQDQRYETVR
ARVSRGSAFVVPGGHPVVEIASSRGSNLQVVCFEINAERNRVWLA
GRNNVIAKLDPPAQELTFGRPARVEVFRAKDQDQDEGFVAGPEQ
QQEHERGDRRRGRDRGDEAVEAFLRMATAAL
    
```

75% homology between Glo-3 and Glo-3B
 93% homology between Glo-3 and Glo-3C

Figure 2: Alignment of the sequences of the 3 types of globulins using NetPhos software.

These globulins have a low carriers of phosphorylation sites. There are many methylation sites. globulins 3B type are potentially three times more methylated, hydroxylated and much more ribosylated ADP than globulins 3 type. The globulins 3C type could be completely phosphorylated and five-fold more than globulins 3B and 3B types (Figure 3). These observations are obtained by the bio-computer analysis of the sequences in the globulins and virtual prediction which it would be appropriate later to confirm by chemical analysis and mass spectrometry.

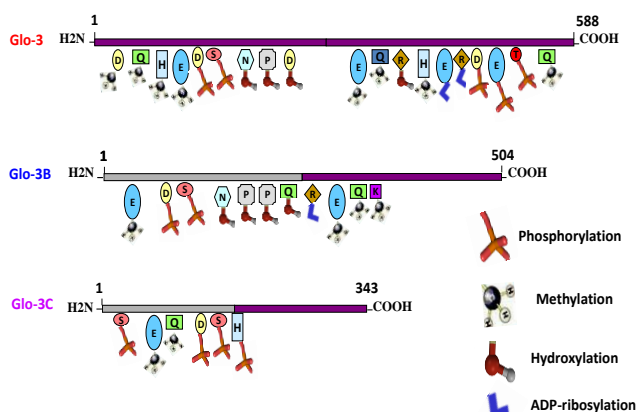


Figure 3: Schematic representation of the hypothetical post-traditional modifications in the sequences of the globulins type 3, 3B and 3C, predicted by FindMod (<http://web.expasy.org/findmod/>) and NetPhos v2.0 might have. (www.cbs.dtu.dk/services/NetPhos/) for the prediction of phosphorylations. The sequences used are those published by Loit *et al*, 2009.

CONCLUSION

The knowledge on this globulin allow it possible to better know the role of these proteins in the aleurone layer which should be useful to address, subsequently, the role of these proteins of the aleurone layer in the cellular functions at the mature stage of the grain such as than in the metabolism of amino acids or sugars or in the functions of regulation of the expression or cell protection largely implicated in the nutritional value of wheat.

Acknowledgements

Authors want to thank the staff of Biology. Department of Sidi-Bel-Abbes University, especially. Biototoxicology Laboratory, and Biochemistry Laboratory in INRA Clermont Ferrand, for valuable assistance in this study

Conflicts of Interest : Authors declare no conflict of interests.

REFERENCES

1. Abecassis. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science*. 2007; 46:327-347.
2. Antoine C, Peyron S, Lullien-Pellerin V, Abecassis J, Rouau X. Wheat bran tissue fractionation using biochemical markers. *Journal of Cereal Science*. 2004. 39:387-393.
3. Barron C, Samson MF, Lullien-Pellerin V, Rouau X. Wheat grain tissue proportions in milling fractions using biochemical marker measurements: application to different wheat cultivars. *Journal of Cereal Science*. 2011; 53:306-311.
4. Gobaa S, Bancel E, Branlard G, Kleijer and P. Stamp, Proteomic analysis of wheat recombinant inbred lines: variations in prolamin and dough rheology. *Journal of Cereal Science*. 2008; 47(3):610-619.
5. Hemery Y, Rouau X, Lullien-Pellerin V, Barron C, Abecassis J. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal Cereal Science*. 2007; 46 :327-347.
6. Loit Evelin, Charles W Melnyk, Amanda J MacFarlane, Fraser W. Scott and Illimar Altsaar Identification of three wheat globulin genes by screening a *Triticum aestivum* BAC genomic library with cDNA from a diabetes-associated globulin. *BMC Plant Biology*. 2009; 9:93. doi:10.1186/1471-2229-9-93.
7. Meziani S, Nadaud I, Gaillard-Martinie B, Chambon C, Benali MH, Branlard G. Proteomic analysis of the mature kernel aleurone layer in common and durum wheat. *Journal of Cereal Science*. 2012; 55(3):323-330. DOI: 10.1016/j.jcs.2012.01.010
8. Neuhoff V, Arold N, Taube D, Enhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis*. 1988; 9:255-262.
9. Rabilloud T. In *Proteome Research: Two-dimensional Gel Electrophoresis and Identification Methods*, Springer, Germany, 1997. pp. 107-126.
10. Rabilloud T. Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics*. 2002; 2:3-10.
11. Regvar M, Eichert D, Kaulich BA, Gianoncelli P, Pongrac K, Vogel-Mikuš Kreft I. Nouveaux aperçus sur les globuloïdes des vacuoles de stockage de protéines dans l'aleurone du blé en utilisant la microscopie à rayons X synchrotron. *Journal of Experimental Botany*. 2011; 62:3929-3939.
12. Surget A, Barron C. *Histologie du grain de blé*, Industrie des Céréales. 2005; 145:3-7.
13. Žilić Slađana. Phenolic Compounds of Wheat. Their Content, Antioxidant Capacity and Bioaccessibility. Slađana Žilić Department of Food Technology and Biochemistry. 2016; 2(3). DOI: 10.15406/mojfpt.2016.02.00037.

HOW TO CITE THIS ARTICLE

Samira M, Mohammed B. Identification of globulins in aleurone layers of wheat species in mature grain. *J Phytopharmacol* 2019; 8(6):303-305.