A PROTEOMIC APPROACH TO VERIFY IN VITRO AND IN VIVO EXPRESSION OF WHEAT GLUTEN PROTEINS


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Wheat storage proteins (gluten proteins) are mainly composed by gliadins and glutenins. Both components are characterised by extended repeated regions rich in glutamine and proline. Gliadins are monomeric proteins, whereas glutenins are polymeric proteins stabilised by disulphide bonds. However, it is possible that gliadins become part of the glutenin fraction when a different distribution of cysteine residues occurs.

Gluten proteins are responsible of qualitative properties of wheat flours and wheat-based foods. Dough viscoelastic properties depend mainly on gliadins and glutenins. Gliadins are considered the main factor triggering the most common human gluten intolerance, the celiac disease, although glutenin subunits seem also involved.

In order to study structure-function relationships of gluten components, and to understand better the genome organisation and the proteome composition of wheat endosperm, we are currently expressing heterologously (in E. coli) gliadin and glutenin genes, to obtain sufficient amounts of single polypeptides to be used in rheological and allergenicity tests. Since the great majority of the genes here used derive from genomic clones, we are verifying if they are actually expressed in planta. To this aim, we have used two-dimensional electrophoresis of heterologously expressed proteins in comparison to gluten patterns of the wheat cultivars used to construct the genomic library, and have performed N-terminal amino acid sequencing and mass spectrometry peptide mass mapping of putative corresponding spots. Moreover, because bacterially expressed heterologous polypeptides may undergo deletions within the proline-glutamine-rich repetitive region, we are using mass spectrometry to verify their correspondence with the expected polypeptides.