

## **CHARACTERIZATION OF WHEAT LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNITS AND THEIR MATURATION PROCESS**

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The glutenin polymers of wheat endosperm are made up of two main types, the high- (HMW-GS) and low- (LMW-GS) molecular weight subunits; subtypes of LMW-GS include LMW-m, LMW-s, and LMW-i, named according to the first amino acid of the mature sequence.

The main difference between LMW-m and LMW-s is the absence in the mature LMW-s type of the expected first 3 N-terminal amino acids (MET-) characteristic of the LMW-m type. According to DNA sequences, however, a nucleotide sequence corresponding to 3 amino acids is present in the sequences encoding both the precursors, although rather than being MET, the precursor sequence of LMW-s is MEN.

According to algorithms that predict the signal cleavage site, the signal peptidase should generate a QMET N-terminal sequence and removal of the N-terminal Q must occur in order to generate the m-type LMW-GS, unless the prediction is incorrect and the N-terminal Q is actually where the signal cleavage occurs. The presence of N instead of T in LMW-s was the basis for our hypothesis that a differential processing of the N-terminal end of the LMW-s sequence occurs such that cleavage at the N residue by an asparaginyl peptidase generates the observed N-terminus of the LMW-s type, similar to the processing that apparently occurs in  $\omega$ -gliadins.

In order to investigate this possibility, we produced transgenic wheat lines, transformed with mutated versions of the LMW-m and LMW-s genes, such that N was substituted for T in the LMW-m gene and T for N in the LMW-s gene, for comparison with their wild type counterparts. Western Blot analyses on 2D gels and proteomic comparisons between the transgenic and untransformed lines, have allowed to identify the transgenic polypeptides. MS-MS analyses have indicated that the processing occurs according to our predictions.