DIFFERENTIAL PROCESSING OF LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS MET- AND SER- TYPES AT THEIR N-TERMINAL END

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wheat, low molecular weight glutenin subunits, N-terminal processing, Nicotiana benthamiana

Gluten is a protein complex that confers unique visco-elastic properties to wheat doughs and is of critical importance in determining end product quality. Gluten is composed by gliadins and glutenins. Gliadins are monomeric proteins in which cysteines, if present, form only intra-molecular disulphide bonds and are classified into α/β-, γ- and ω-gliadins according to their mobility in lactic acid PAGE. Glutenins form polymers whose subunits are joined together by disulphide bonds. After chemical reduction, they release high and low molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively) whose amount, structure and interactions into the polymeric network strongly influence technological properties of wheat doughs. Whereas functional and structural properties of HMW-GS have been extensively investigated, LMW-GS are much less characterized due to their greater number and heterogeneity. On the basis of their N-terminal amino acid sequences, LMW-GS have been classified into two main groups, namely LMW-s (or LMW-Ser) and LMW-m (or LMW-Met) types. LMW-s are the most common and their amino acid sequences start with SHIPGL-; conversely LMW-m show more various sequences represented by METSHIPGL-, METSRIPGL-, METSCIPGL-.

It has been hypothesized that the substitution of a threonine at position 23 of the immature polypeptide by an asparagine residue could determine a differential processing at the N-terminal end of LMW-s type sequences, that might generate the cleavage of the peptide MEN by an asparaginyl endoprotease. In order to investigate the correctness of this hypothesis, we have expressed two LMW-GS in *Nicotiana benthamiana* by using an episomal vector based on PVX (Potato Virus X). The two LMW-GS expressed are represented by *wild types* LMW-m and LMW-s, along with the mutated forms at position 23. In particular, in the LMW-m type, the threonine at position 23 was substituted by an asparagine and in the LMW-s type the asparagine was conversely replaced by a threonine. Preliminary results give indication that the N-terminal amino acid sequence of the mutated LMW-m type (T23N) might be SCISGLERWQ- whereas the sequence of the *wild type* LMW-m is METSCISGLE-, thus supporting the hypothesis that the presence of an asparagine in position 23 causes a differential processing at the N-terminal end of the mature polypeptide. This work is still in progress and we are currently purifying the wild type and mutated LMW-s type in order to determine their N-terminal amino acid sequence.