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INTERCHAIN DISULFIDE BOND FORMATION IN THE ASSSEMBLY OF HIGH- AND LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNITS

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Tritucum aestivum, Triticum durum, High-Molecular-Weight Glutenin Subunit, Seed Storage Proteins, Gluten Proteins

Wheat seeds are one of the most important protein and energy sources for human nutrition. In addition, wheat seed proteins have unique functional characteristics that make wheat flour suitable for bread and pasta making. One major group of gluten proteins is constituted by the polymeric glutenins, consisting of Low-Molecular-Weight (LMW) subunits, High-Molecular-Weight (HMW) subunits, and "aggregated" gliadins. Interchain disulfide bonds play a crucial role in stabilizing the glutenin polymer but the organization of the different subunits remains largely unknown. To gain insight into the mechanisms controlling the assembly of glutenin polymers, we have expressed glutenin subunits, individually or in combination, in tobacco protoplasts. Using this system we have compared the assembly of two HMW subunits, Bx7 from *T. aestivum* cv Glenlea and Bx20 from *T. durum* cv Bidi17. The over-expression of Bx7 is associated with good pasta-making phenotype, while Bx20 has an opposite effect. The two proteins have similar amino acid sequence, but differ in the number of cysteine residue. The Bx20 precursor is a large protein of 795 aminoacids, and contains two cysteine residues, one in the N-terminal and one in the C-terminal non-repetitive domains (C31 and C783). The Bx7 subunit has 795 aminoacids, with four cysteines, three in the N-terminal (C31, C38 and C53), and one (C783) in the C-terminal domain. The role of individual cysteine residues in the formation of intermolecular disulfide bonds was studied through mutagenesis and substitution with the non polar aminoacid alanine. Bx20 mutants containing a single cysteine residue could still form homodimers, indicating that the protein can assemble via C31-C31 and C783-C783 interchain disulfide bonds. In addition, the two single cysteine mutants were able to dimerize with a LMW-GS, indicating that both cysteine residues in the Bx20 proteins can be involved in the interaction with this second class of gluten proteins. In the Bx7 protein, the C783A mutation was sufficient to block polymer formation, the mutant protein being recovered in monomeric and dimeric form. This indicates that the N-terminal domain of the protein, although it contains three cysteine residues, cannot by itself support polymerization, possibly because of the presence of an intrachain disulfide bond and/or because of a steric hindrance phenomenon. These results raise the possibility that, notwithstanding the different number of cysteine residues, both Bx20 and Bx7 subunits can only form linear, rather than branched polymers.