

STUDIES ON DURUM AND BREAD WHEAT LINES WITH LOW-AMYLOSE STARCH

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Starch is the major storage constituent and it is composed of two different glucan polymers: amylose and amylopectin. Amylose is a linear polymer of α -1,4 linked glucose residues with very few α -1,6 branches and it is about 20-30% of total starch; whereas amylopectin exists as shorter chains of α -1,4 linked glucose residues that are connected by α -1,6 glycosidic linkages resulting in a branched structure and constitutes the remaining 70-80% of starch. The relative amounts of amylose and amylopectin influence the physical-chemical properties of starches. By altering levels of key enzymes for the regulation of starch synthesis, it is possible to generate novel starches with new unique properties. The enzymes directly involved in amylopectin biosynthesis are starch synthases (SSs) and starch branching enzymes (BEs). The waxy protein is a granule-bound starch synthase responsible for amylose synthesis; in bread wheat (genomic formula AABBDD) three different isoforms are present which are encoded by three genes designated as *Wx-A1*, *Wx-B1* and *Wx-D1* located, respectively, on chromosome arms 7AS, 4AL and 7DS. In durum wheat (AABB) two isoforms are present, associated to the *Wx-A1* and *Wx-B1* loci.

Partial *waxy* mutant lines, characterised by the lack of one or two waxy proteins, have been identified by extensive electrophoretic analysis of durum and bread wheat. Crossing of these materials has permitted the combination of different *null* alleles detected both in a bread wheat line (N11) and in a durum wheat cultivar (Svevo) with the production of the entire set of partial lines along with the total waxy. Comparison of partial waxy lines (single and double *null*) with wild-type genotype has showed that amylose content is not linearly proportional to the number of the functional *waxy* genes. In fact there is a difference of 1-7% amylose content between wild-type and partial *waxy* mutants, whereas the total *waxy* genotype presents a drastic reduction of amylose content (0-1% of total starch). To investigate if the functional *waxy* gene compensated for the lack of other isoforms by producing more transcript and consequently more enzyme or increasing enzyme activity, a set of analyses has been performed on developing endosperms, both at the transcript and protein level.

In order to achieve this, gene specific primers have been identified and used for RT-PCR analyses on wheat kernels collected at different development stages.

The effects of the different waxy mutations on starch-pasting properties have been assessed by RVA (Rapid Visco Analyzer) analysis.