A STRATEGY FOR INCREASING AMYLOSE CONTENT IN DURUM WHEAT BY THE OVEREXPRESSION OF A WAXY GENE

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Reserve starch provides a food energy source for the human population. It is composed of two different glucan polymers: amylose and amylopectin. Amylose is a linear polymer and it constitutes about a third part of total starch, whereas amylopectin results in a branched structure and constitutes the remaining starch. The waxy protein is a granule-bound starch synthase responsible for amylose synthesis; in durum wheat (genomic formula AABB) two different isoforms are present which are encoded by two genes designated as Wx-A1 and Wx-B1 located on chromosome arms 7AS and 4AL respectively.

The amylose/amylopectin ratio influences the physical-chemical properties of starches. By altering the regulation of starch biosynthesis, it is possible to modify the amounts of amylose and amylopectin and to produce starches with new unique properties. High amylose starches are particularly interesting because they have an increased content of resistant starch that has beneficial effects on human health. The nutritionists believe that the resistant starch has a role similar to fibres inside the intestine preventing diseases as colon cancer, diabetes and obesity.

In order to produce transgenic wheat lines with high-amylose starch, the Wx-B1 gene has been isolated by RT-PCR from immature (21 days after anthesis) wheat kernels of the durum wheat cultivar Svevo and cloned in a vector for biolistic transformation. The construct contains an endosperm-specific high-molecular-weight glutenin promoter. Immature embryos of two durum wheat genotypes: Svevo and Svevo waxy (low amylose mutant lacking both Wx-A1 and Wx-B1 proteins) have been transformed. Eighteen transgenic lines have been regenerated (5 lines from Svevo and 13 lines from Svevo waxy) and screened by PCR using primer pairs specific for the construct. The presence of the transgene has been confirmed also by Southern blot analysis. To investigate if the Wx-B1 protein was overexpressed in putative positive plants, SDS-PAGE and densitometric analysis has been performed on the starch granule-bound proteins.

From our data it results that only two lines (generated by transformation of Svevo) overexpress the waxy protein. The analysis of the Svevo waxy transgenic has shown that some lines restored the wild type phenotype, although the Wx-B1 protein has not been found on the granules.