

## PRODUCTION OF HIGH AMYLOSE WHEAT LINES BY AFFECTING GENES INVOLVED IN AMYLOPECTIN BIOSYNTHESIS

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Starch is the major component of human diet, because it is present in considerable amount in common foods as pasta, bread, cakes, snacks, couscous, noodles. In the recent years, the production of wheat lines with an increased amylose content has received a great attention for its positive effects on human health. In fact, a positive correlation exists between the increase of the amylose portion in flour or semolina and resistant starch in foods. Resistant starch has a role similar to fibre preventing various human diseases as colon cancer, obesity and diabetes.

Reserve starch is composed from two glucan polymers, amylose and amylopectin that are synthesized beginning from a common substrate (ADP-glucose), but with different pathways. A granule bound starch synthase (GBSSI) or waxy protein is involved in amylose biosynthesis whereas the amylopectin is produced by the concerted action of different starch synthases (SSs), branching (SBEs) and debranching enzymes (DBEs).

In this work, classic and biotechnology approaches have been used to increase amylose content. A reverse genetic strategy (TILLING) has permitted to identify null and novel variants for *Sgp-1* genes by a screening of a mutagenised bread wheat population. Moreover, natural mutants lacking SGP-1 isoforms (corresponding to SSII) have been identified and crossed with the durum wheat cv. Svevo and a bread wheat line. Sets of partial and complete *Sgp-1* null lines have been obtained both in durum and bread wheat.

In these materials the amylose content was increased up to 43 % of total starch, whereas partial mutants showed an amylose content in a range between 26 and 35%. Starch properties were investigated by Rapid Visco Analyzer (RVA) and resulted deeply altered in *Sgp-1* null sets.

RNA interference strategy was applied to silence genes coding starch branching enzymes IIa (SBEIIa). The amylose content was strongly increased in transgenic lines in comparison with untransformed control (26,5%). Amylose value varied between 32.4 and 75%. SEM analysis showed deep alterations of starch granules. The granules in the lines with reduced *SBEIIa* expression were deformed with irregular shape, looked deflated and were smaller than in the control lines. Viscosity properties of starch was measured by RVA. Significant variations were observed for all parameters, which resulted significantly lower than the control lines. To verify whether the silencing of *SBEIIa* played additional effects on the expression of other genes involved in starch biosynthesis, semi-quantitative RT-PCR was performed on *GBSSI*, *SSI*, *SSII*, *SSIII*, *SBEI*, *SBEIIb*, *ISO1* and *LD* genes. Compared with the normal parent, the transcript level of the genes encoding *GBSSI*, *SSIII*, *LD* and *Iso1* was increased in transgenic lines, whereas the accumulation of transcript corresponding to *SSI*, *SSII*, *SBEI* and *SBEIIb* genes did not show any significant change.

The increase of mRNA level of affected genes were also investigated by quantitative Real-Time RT-PCR.