

SILENCING OF BRANCHING ENZYME IIa BY RNAi: A STRATEGY TO INCREASE AMYLOSE CONTENT IN DURUM WHEAT SEEDS

SESTILI F.*, JANNI M.*, DOHERTY A.**, IMPERATORI F.*, BOTTICELLA E.*,
PAOLETTI F.*, JONES H.**, D'OVIDIO R.*, LAFIANDRA D.*

*) Department of Agrobiological and Agrochemistry, University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo (Italy) - lafiandr@unitus.it

**) Rothamsted research Harpenden, Herts, AL5 2JQ (UK)

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Starch constitutes the major component of wheat seed, representing more than 70% of its dry weight. Reserve starch is composed of two polymers: amylose and amylopectin. Both are made from α -glucose, but amylose is linear, whereas amylopectin is a very branched polymer. Several approaches are being used in the attempt to modify amylose/amylopectin ratio; for instance efforts are addressed to increase amylose content due to the beneficial effects on human health.

Amylose and amylopectin biosynthetic pathways have a common substrate (ADP-Glucose), so recent works have shown that it is possible to alter the two polymers ratio by modulating the activity of enzymes involved in starch synthesis.

A transgenic approach has been undertaken in this work in order to increase amylose content. In particular starch branching IIa genes (SBEIIa) have been silenced by RNA interference. Two different durum wheat cultivars, Svevo and Ofanto, have been transformed by biolistic and Agrobacterium-mediated genetic transformation, respectively. The two constructs contain an endosperm-specific high-molecular-weight glutenin subunit gene (Dx5) promoter and terminator.

DNA fragment containing sequence corresponding to exons 1, 2, and 3 of the wheat *SBEIIa* gene has been isolated by RT-PCR and cloned in sense and antisense direction into the vectors used for the silencing. The two complementary fragments are linked by a sequence corresponding to intron 3, that plays a role of loop.

Sixty one transgenic lines have been regenerated (48 lines from Svevo and 13 lines from Ofanto) and screened by PCR using specific primer pairs for the Dx5 promoter.

The silencing of the SBEIIa genes has been investigated by immunoblotting with an antibody which recognizes SBEIIa proteins.

Unexpectedly, two new starch granule-bound proteins have been identified on SDS-PAGE separation of transformed lines. Moreover staining intensity of the other starch granule proteins (Sgp-140, Sgp-145, Sgp-1, Sgp-3 and waxy) is strongly increased in transgenic lines compared to the control. The new proteins are under investigation through mass spectrometry and N terminal sequencing. Preliminary analysis of T₁ lines indicated increase of amylose content in transgenic seeds.