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COMPARATIVE PROTEOMIC ANALYSIS OF METABOLIC AND STARCH GRANULE-ASSOCIATED PROTEINS IN WHEAT KERNEL OF A HIGH AMYLOSE TRANSGENIC LINE AND ITS CORRESPONDING UNTRANSFORMED CULTIVAR

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Wheat is widely cultivated in the world and represents one of the most important cereal for the human diet. The components that mainly influence semolina and flour quality are the proteins and the starch. Starch is of remarkable interest because it can be used for various applications in food and non food industry. It is composed by amylose and amylopectine, whose relative amounts affect its chemical-physical properties and final uses, In the last years various studies have been performed to clarify the mechanisms that regulate starch biosynthesis, in order to produce starches with specific properties, such as a higher amylose content, that has beneficial effects on human health.

In our laboratory, high amylose transgenic lines of durum wheat have been produced through the silencing of the Starch Branching Enzyme IIa (*SBEIIa)* genes. In this work, a 2D electrophoretic comparison between the kernel proteome of a transgenic line of the durum wheat cultivar Svevo with silenced *SBEIIa* genes and the corresponding untransformed line have been performed. In particular, two developing stages have been considered: the dough (15 dpa) and ripening (physiological maturity) stages.

As for the mature stage, the differentially expressed proteins have been identified by the software SameSpots Progenesis (Nonlinear Dynamics, UK) and characterized by MS/MS. The analysis of proteins associated to starch grains have revealed three polypeptides (a phosphorylase, a b-amylase and a ramification enzyme class I (SBEI)), that show a higher volume in the transgenic line with respect to cv. Svevo. This could be the resultof a compensating effect of the absence of the isoform SBEIIa. Moreover, four protein spots are present exclusively in the transgenic line and absent in Svevo. MS/MS analysis identified these polypeptides as Granule Bound Starch Synthase (GBSS) fragments.

Based on the analyses of proteins of the soluble fraction of the mature wheat kernel, corresponding to the so-called metabolic proteins, seven spots whose volumes are lower in the transgenic line respect to the control, have been also characterized by MS/MS. Among these proteins, some are involved in carbohydrate metabolism (glyceraldeyde 3-phosphate dehydrogenase, fructose bi-phosphate aldolase and b-amylase); others have a defense role against biotic and abiotic stresses (a-purotionine, a-amylase inhibitor, 3C globulin, HSP70), and these latter are also known for triggering adverse reaction in sensitive individuals.

The analyses relative to the dough stage of wheat kernels are under investigation.