

CHARACTERIZATION OF BREAD WHEAT STARCH MUTANTS BY TRANSCRIPTOME ANALYSES TARGETED TO GRAIN METABOLIC PATHWAYS

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Reserve starch, the major component of wheat kernel, consists of amylose and amylopectin. The variation of their ratio (about 1:3 in normal wheat) has a potential impact on nutritional and technological characteristics of wheat-derived foods. Previously, mutations in homeologs of three key genes involved in starch biosynthesis (*granule-bound starch synthase I*, *GBSSI*; *starch synthase IIa*, *SSIIa*; *α-glucan-branching*, *SBEIIa*) have been identified and pyramided obtaining three complete null bread wheat lines, respectively named Cad-*GBSSI*^{*}, Cad-*SSIIa*^{*} and Cad-*SBEIIa*^{*}. These genotypes have altered amylose/amylopectin ratios, starch properties, and polysaccharide compositions. In order to refine the characterisation of starch metabolism and to bring out transcriptional divergences underlying pleiotropic effects, the grain transcriptomes of Cad-*GBSSI*^{*}, Cad-*SSIIa*^{*} and Cad-*SBEIIa*^{*} mutants were compared to the wild-type at 15 and 25 days post anthesis (dpa). First, the principal component analysis of RNA-seq data scored similar gene expression between replicates and biological variability of the mutants. At 15 dpa, Cad-*GBSSI*^{*}, Cad-*SSIIa*^{*} and Cad-*SBEIIa*^{*} diverged for 1114, 1737 and 335 genes compared to controls, suggesting a stronger and precocious effect of the *SSIIa* loss of function. The transcriptome of all mutants encompassed over 3000 DEGs at 25 dpa, suggesting a later responsive boost. As for the starch pathway, DEG profiles were consistent with the patterns of over 30 key genes that were targeted in a previous work. DEG

analyses also pointed to gene sets that were modified recurrently or specifically in the mutants. The commonly altered genes included ca. 60 sequences related to carbohydrate pathways, while 658, 420 and 1282 were respectively peculiar for the Cad-GBSSI^{*}, Cad-SSIIa^{*} and Cad-SBEIIa^{*} genotypes. Moreover, these mutants shared common enriched GO-terms involved in nitrogen, glutamate/glutamine metabolism and proteolysis processes that further supported the alteration of C/N equilibria. Interestingly, GO-term analysis also pointed at some collateral effects that recurred in the three mutants and regarded the functioning of both chloroplast and photosystems, suggesting some interference with photosynthesis, a well-known process in immature seeds.