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TAILORING STARCH BIOSYNTHESIS IN WHEAT HITS CRUCIAL KERNEL METABOLIC PATHWAYS AS DISCLOSED BY MULTIPLE OMICS APPROACHES

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Engineering metabolic pathways of key compounds is one of the sustainable ways to enhance the nutritional value of food crops. As for staple crops like wheat, thoroughly consumed worldwide, even modest increases in good components can significantly contribute to fighting serious diet-related concerns in human health. Among nutrients, wheat is mainly a major source of carbohydrates in the form of starch; moreover, it partially covers protein demand and, to a less extent, provides essential micronutrients overall in whole grain-based food. Engineering starch biosynthesis by targeting key genes of the amylopectin pathway shaped new wheat genotypes significantly enhanced in a healthy valuable starch fraction, named resistant starch, (RS), providing benefits like those related to dietary fibers with negligible impact on sensorial food properties. Here, a set of two bread wheat lines, sharing the same varietal background ('Cadenza'), knocked- down in two genes coding for a starch synthase (SSIIa- Cad-SSIIa*) and a branching enzyme (SBEIIa- Cad-SBEIIa*) involved in the build-up of the amylopectin structure, resulted in a valuable increase in amylose and RS content. The previous characterization of these genotypes showed that altered starch biosynthesis resulted in changes at the phenotypic,

metabolic and gene expression levels in the mature seed, suggesting a close relationship between starch and several metabolic fluxes.

Given the above, a combined metabolomics/transcriptomics approach was used to investigate the nature and mechanisms governing these interactions in the immature caryopses of mutants vs the control 'Cadenza'. The results highlighted the occurrence of shared enriched pathways at the transcript and metabolomic levels in both Cad-SSIIa* and Cad-SBEIIa*. Specifically, a set of differentially expressed genes (DEGs) regarded the function and structure of chloroplasts and photosystems; consistently, a significant difference was measured in the levels of metabolites involved in chlorophylls, quinol, quinone, and tocopherols biosynthesis. In addition, electron microscopy evidenced modifications of chloroplast structure and thylakoid membranes organization. A second notable change emerged in nitrogen and sulfur metabolisms by enriched GO terms and crucial related DEGs in both lines, which found coherent abundance variation of several metabolites involved in amino acid and glutathione metabolism, supporting mechanisms of C reallocation and C/N/S partitioning related to mutations in the starch pathway.