Title:	Characterization of Wheat Granule-Bound Strach Synthase I Novel Alleles and GBSSI-PTST1 Interaction Affecting Amylose Biosynthesis
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Abstract:	Abstract: Starch, comprising amylose and amylopectin is synthesized by the coordinated activities of starch pathway enzymes. Amylose by virtue of its chemical composition offers several health benefits. Granule-Bound Starch Synthase (GBSSI) is the most abundant starch- bound protein and only known starch synthase to elongate linear amylose chains. Despite having comprehensive knowledge of the starch biosynthesis pathway, the allelic variation in GBSSI and their effect on amylose content is least explored. The present study identified a novel allele of GBSSI from EMS-induced wheat mutants. The GBSSIL.539P was found to contribute to high amylose content in wheat mutant ‘TAC 75’. GBSS1.L539P showed improved activity than wild GBSSI in vitro and in yeast cells. Structural analysis and molecular docking experiments revealed that the mutant residue is distant from the catalytic active site of enzyme and had no direct interaction with active site residues. our results indicated that the distant residue affected GBSSI activity by affecting its starch-binding ability and can be a good target for site-directed mutagenesis to modulate grain amylose content. As, GBSSI lacked any dedicated Carbohydrate-binding module (CBM) and no targeting mechanism have been identified crucial for localization of GBSSI to starch granules in wheat endosperm. Using homology-based approach we identified Protein targeting To Starch 1 (TaPTST1), containing potential coiled-coil structure that interacted with GBSSI in vitro and in planta. TaPTST1 was found to have N-terminal CBM mediated interactions with starch, amylose and amylopectin with different affinities. Thus, the present study reported TaPTST1, a homolog of AtPTST1 as a functional protein of starch biosynthesis, potentially involved in targeting GBSSI to starch granules in wheat endosperm.
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