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Mining of loss-of-function alleles on starch synthesis gene *TaSSIVb-D* by TILLING and characterization of their effects in wheat

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TILLING (Targeting-Induced Local Lesions in Genomes) is a powerful approach for novel allele identification and has been used to screen mutagenized plant populations for novel alleles of target genes. Starch synthesis IV (SSIV) is one of the important starch synthase isoforms for transient starch synthesis, but its impact on wheat starch synthesis has not yet been reported due to the lack of mutant lines. Using the TILLING approach and a EMS mutagenized population containing 3058 M2 individuals, we identified 54 mutations in the wheat gene *TaSSIVb-D*, with a mutation density of 1/165 Kb. Among these mutations, 26 are in the coding region, including 1 nonsense mutation (E054-13), 15 missense mutations and 10 silent mutations. Using SIFT program, 3 missense mutations and 1 nonsense mutation were predicted to have severe impacts on protein function. Results of RT-qPCR, transmission electron microscope and chlorophyll fluorescence analysis showed that, *TaSSIVb-D* was significantly down-regulated without compensatory increases in the homoeologous genes *TaSSIVb-A* and *TaSSIVb-B*. Altered expression of *TaSSIVb-D* reduced granule number per chloroplast compared with wild type and photosynthesis was affected accordingly. These results indicate that TILLING approach and our mutagenized population allows efficient identification of novel loss-of-functional alleles of target genes.

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