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CAS9 ENDONUCLEASE-MEDIATED MODIFICATION OF THE *BRASSINOSTEROID INSENSITVE 1* GENE OF WHEAT WITHOUT GENOMIC INTEGRATION OF RECOMBINANT DNA

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Site-directed genome modification technology facilitates the experimental validation of gene function and can speed up plant breeding by producing new genetic variability or by genocopying valuable alleles from one accession to another. However, its application is particularly challenging in wheat owing to high gene redundancy and cumbersome gene transfer and tissue culture procedures. Here, we report gene-specific induction of mutations in the *BRASSINOSTEROID INSENSITIVE 1* (*Bri1*) gene of wheat using a RNA-guided Cas9 endonuclease platform which relies on components of a microbial immune system called clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas9). *Bri1* is known from barley, where its mutation causes a reduction in plant height, which itself is an important trait in modern cultivars. However, it is not known yet whether the modification of *Bri1* is useful to generate new variability of plant height in wheat as well. Site-directed genome modification involves the guidance of Cas9 endonuclease by a target gene-specific guide (g)RNA to a genomic DNA motif of choice, where the target is cut so that error-prone DNA repair is induced, which entails the occurrence of nucleotide insertions and/or deletions. Our approach aims to generate target gene-specific mutant wheat plants without genomic integration of gRNA and Cas9-coding DNA, which is achieved through regeneration of doubled haploids from wheat x maize crosses. To this end, we use transgenic maize lines whose sperm transmits gRNA and Cas9 endonuclease genes as well as their products to the hybrid zygote. The gRNA/Cas9 coding sequences carried by the maize genome are then removed during early embryogenesis via uniparental genome elimination, which results in haploid mutant wheat plants that do neither carry any maize genetic material nor any transgene-coding DNA. Seedlings from rescued haploid wheat embryos can be subjected to colchicine treatment for whole genome duplication so as to generate fertile mutant plants. Our proof-of-concept constitutes a promising step towards the establishment of genotype-independent site-directed genome modification in wheat.