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Advancing gene editing: multiplex mutagenesis in hexaploid triticale

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The presence of several sets of chromosomes in polyploid crops is a serious problem for the application of gene and genome editing systems. Efficient CRISPR/Cas-based mutagenesis of series of genes involved in the grain starch biosynthesis of hexaploid triticale has been developed. Triticale (×*Triticosecale*), is a hybrid of rye (*Secale*) and wheat (*Triticum*) and consists of three subgenomes. Four genes were targeted and to ensure efficient editing of all subgenomes, a trio of guide RNAs for each target genes were designed. To enable simultaneous editing of 36 genetic loci at once (three sgRNAs × four genes × three subgenomes), an expression cassette was constructed, assembled as an array of twelve sgRNAs. The polysitron vector was delivered to morphogenic calli using a gene gun [1] together with a vector encoding Cas9 nuclease [2] to induce mutations. A number of transgenic plants of spring and winter triticale carrying both Cas9 and sgRNAs inserts have been generated. The efficiency of native gene editing varied depending on the target gene and sgRNA activity. Using a trio of sgRNAs for each target gene, we successfully mutated all three subgenome copies, thereby modifying seed starch synthesis. It can be expected that the described approach will make an important contribution to the future breeding of polyploid crops to produce various combinations of new genetic alleles encoding desired traits.

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Keywords: CRISPR-Cas9; polyploid crops; Triticale.

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