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Development and validation of an effective CRISPR/Cas9 vector for efficiently creates specific mutations at multiple loci using one sgRNA and transgene-free mutants in a wide range of plant species

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CRISPR/Cas9 is a valuable tool for both basic and applied research that has been widely applied to different plant species. Nonetheless, a systematical assessment of the efficiency of this method is not available for the allotetraploid Brassica napus—an important oilseed crop. In this study, we examined the mutation efficiency of the CRISPR/Cas9 method for 12 genes and also determined the pattern, specificity and heritability of these gene modifications in B. napus. The average mutation frequency for a single-gene targeted sgRNA in the T0 generation is 65.3%. For paralogous genes located in conserved regions that were targeted by sgRNAs, we observed mutation frequencies that ranged from 27.6% to 96.6%. Homozygotes were readily found in T0 plants. A total of 48.2% of the gene mutations, including homozygotes, bi-alleles, and heterozygotes were stably inherited as classic Mendelian alleles in the next generation (T1) without any new mutations or reversions. Moreover, no mutation was found in the putative off-target sites among the examined T0 plants.

In order to speed up mutant plants isolation, then we inserted a fluorescence tag (sGFP) driven by the constitutive 35S promoter into an available CRISPR/Cas9 vector pKSE401 to facilitate a visual screen of mutants. This modified vector was named pKSE401G and tested in several dicot plant species. Consequently, GFP-positive plants were readily identified through fluorescence screening in all of these species. Among these GFP-positive plants, the average mutation frequency ranged from 20.4% to 52.5% in Arabidopsis and B. napus with stable transformation, and was 90.0% in strawberry and 75.0% in soybean with transient transformation, indicating that the editing efficiency resembles that of the original vector. Moreover, transgene-free mutants were sufficiently identified in Arabidopsis in the T2 generation and B. napus in the T1 generation based on the absence of GFP fluorescence, and these mutants were stably transmissible to next generation without newly induced mutations. Collectively, pKSE401G provides us an effective tool to readily identify positive primary transformants and transgene-free mutants in later generations in a wide range of dicot plant species. These findings open many doors for biotechnological applications in oilseed crops.