

Hongtao Cheng¹Qiong Hu¹¹ Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, China**Background:**

Single nucleotide change is usually associated with agronomic traits and was applied as the important target for genetic improvement of crops. Base editors are recently developed technology, based on CRISPR-Cas9 system and cytidine deaminase or adenine deaminase, which do not require double-DNA-stranded breaks and can enable specific base conversion at the target site. Base editing method has become a promising tool for precise genetic modification of important agronomic traits. However, it's still hard to establish base editing system in oilseed rape with high efficiency. A3A-PBE consisting of human APOBEC3A cytidine deaminase and Cas9 nickase was demonstrated to be more efficient in converting C to T mutations with larger base-editing window.

Objective:

We try to establish and develop efficient base editing system in allotetraploid *Brassica napus* by using A3A cytidine deaminase fused with a Cas9 nickase and uracil glycosylase inhibitor. We want to create herbicide resistance and semi dwarf mutants, which are important agronomic traits for mechanic production in oilseed rape, by utilizing this system. Furthermore, we try to develop base editing system with extended PAM in oilseed rape.

Methods:

A3A-PBE system consisting of human A3A cytidine deaminase fused with a Cas9 nickase and uracil glycosylase inhibitor was used to construct the A3A-PBE vector. We designed three sgRNAs to target ALS, RGA and IAA7 genes, respectively. Genetic transformation mediated by *Agrobacterium* in oilseed rape was conducted. Semi-winter type oilseed rape variety Zhongshuang 6 was used as the transgenic receptor. Potential off-target sites were predicted by online tool CRISPR-GE. The potential off-targets were amplified by specific primers and used to perform Sanger sequencing.

Results:

Base-editing efficiency of the A3A-PBE was demonstrated to be more than 20% for all the three target genes. Target sequencing results revealed that the editing window ranged from C1 to C10 of the PAM sequence. Base-edited plants of ALS conferred high herbicide resistance, while base-edited plants of RGA or IAA7 exhibited decreased plant height. All the base editing could be genetically inherited from T0 to T1 generation. Several Indel mutations were confirmed at the target sites for all the three sgRNAs. Furthermore, though no C to T substitution was detected at the most potential off-target sites, large-scale SNP variations were determined through whole-genome sequencing between some base-edited and wild-type plants.

Conclusions:

A3A-PBE base-editing system could effectively convert C to T substitution with high-editing efficiency and broadened editing window in oilseed rape. Mutants for ALS, IAA7 and RGA genes could be potentially applied to confer herbicide resistance for weed control or with better plant architecture suitable for mechanic harvesting.