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Background:

Rapeseed is the main source of vegetable oil in China, and the annual average yield of rapeseed oil accounts for about 55% of the total oil production of oil crops. Rapeseed is a complex allopolyploid crop ($2n = 38$, AACC), with a complex genome, multiple homologous copy sequences, and often redundant or differentiated functions between different gene copies, which poses challenges for using conventional genetic manipulation to study gene function and subsequent molecular genetic improvement.

Objective:

Gene editing technology can artificially introduce targeted gene sequence deletions, insertions, or replacements to create heritable genetic variations, thereby achieving targeted and precise improvement of target traits. In addition, mutation sites of gene variations can be separated from transgenic insertion sites in offspring, producing mutation materials that do not contain transgenic elements, which has a significant impact on accelerating the cultivation of biological new products.

Methods:

we have developed an efficient genetic transformation system for rapeseed, with receptor materials including common rapeseed backbone parent materials such as ZhongShuang 6, ZhongShuang 11, and Zhongyou821. On this basis, we optimized and established rapeseed editing technology, which can achieve simultaneous mutation of multiple gene copies in rapeseed with a mutation efficiency of about 20%.

Results:

Using CRISPR/Cas9 genome editing technology, we analysed the function of homologous copies of genes related to rapeseed pod shattering, obtaining mutant materials such as bnshp1/shp2, bnind, and bnalc, which exhibited significantly improved resistance to pod shattering. Furthermore, we used gene editing technology to simultaneously mutate the six copies of the BnMOL6 gene, increasing rapeseed resistance to white powdery mildew and Sclerotinia. Editing the four copies of the BnVTE4 gene clarified the impact of different gene copies on α -tocopherol biosynthesis. Currently, most gene editing technologies use Agrobacterium-mediated genetic transformation to introduce genome editing vectors into plant cells. In order to overcome the dependence of genome editing technology on genetic transformation, we developed a gene editing method mediated by a double haploid induction system which can directly edit the genome of rapeseed via pollination and obtain mutant materials without transgenic elements. This provides a foundation for the rapid application of gene editing technology in breeding materials that are difficult to genetically transform.

Conclusions:

Genome editing technology is a powerful tool for studying gene function and creating new rapeseed materials. The genome editing technology mediated by the double haploid induction system can effectively break through the limitations of receptor material genotype on the application of genome editing technology, rapidly accumulating elite gene mutations into breeding materials, and providing technical support for the cultivation of high yielding, high quality, and multi-resistant new rapeseed varieties.