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Enhancing low seed glucosinolate breeding by editing a transporter gene lacking natural variation in *Brassica napus*

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Background:

A major challenge in current genetics study is to identify functions of genes with rare or no genetic variation through forward genetics approaches, such as quantitative trait locus mapping and association study in germplasm, and particularly in polyploid crops, it is difficult to study functional differentiation of duplicated genes.

Objective:

Here, we report a causal gene with rare mutation in glucosinolate transport and creation of a genotype of low seed glucosinolate for quality and resistance breeding of polyploid *Brassica napus* canola, the second largest global source of edible oil and protein meal.

Methods:

We provided a method to identify functions of genes with rare or no genetic variation in natural populations, we used genome-editing to create a low seed glucosinolate resource that is rare in the germplasm.

Results:

We comprehensively identified all homologs of A. thaliana GTRs from 12 de novo assembled *B. napus* genomes and those from one high glucosinolate cultivar ZY821 were divided into three subclades showing sequence differentiation of GTR1, GTR2, and GTR3. The expression patterns of all these 20 BnaGTRs are very complicated and there was no clear cue to judge their contribution to glucosinolates except for BnaA06.GTR2 showing the highest expression level in developmental silique wall, subsequently, whose genomic regions did not show significant association in the GWAS of seed glucosinolate content of our 312 diverse *B. napus* germplasm accessions. We then conducted CRISPR/Cas9 against BnaA06.GTR2 and significantly reduced the seed glucosinolate content compared to the wild type (ZY821).

Conclusions:

We provided a method to identify functions of genes with rare or no genetic variation in natural populations, we used genome-editing to create a low seed glucosinolate resource that is rare in the germplasm, and we proved BnaA06.GTR2 with rare natural mutation is crucial in seed glucosinolate accumulation in polyploid *B. napus*.