#028

Gene knock-out by CRISPR-Cas9 and EMS-induced point mutations on SEED FATTY ACID REDUCERS increase the seed oil content in rapeseed (Brassica napus)

Nirosha L. Karunarathna
Hans-Joachim Harloff, Christian Jung

Plant Breeding Institute , Christian-Albrechts-University of Kiel, Kiel, Germany

Rapeseed (Brassica napus) is one of the major oil crops grown worldwide as an important source of edible vegetable oil and protein-rich meal. Many attempts have been made to understand the oil biosynthesis aiming to increase the oil content in rapeseed. However, little attention has been paid to the understanding of lipid degradation that leads to a reduction of oil accumulation in seeds. Seed Fatty Acid Reducer (SFAR) genes belong to the GDSL lipases/esterases family, and their disruption leads to a seed fatty acid increase in maturing seeds of Arabidopsis thaliana (Chen et al. 2012). In this project, we aim at the knock-out of BnSFAR genes in rapeseed to increase its seed oil content.

We identified 12 homoeologous genes in the rapeseed genome for the five Arabidopsis genes SFAR1 to SFAR5. The CRISPR-Cas9 system was employed to generate mutations in all 12 paralogs using a common subfamily-specific target region. Agrobacterium-mediated hypocotyl transformation was used, and the transformation efficiency ranged between 0.2 and 1.1%. InDel mutations were found in BnSFAR4 and BnSFAR5 paralogs in transgenic T2 plants which have four and two paralogs in the rapeseed genome respectively. BnSFAR4 showed full gene editing in all paralogs while BnSFAR5 showed partial editing on both paralogs. Two independent mutant families were selected for each gene family to perform phenotyping. We observed a significant increase in oil content of 3-5% in bnsfar4 mutant T3 seeds compared to wild-type plants. Phenotyping of bnsfar5 mutants is undergoing. Moreover, we screened mutations in an EMS mutated winter rapeseed population and revealed nonsense and missense mutations on SFAR1 and SFAR4 paralogs. They were combined to produce double mutants, and phenotyping of double mutants also showed an increase in oil content.