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Poster Communication Abstract – 3.08

CRISPR-CAS9 SYSTEM TO MODULATE GLUCOSINOLATE (GLS) CONTENT IN CAMELINA SATIVA

PONZONI E.*, BRAMBILLA I. M.*, GALASSO I.*

*) Institute of Agricultural Biology and Biotechnology – CNR, Milan (Italy)

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In agriculture, toxic defence compounds in edible parts of crops (e.g. seeds, leaves or tubers) reduce their nutritional value. *Camelina sativa* (L.) Crantz is an important *Brassicaceae* oil crop with a number of excellent agronomic traits including low water and fertilizer input, strong adaptation and resistance. The main content of camelina seed is the oil (40%) that is rich in α-linolenic (omega-3, >35%) acid and tocopherols (800–1000 ppm). The seedcake (by-product generated after the seed oil extraction) is rich in proteins (30–40%), carbohydrates, phytochemicals and in a considerable amount of residual oil (10–15%), therefore it has been suggested as a new potential ingredient in livestock rations. However, exploitation of this seedcake may be limited by the presence of glucosinolates (GLSs), compounds that after hydrolysis produces different catabolites (e.g., isothiocyanates, thiocyanates, epithionitriles and nitriles) with detrimental and antinutritional characteristics. For this reason, genetic modulation of the GLS content in the seed will improve the nutritive value of this crop in the animal feed diet.

Recently, it has been demonstrated that the defence GLS compounds are translocated to seeds by transporters (GTRs) belonging to the NRT/PTRs family. In fact, mutations in these genes reduced the glucosinolate content in *Arabidopsis thaliana* seeds, but translation of loss-of-function phenotypes into *C. sativa* is challenging because camelina is an allohexaploid crop.

Genome editing allows the targeting of multiple copies of functional genes at once and this might be especially useful in plants as C. sativa with multiple chromosome sets. Therefore, CRISPR/Cas9 gene editing technology was used to generate mutations in the three homoeologous GTR2 (GTR2A,

GTR2B, GTR2C) genes, encoding a GLS transporter in camelina. Two different guide RNAs were designed following the guide RNA architectures (20nt-NGG). Camelina, cv. Calena, was transformed using the floral dip method with vectors containing Cas9/sgRNA constructs targeting the camelina GTR2 genes. Transgenic plants were self-pollinated, and leaves and seeds were collected from sequential generations of plants to determine by PCR/restriction enzyme analyses and DNA sequencing if gene editing events had occurred at the gene target sites. Twelve mutant events for the target genes were generated. Mutation genotypes with different indels events were found in two out of three GTR2 genes of the transgenic plants. But since the presence of Cas9/sgRNA in the samples could cause new mutations in subsequent generations, the T3 generation is under analysis in order to achieve the low-GLS phenotype.

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