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EMS- and CRISPR-Cas9 mediated mutagenesis in oilseed rape (Brassica napus)

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Brassica napus L. (oilseed rape) is an important dual use crop for oil production and as a valuable protein resource. Our projects are focused on yield potential, oil content and antinutritive compounds such as phytic acid and glucosinolates. Functional genomic studies by genetic modifications or mutagenization in rapeseed are difficult, as it is an allotetraploid species and for each Arabidopsis gene usually 2-9 homoeologs can be found that either have the same function or have undergone neo- or subfunctionalization. In different projects during the last years, we used two reverse genetic approaches to elucidate gene function and to generate prototypes with new qualities for rapeseed breeding. TILLING (Targeting Induced Local Lesions IN Genomes) was performed in an EMS mutagenized Express617 winter rapeseed population and simultaneous knock-out of homoeologous genes was carried out by a CRISPR-Cas9 approach. We will highlight advantages and disadvantages of both techniques for four traits: silique shatter resistance, oil content and phytic acid and glucosinolate accumulation. In the case of silique shatter resistance, two major transcription factors, INDEHISCENT and ALCATRAZ have been successfully knocked out, oil content was significantly increased by mutagenization of the Seed Fatty Acid Reducer (SFAR) 4 gene, whereas in the case of phytic acid and glucosinolate content major biosynthesis genes and transporters like MRP5, ITPK, GTR2 and CYP79 were modified and first results for a reduction of phytic acid have been obtained. The challenge for a successful strategy is the simultaneous knock-out of all functional gene copies, which can be realized by crosses of TILLING single mutations or multiple targeting by CRISPR-Cas9. We discuss ways how to cope with major drawbacks of both approaches like backcrossing strategies to decrease background mutation load in TILLING and how to avoid off-target effects and poor editing efficiency in CRISPR-Cas9.