High throughput reverse genetic tools for knocking out several genes of the phytic acid pathway in *Brassica napus*

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Brassica napus L. (oilseed rape) is an important oil crop in the temperate regions. After oil extraction, the seeds are used as a feed for livestock due to their rich protein and balanced amino acid content. However, the meal is not utilized efficiently because of the presence of high quantities of anti-nutritive substances. One of those is phytic acid, accounting for 2-4 % in oilseed rape cultivars. Phytic acid is an important source of inorganic phosphate for plants and is involved in many biological functions. It is still not known to what extent it is required to maintain the basic physiological functions. Identification of low phytic acid mutants may not only provide valuable information for understanding the biological function of the genes involved in the phytic acid pathway, but also result in improved seed quality. However, mutational analyses in oil seed rape is challenging due to polyploidization. Since gene functions are often encoded by several paralogs, more than one gene has to be knocked out to study the underlying effect. In this project, we adopted two different strategies for the mutational analysis. One approach is TILLING by sequencing and the other is genome engineering by using Streptococcus pyogenes Cas9 endonuclease. We chose most of the functional paralogs of seven crucial gene families (ITPK, MIPS, MIK, IMPK, PGK2, MRP5, IPK1) of the pathway. We were able to establish a high throughput mutant screening by sequencing, which resulted in an average mutation density of 1/18 kb in all the targeted genes. Furthermore, by targeting the conserved regions of two subfamilies of the ITPK gene family we obtained gene editing in the spring rapeseed cultivar Haydn by using hypocotyl transformation.