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Discovering novel phytic acid mutants in oilseed rape for future breeding

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Phytic acid is a major phosphorous storage compound in seeds of various plant species. Brassica napus L. (oilseed rape) has 2-4% of phytic acid content in their seeds. Being a major producer of oil in the temperate regions, the meal of oilseed rape seeds also serves as well balanced diet for animals. However, the negative charge of phytic acid reduces the bio-availability of essential minerals from the diet. Furthermore, the lack of phytase in mono gastric animals results in excretion of undigested phytic acid, which causes eutrophication. Phytic acid is synthesized by two independent pathways consisting of 9 gene families. The active role of intermediates of the pathway in various other biological pathways makes it critical to aim for complete knockouts. Our project aimed at discovering novel low phytic acid mutants in oilseed rape with no pleiotropic effects. The decision of choosing the candidate genes is a key in polyploids due to the presence of multiple gene copies. Furthermore every enzyme of the pathway is encoded by several subfamilies. After mRNA expression analysis in seeds, we chose two major genes of seven gene families for our knock out strategies. We implemented TILLING by sequencing, which resulted in an average mutation density of 1/18 kb in all the targeted genes. Although, EMS derived mutants are considered as non GMO with the European regulations, TILLING mutants' takes longer time for pyramiding the mutated genes. Furthermore, we could not apply TILLING by EMS for one gene family BnITPK, because of its high copy number in oilseed rape. Therefore, we used CRISPR-Cas9 by using a conserved target site for simultaneous knock of several paralogs of the BnITPK gene family. Finally, in our study we could identify novel phytic acid mutations with reduced phytic acid content, which could be used as animal feed. Apart from the use as an animal feed, phytic acid mutants may provide as valuable information for understanding various other myo-inositol dependent pathways.