CRISPR/Cas9 induced point mutations in genes active during male reproductive development

Multiple genes with partly redundant functions govern many developmental processes in plants. In cases where the genes are genetically linked, functional analyses have been very challenging due to difficulties in generating double mutants through conventional crosses. The CRISPR/Cas9 technology provides an opportunity to induce point mutations in genes that are located closely together on the chromosomes. In the current project we have induced point mutations in a gene, *A120*, which is active during stamen development. Subsequently we will induce point mutations in neighbouring genes. The long-term goal of this project is to increase our basic understanding of male reproductive development in flowering plants. This is of importance for *e.g.* hybrid seed production in many of our crops plants.

Primary transformants are always heterozygotes with respect to a CRISPR/Cas9 transgene. In consecutive generations it is possible to segregate away the transgene while keeping the mutated gene. Hence, no traces of recombinant DNA are present in the plants that will be used in our field trials. The only difference from wild type will be the induced mutations in the *A120* gene. A complementation test in which the wild type of *A120* allele is introduced into an *a120*-mutant background will also be performed.

We asked the Swedish Board of Agriculture if approvals for GMO-field trials are necessary for all or any of these three types of *a120*-mutants: *i*) Primary transformants with point mutations in the *A120*-gene and the CRISPR/Cas9 transgene present, *ii*) Plants with mutations in the *A120*-gene and no CRISPR/Cas9 transgene present, *iii*) *a120*-mutant plants complemented with the wild type allele. The answer from the competent authority was that applications for GMO-field trials are necessary for the first and third type of *a120*-mutants while the second category should not be considered as a GMO. Hence, no applications for GMO field trials are necessary for plants harbouring CRISPR/Cas9 induced point mutations provided that the CRISPR/Cas9 transgene has been segregated away.

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Further reading:
Exam work: Eriksson, Ida, 2015. *CRISPR Cas9 system for plant genome editing within the European Union.* http://stud.epsilon.slu.se/8549/