ViciaMut:
DNA-free induction of sequence specific **mut**ations in *Vicia faba* using the CRISPR/Cas system

"Crops for the future"

Bundesministerium für Bildung und Forschung

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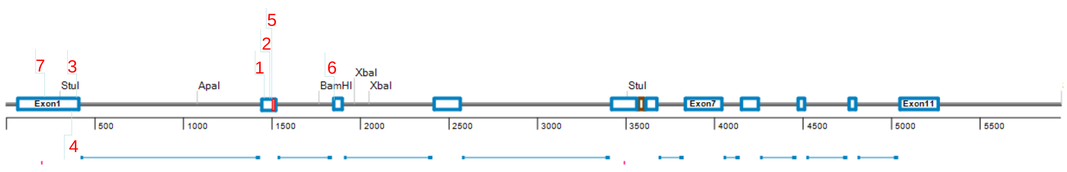
Why genome editing for Vicia faba?

- **Faba bean** (*Vicia faba* L.), a domestic grain legume in Germany, is a **major food and feed legume because of the high nutritional value of its seeds, which are rich in protein and starch**
- Legumes are architects of soil health
- Cultivation of Faba bean is still limited due to a range of factors such as the high susceptibility to diseases and pests and the sensitivity of the crop to adverse environmental conditions.
- **Plant breeding has to cover the need for appropriate faba bean cultivars with high yield stability in a clear time frame**

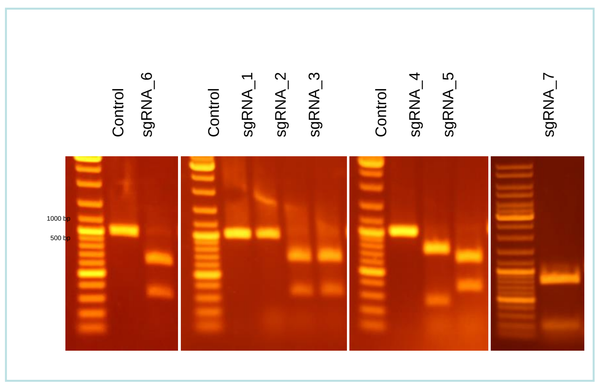
Target gene



Genomic structure of the **Phytoene desaturase** (VfPDS) gene and location of the designed sgRNAs

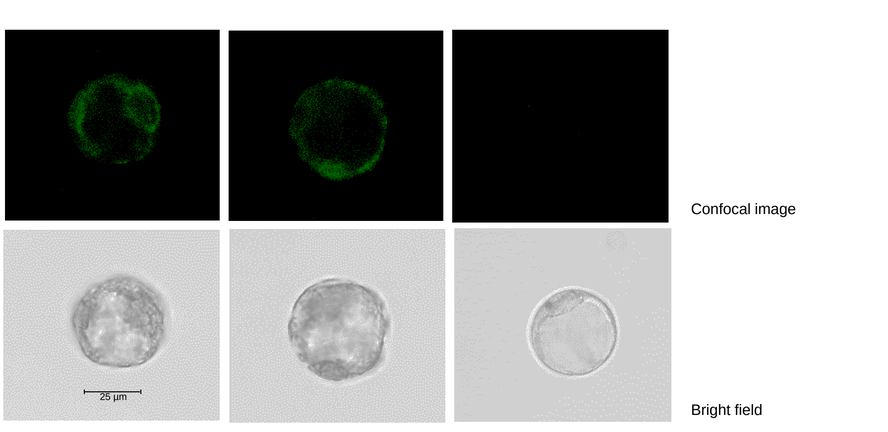
#	Sequence	PAM	Site	Function *in vitro*
sgRNA_1	TCACAAGCCTATATTGCTGG	AGG		–
sgRNA_2	CTGGAGGCAAGAGACGTTCT	AGG		+
sgRNA_3	GTGTTAAGAGAAATGGGTTT	AGG		+
sgRNA_4	GCGAGGAGAAGCACGAAAGG	TGG		+
sgRNA_5	GAGGCAAGAGACGTT**CTAG**G	TGG	**BfaI**	+
sgRNA_6	GGAAAGATGAAGACGGAGAC	TGG		+
sgRNA_7	CAACCTAAAGTTAA**GGCCC**A	TGG	**SauI96**	+

Listing of designed sgRNAs
to target the PDS gene of *Vicia faba*

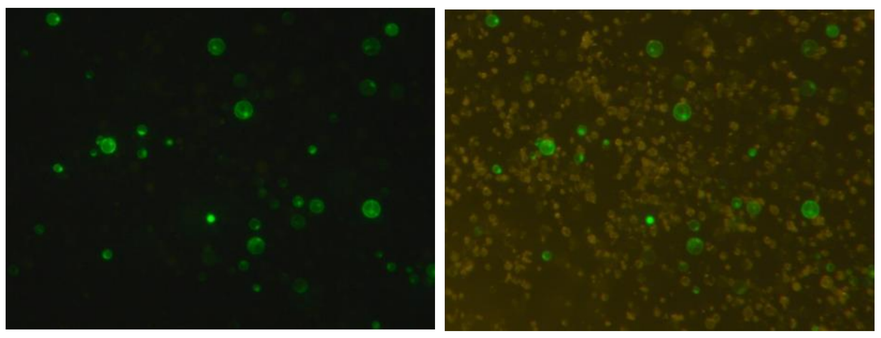


Results of *in vitro* digestion assay

Protoplast system



Epicotyl derived protoplast culture



Plasmid 35S::GFP, 48 h post transfection

Confocal image

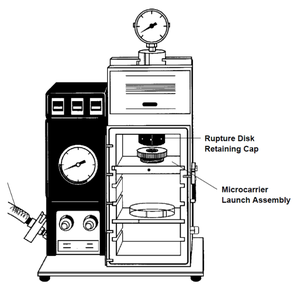
Bright field

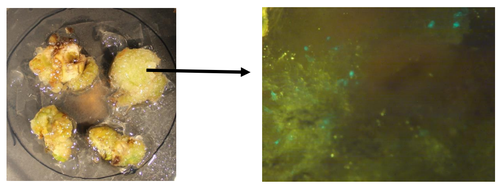
RNP [GFP Cas9 NLS], 24 h post transfection

The **aim** of our project is the development of an efficient **DNA-free** technique to introduce sequence specific mutations based on **biolistic delivery** of ribonucleoprotein complexes of the CRISPR/Cas system in embryonic explants derived from mature seed from faba bean. The axis of the embryo and its derived tissues has been described as highly morphogenic. Direct shoot organogenesis could already be induced from meristematic cells derived from embryo axes of faba bean. The potential of the CRISPR/Cas-system for *Vicia faba* by our proposed method will be proven by targeting sequence specific mutation in the phytoene desaturase gene. The disruption of the phytoene desaturase gene results in a visual detectable albino genotype. Our proposed method of genome editing for faba bean will pave the way for more efficient breeding processes of this crop. Currently we are analysing the efficiency of the sgRNAs in protoplasts of *Vicia faba*. A transfection method was established. Induced Mutations are analysed by PCR based method or targeted deep sequencing.

Bottleneck for the application of genome editing is, however, the recalcitrance of faba bean regarding tissue culture techniques. In general, *Vicia faba* tissue displays a **low morphogenic potential** and has a tendency to browning. Nevertheless, we were able to establish an efficient regeneration protocol for faba beans using explants of the embryogenic axis of mature seeds according to the method from Anwar et al (2011). After RNP delivery and regeneration of shoots with putative targeted mutations of the PDS gene homozygous and heterozygous mutations have to be selected and analysed via PCR. Therefore a high throughput DNA isolation for faba bean will be established.

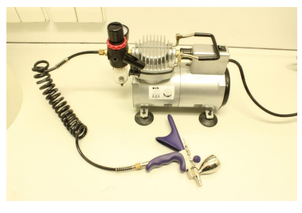
Delivery of RNPs

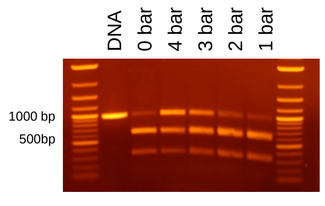




Plasmid 35S::GFP
3 d post bombardment

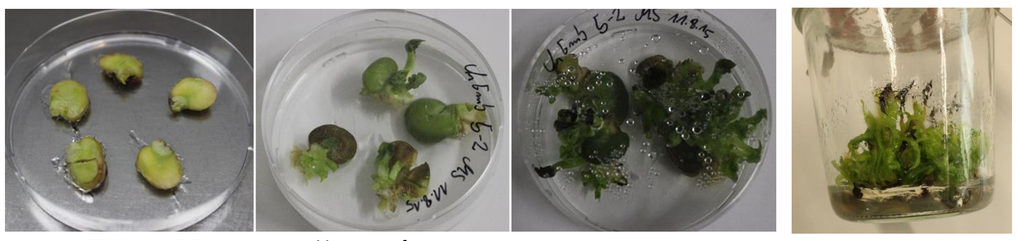
- **Particle Bombardement**
- **‚high pressure' spraying[1)]**
  - Applicable under sterile conditions
  - Adjustable 1-4 bar
  - Successful delivery of RNA



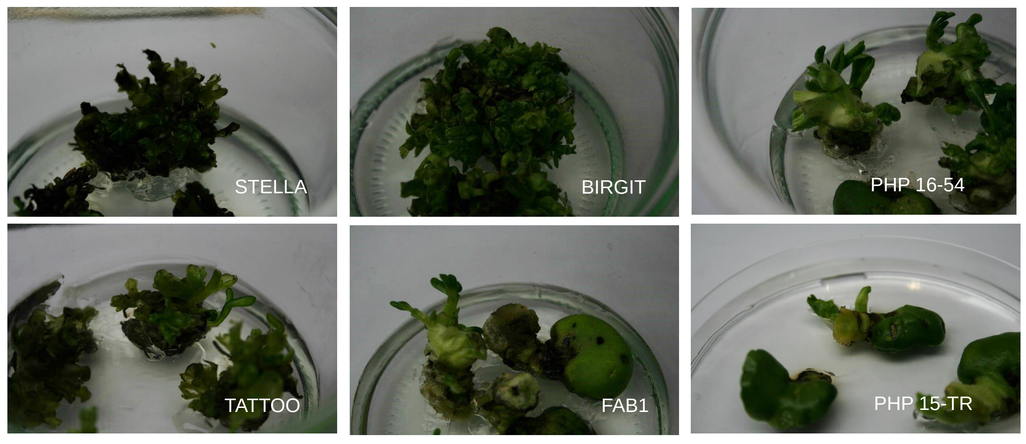


Functionality test of Cas9
after pressure treatment

Target tissue



Formation of morphogenic callus material from explants of the mature embryo



Screening for genotypes with high morphogenic potential

References: Anwar F, Alghamdi SS, Ammar MH, Siddique KHM (2011) An efficient in vitro regeneration protocol for faba bean (*Vicia faba* L.) Journal of Medicinal Plants Research, 5(28): 6460-6467.
[1)]Dalakouras, A., Wassenegger, M., McMillan, J., Cardoza, V., Maegele, I., Dadami, E., Runne, M., Krczal, G. and Wassenegger, M. (2016) Induction of silencing in plants by high-pressure spraying of in vitro-synthesized small RNAs. Frontiers in Plant Science, 7.