Testing of CRISPR-Cas technologies

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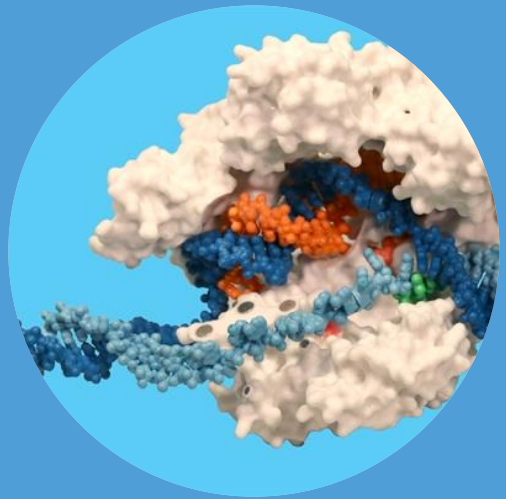

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100years
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New project on CRISPR-Cas technologies

Rationale:

Generally we use CRISPR-Cas as a tool. Not as aim.

We prefer to use the most efficient CRISPR-Cas elements.

Many CRISPR-Cas variants for gene editing. Limited time to test.

A new 4 years project, dedicated to testing CRISPR-Cas components in plants.

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Project proposal.

- Last Friday granting letter received!
- No results yet.
- 30 minutes presentation?

4-year project

- Size: 1,440 kEuro
- 9 (plant breeding) companies involved

- Testing will be done mainly by Wageningen UR

Four Work Packages (WPs)

WP1: Comparison of efficiencies of the building blocks

1.1 Cas genes

- Different *Cas9* genes
- Different *Cpf1* genes, including *Mad7, TT-Cpf1*
- Type I CRISPR-Cas3 system

Testing mainly in protoplasts of tomato

WP1: Comparison of efficiencies of the building blocks (cont.)

1.2 Promoters:

- promoters of *Cas* genes
- promoters of gRNAs (U6-1, U6-26, etc.)

WP1 (cont.)

1.3 Multiplexing gRNAs

Should each gRNA have its own promoter?

Or is it better to use one promoter, and separate the gRNAs subsequently?

- **1.4 Testing in other crops**

‘In kind’ contribution from companies.

WP2. Delivery systems

Ribonucleoprotein (RNP) delivery (DNA-free)

In vitro synthesised gRNA and nuclease

Transfection of tomato protoplasts

Virus-induced mutagenesis

Nanoparticles as vehicles

WP3. Different types of gene editing

3.1 **Expression modulation through deleting domains of promoters**

- Multiple gRNAs -> different large deletions
- Test for *FT* gene -> (very) early flowering when higher expressed

3.2 **Modifying chromatin state (epigenetics)**

- deadCas9 fused to transcription activators (e.g. DNA demethylases) or transcription suppressors (e.g. DNA methylases, histone modifiers).

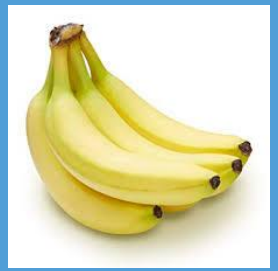
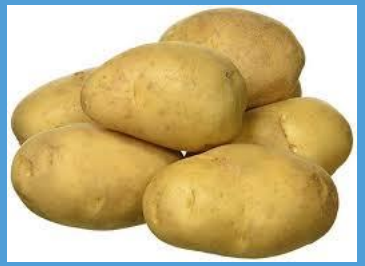
3.3 Genome editing via homology-directed repair (HDR)

Template sequences introduced into plant using geminiviral replicons (Dahan-Meir et al., 2018).

Flexible program.

WP4: Mutagenesis in polyploid species

Many crops are polyploid


Knocking out all alleles very difficult or impossible for classical mutagenesis (especially for allopolyploids).

Targeted mutagenesis THE solution.

Potato as model for this WP.

Co-workers

- Gerco Angenent
- Jan Schaart
- Ruud de Maagd
- Henk Schouten
- Several technicians (t.b.d.)

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