Hudson River

Protoplast-CRISPR technology in strawberry

Lena Maas, Head of R&D, Hudson River, NL

Strawberry congress
Antwerp - 17.09.2025

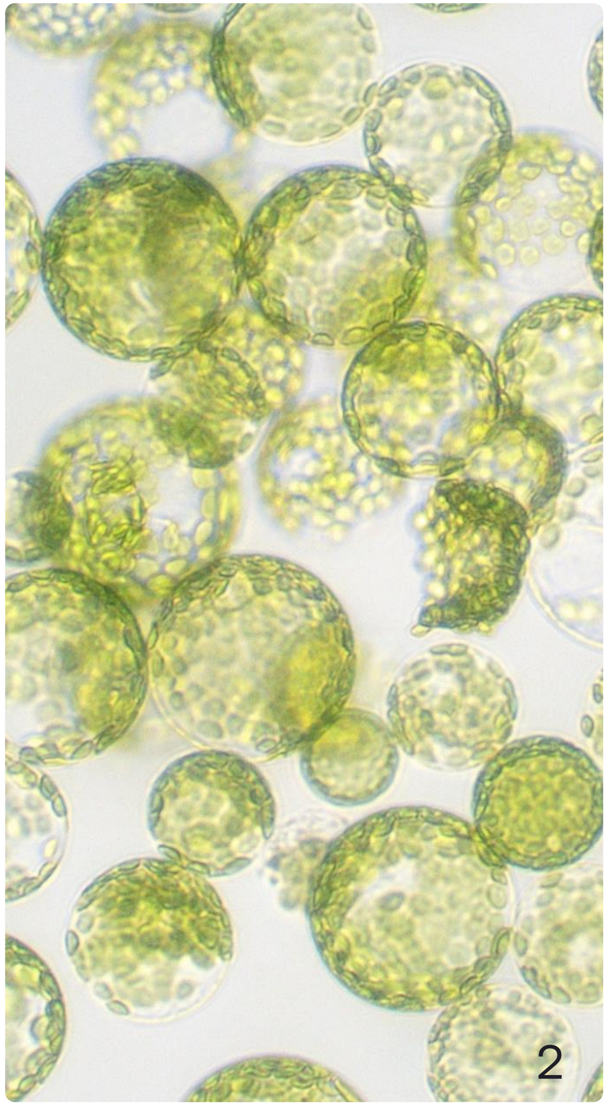
Hudson River at a glance

CRISPR for breeding

Independent and in partnerships

Focus on strawberry & tomato

- **19 employees (~80% R&D)**
- 350 m^2 lab,
- 300 m^2 office
- **Independent!**
  Backed by angel and strategic investors



Vision

- Become a global leader in CRISPR plant breeding, bridging the gap between innovation and commercial impact

Mission

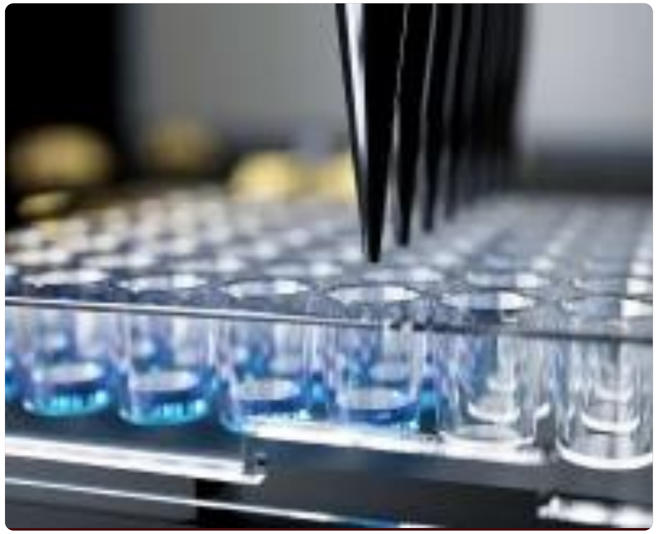
- Developing new **plant traits** by combining cutting-edge gene editing with deep expertise in **plant regeneration** – turning genetic concepts into verified plant performance

- **Trait-development partners**

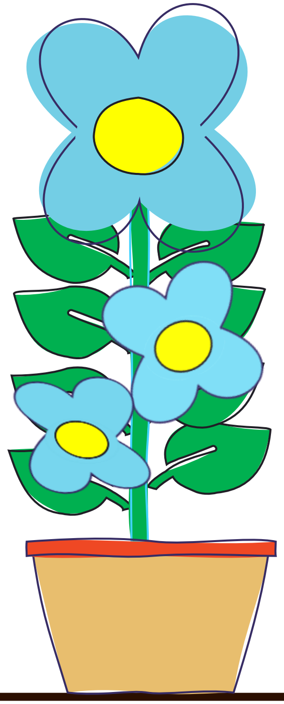
plantik

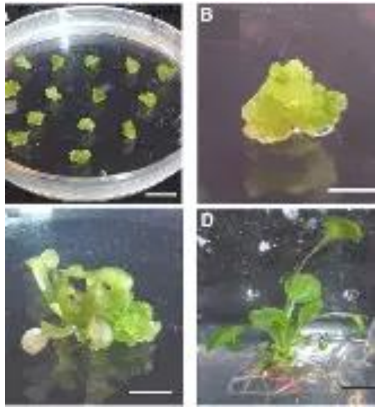
Resurrect bio

computomics®
AI TECHNOLOGY FOR GROWTH

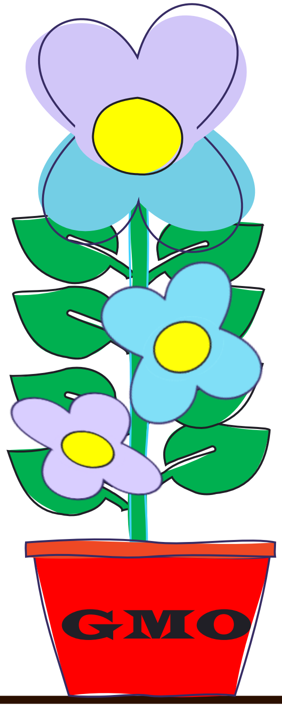


Transgenic CRISPR approach not suitable for all crops and markets

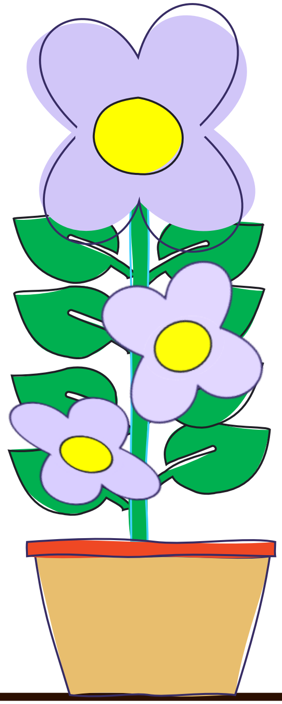




Transgenic route
to CRISPR editing



Cross out transgenes
&
remove heterogenity

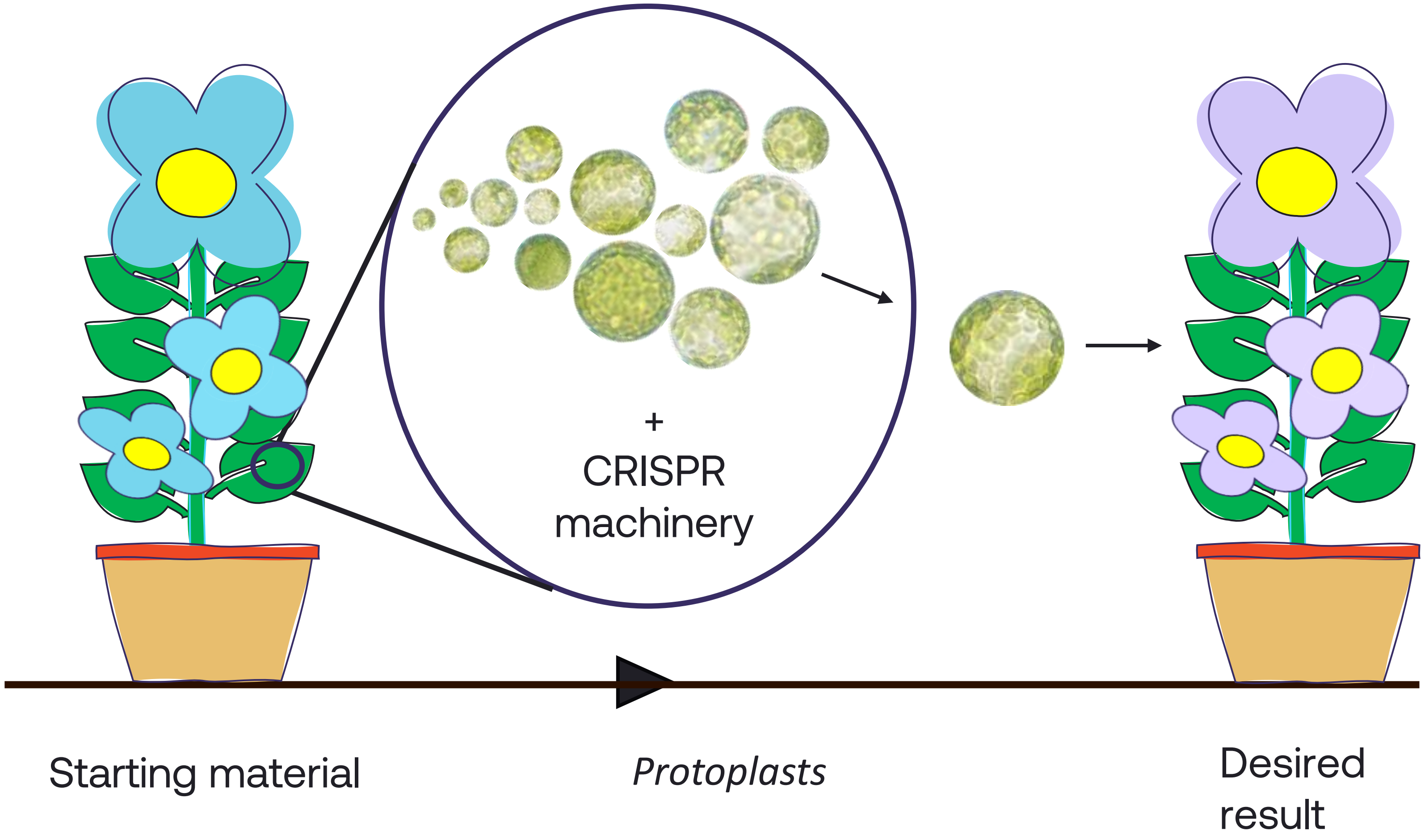


Starting material

Result

Desired gene-edited plant

Hudson River's transgene-free CRISPR pipeline: BioMaas



Advantages of BioMaas :

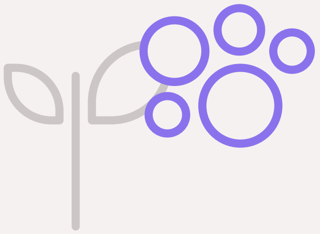
- Efficient, fast and scalable
- No transgenes; no need for crossing out
- Suitable for a broad range of crops
- Commercially attractive CRISPR licence (Inscripta MAD7™)

BioMaas Platform Workflow

01.

Protoplast Isolation

Single plant cells without cell walls are the starting point of the BioMaas Workflow



02.

Genetic Inventory and Guide Design

Genetic inventory

Editing strategy

Guide design

03.

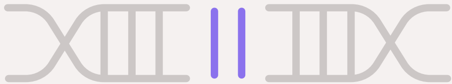
Editing

In house-produced CRISPR

Pre-assembly with guide

Delivery to isolated plant cells

Optimized conditions for in vivo editing



04.

Plant Regeneration

Single cell > callus > plants

High-throughput screening to identify ideal regeneration conditions

Plant Regeneration

Single cell > callus > plants

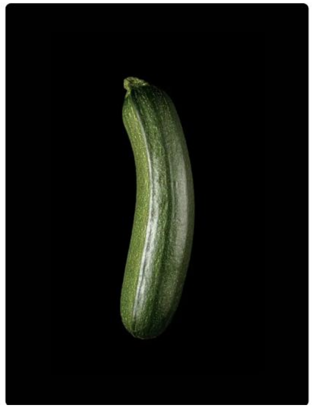
High-throughput screening to identify ideal regeneration conditions

Plant Regeneration is the biggest bottleneck

Multi-crop regeneration platform: enhancements through automation, regeneration enhancers and improved hydrogels

- Experience with >40 commercial varieties of 20 crop species
- “Cross-pollination” effect – applying our knowledge base to new species
- Successful regeneration of multiple recalcitrant varieties
- 100% success rate on protoplasting, CRISPR editing and callus formation

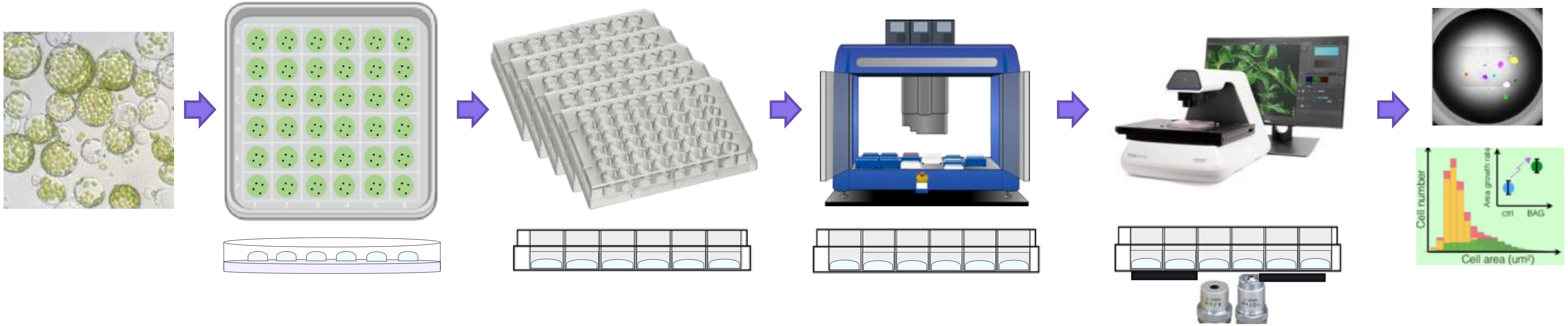




Regeneration – the biggest challenge for CRISPR in any crop/variety

Protoplast isolation → Embedding in hydrogel → Transfer to plates → Screening of media compositions → Microscopy → Image analysis and interpretation

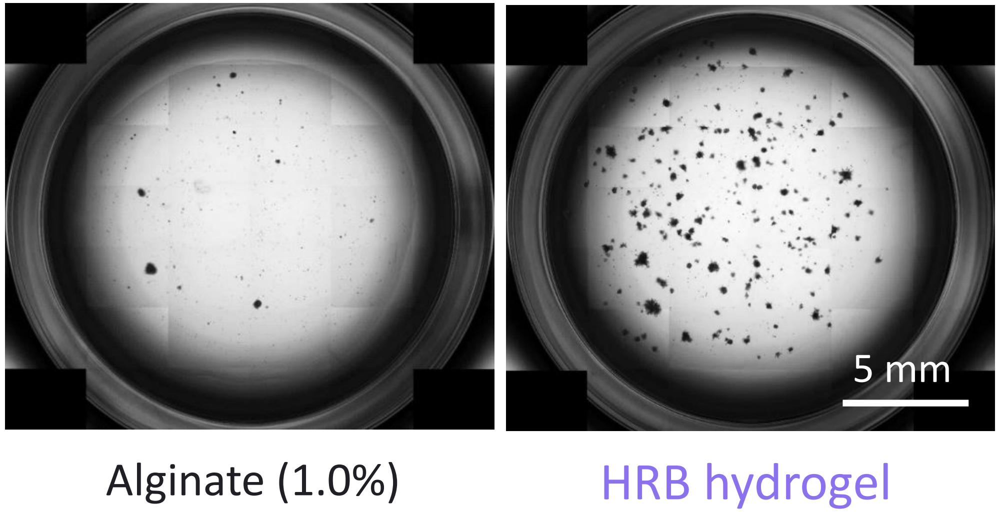


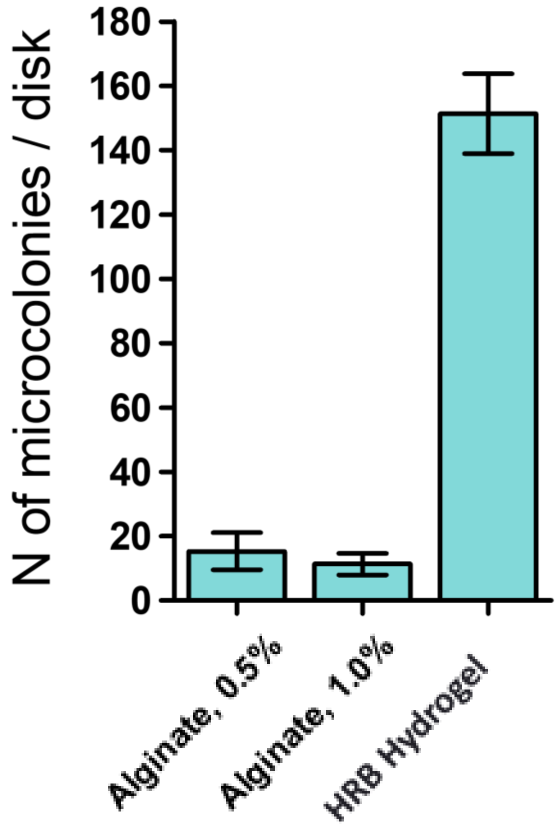
2 days

2 to 4 weeks

The development of the Hudson River hydrogel allows efficient regeneration from individual cells and with that economic scalability

Development from callus from individual cells
‘proto-calli’

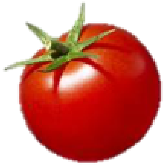




BioMaas protocols

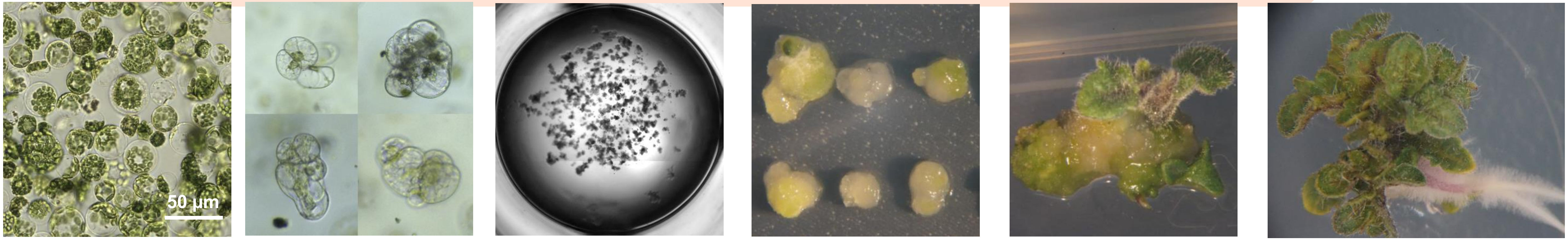
>18 species, 40+ commercial varieties

Single cell regeneration of our focus crops



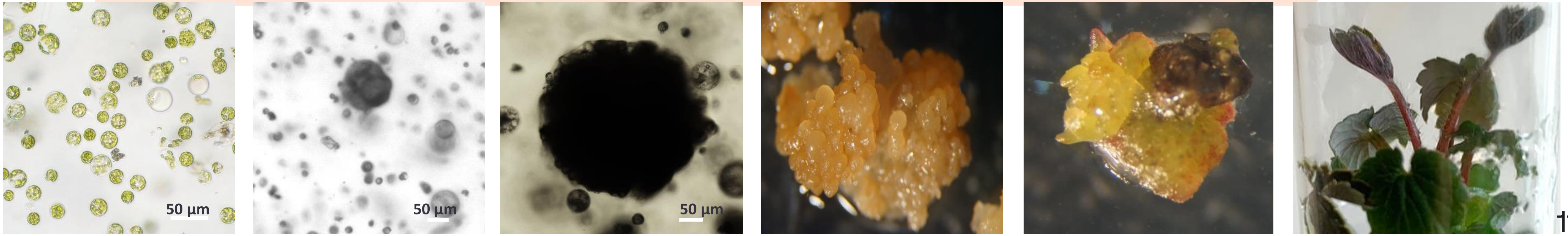
3-4 months to shoots; 9-12 months to edited plants

Protoplast → **proto-colony** → **micro-proto-callus** → **proto-callus** → **proto-callus-shoot** → **Edited plantlet**



6-8 months to shoots; 18 months to edited plants

Protoplast → **proto-colony** → **micro-proto-callus** → **proto-callus** → **proto-callus-shoot** → **Edited plantlet**



Hudson River

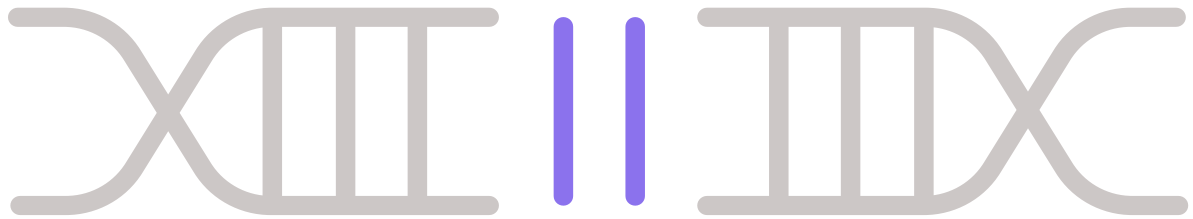
CRISPR editing

In house-produced CRISPR

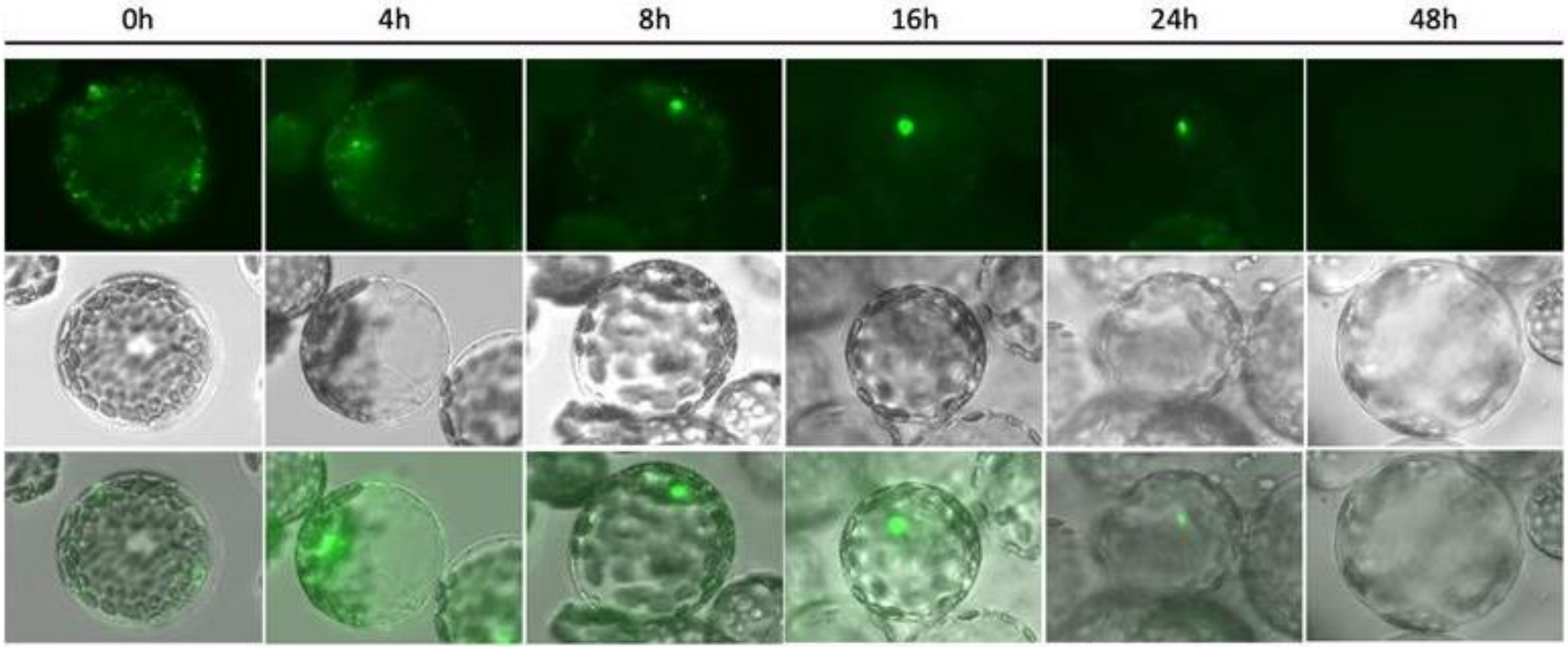
Pre-assembly with guide

Delivery to isolated plant cells

Optimized conditions for in vivo editing



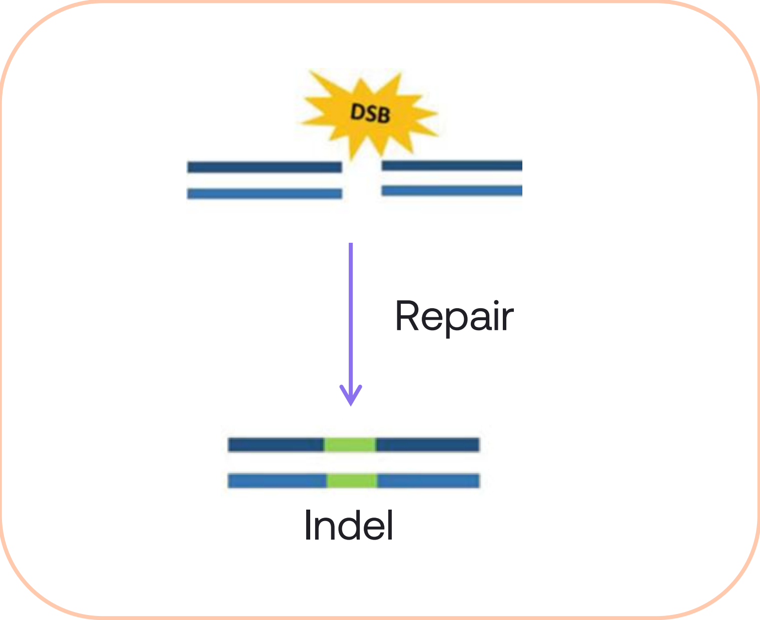
CRISPR protein delivered to protoplasts edits the genome in a brief window of time

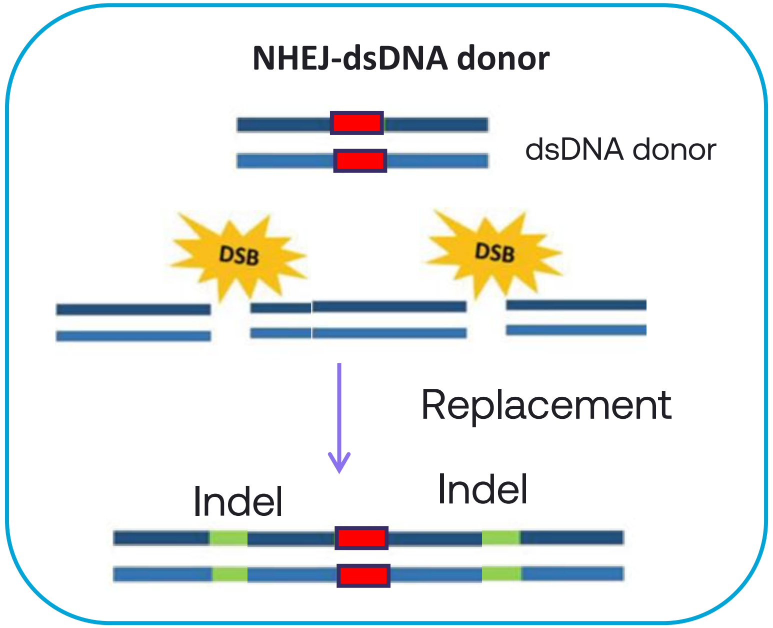


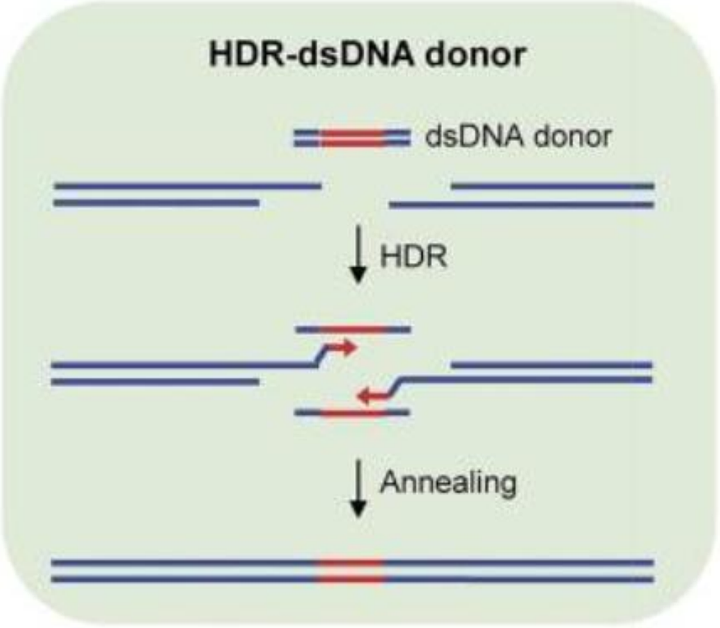
Nuclear accumulation of GFP-tagged MAD7 RNPs in *Nicotiana benthamiana* protoplasts over time

Different editing approaches for distinct goals

No Donor DNA	With Donor DNA	







Edit:

- Knock out
- In frame deletion

- Controlled DNA insertion

- precise sequence replacement

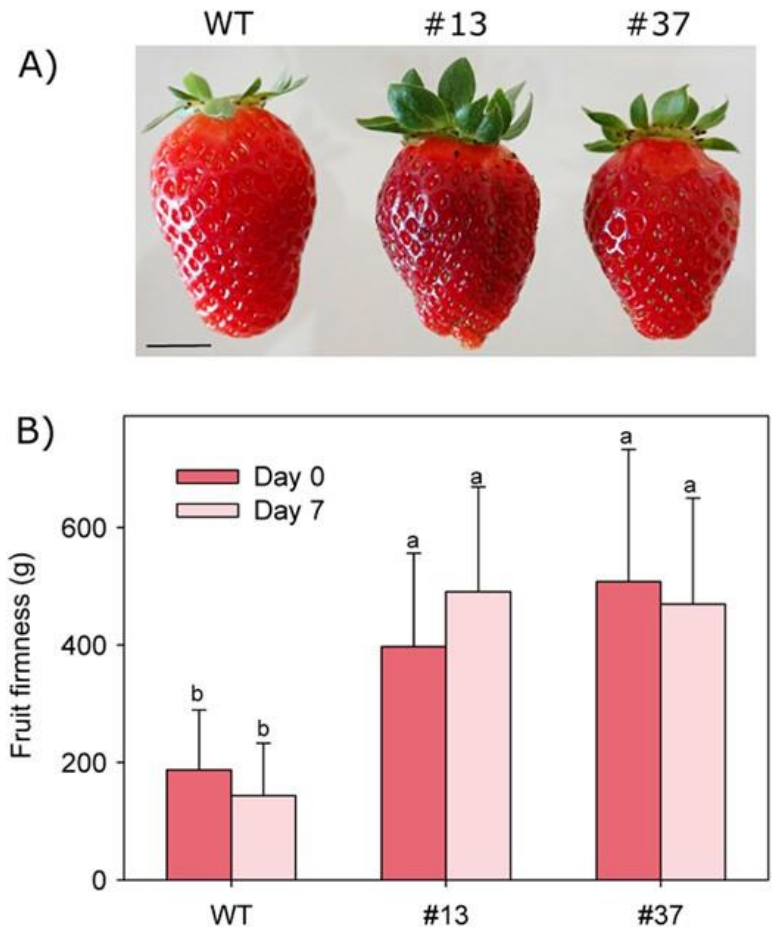
Expected efficiency: >80% <10% <1%

Fruit firmness (Retailer trait)

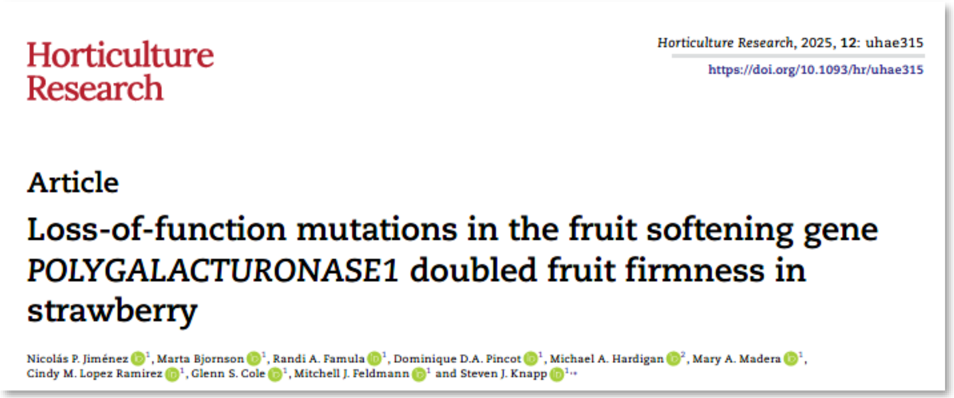
PG1 - PolyGalacturonase 1

- Encoded enzyme breaks down cell wall pectins during fruit ripening
- Soft fruits: shorter shelf life and more susceptible to *Botrytis*

- PG1 null mutants show increased firmness and resistance to *Botrytis* (López-Casado *et al.*, 2023)
- Natural loss-of-function of PG1-6A1 has been critical in breeding for firmer strawberries (Jiménez *et al.*, 2025)



López-Casado *et al.*, 2023


Horticulture Research

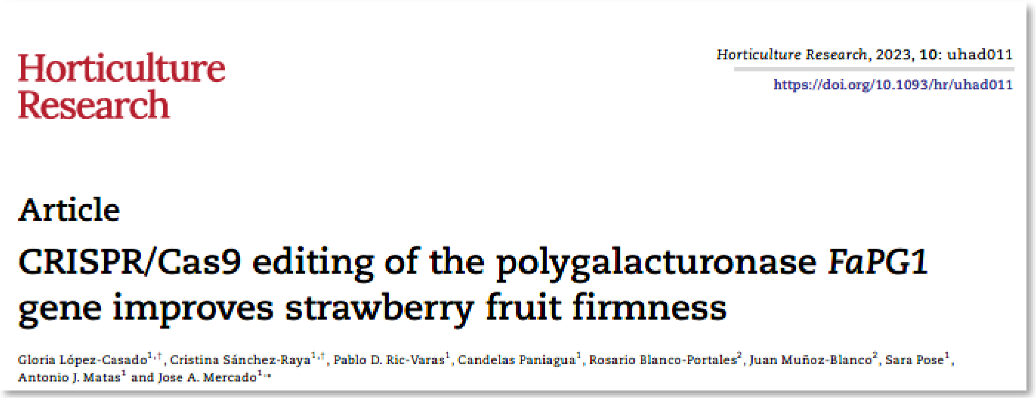
Horticulture Research, 2025, 12: uhae315

https://doi.org/10.1093/hr/uhae315

Article

Loss-of-function mutations in the fruit softening gene *POLYGALACTURONASE1* doubled fruit firmness in strawberry

Nicolás P. Jiménez[1], Marta Bjornson[1], Randi A. Famula[1], Dominique D.A. Pincot[1], Michael A. Hardigan[2], Mary A. Madera[1], Cindy M. Lopez Ramirez[1], Glenn S. Cole[1], Mitchell J. Feldmann[1] and Steven J. Knapp[1,*]


Horticulture Research

Horticulture Research, 2023, 10: uhad011

https://doi.org/10.1093/hr/uhad011

Article

CRISPR/Cas9 editing of the polygalacturonase *FaPG1* gene improves strawberry fruit firmness

Gloria López-Casado[1,†], Cristina Sánchez-Raya[1,†], Pablo D. Ric-Varas[1], Candelas Paniagua[1], Rosario Blanco-Portales[2], Juan Muñoz-Blanco[2], Sara Pose[1], Antonio J. Matas[1] and Jose A. Mercado[1,*]

Flavour in Strawberry - two possible roads to a product (Consumer trait)

1. Use CRISPR to stimulate flavor compound production in modern commercial varieties
    1. Knock-out or knock-down of competing pathways or repressors
    2. Modify key genes to gain functionality (expression, activity)

+ flavor

2. Use CRISPR to improve varieties with great flavor and make them more suitable for commercialization
    1. E.g. Mara des Bois (ever bearer): exceptional taste & sweetness, but small soft fruits which hampers large scale commercial sales > through CRISPR we can improve fruit firmness, fruit size and disease resistance

+ firmness
+ fruit size

Fungal resistant strawberry (Grower trait)

- Most strawberry varieties are susceptible to Strawberry Powdery Mildew (*Podospaera aphanis*)
- All aerial parts of the plant (leaf, runner, flower, fruit) are affected and severe infections cause yield losses up to 30%
- The disease is usually managed by spraying synthetic fungicides, but Powdery mildew starts to develop fungicide resistance and becomes more difficult to manage. Also, the use of synthetic fungicides is increasingly regulated.
- We aim to combine targets for Powdery mildew with targets for downy mildew and Botrytis, creating strawberry varieties with a rounded anti-fungal trait package.

Tapia et al. 2021 doi 10.1038/s41438-021-00587-y

What does CRISPR offer?

- For some (specifically complex and/or vegetatively propagated) crops, complex traits come into reach, that could not be realised via classical breeding
- Quicker translation of findings in other plant species or wild relatives to commercial varieties
- Utilising CRISPR as a research tool to identify the contribution of genome edits to the favourable traits in commercial varieties can bring breeding programs to a higher level
- Growers switch more easily to an upgraded variety they know (keep the variety name with and small addition!), rather than to a completely new variety

Resurrect bio

(Re)-introduction of disease resistance genes

plantik

Heat tolerance

BETTERSEEDS

Drought resistance

When to start? And what is needed?

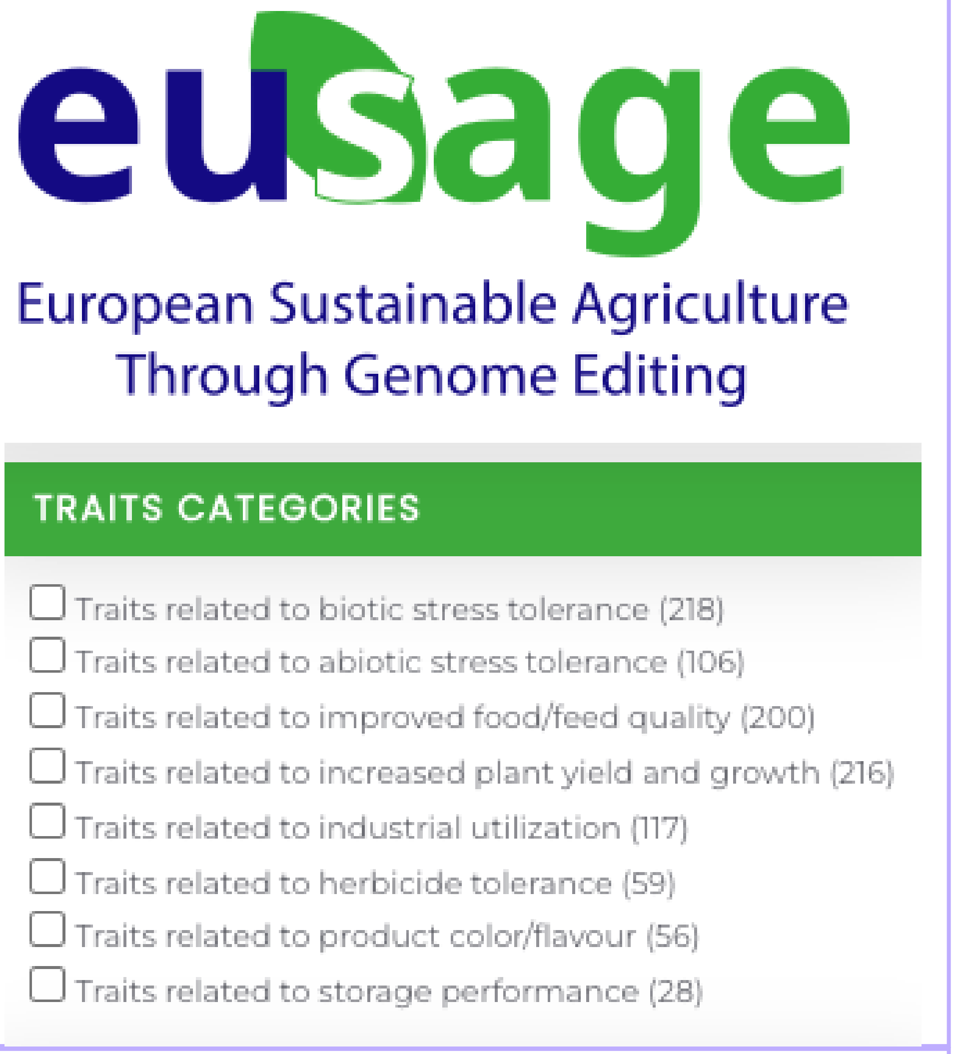
Urgency: competition from USA/China

If we do not continue our development in this field, competitors from more favourable regulatory regions will have a head start after Europe finally deregulates.

Long-term commitment – a layer of breeding here to stay

Firstly, setting up regeneration protocols is the key. For strawberries this required a couple of years. Often the protocol also needs to be adapted for particular varieties.

Secondly, traits are under continuous development. There are simple and multi-gene traits. Some will require precise editing, a still challenging pursuit. And most importantly, the effect of a genome edits can vary based on the genomic background (the variety) and as such, to utilise CRISPR in the most efficient way, it should be a standard part of the breeding pipeline.



https://www.eu-sage.eu/genome-search

The knowledge base on the effect of directed genome edits on crop traits is expanding rapidly!

Thank you for your attention!

Visit us in Wageningen, NL or on
hudsonriver.bio