



Crop Transformation

Group
Penny Hundleby



Providing GM and gene editing resources to support UK and International Research

Focus crops: Wheat, Barley, *Brassica oleracea* and *B. napus*

Other crops: potato, tomato, N. benthamiana, primula, Medicago (in the future – Pea)

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B. oleracea: AG DH1012 (DH line from the A12 x Green Duke mapping population)

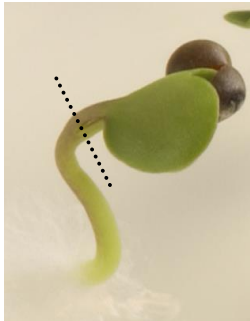


Provide access:

Protocols
Germplasm
Constructs
Training

Access Genome sequence

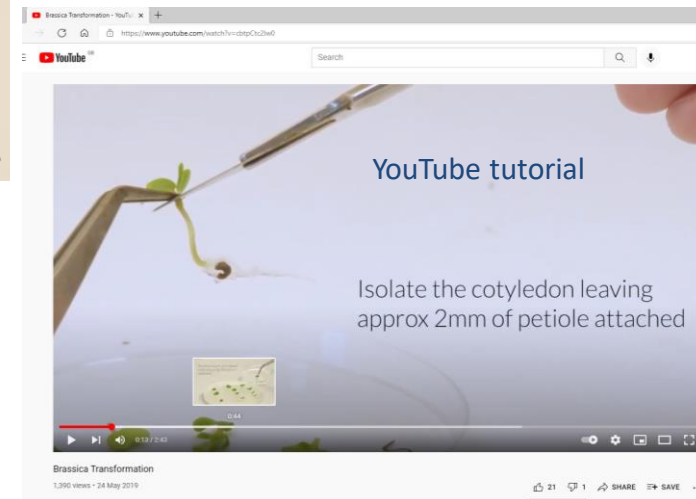
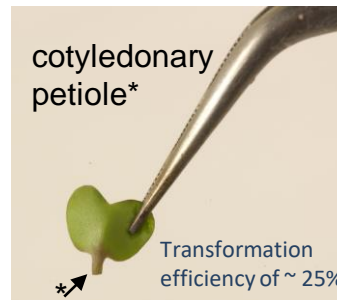
excised here



transfer to co-cultivation medium



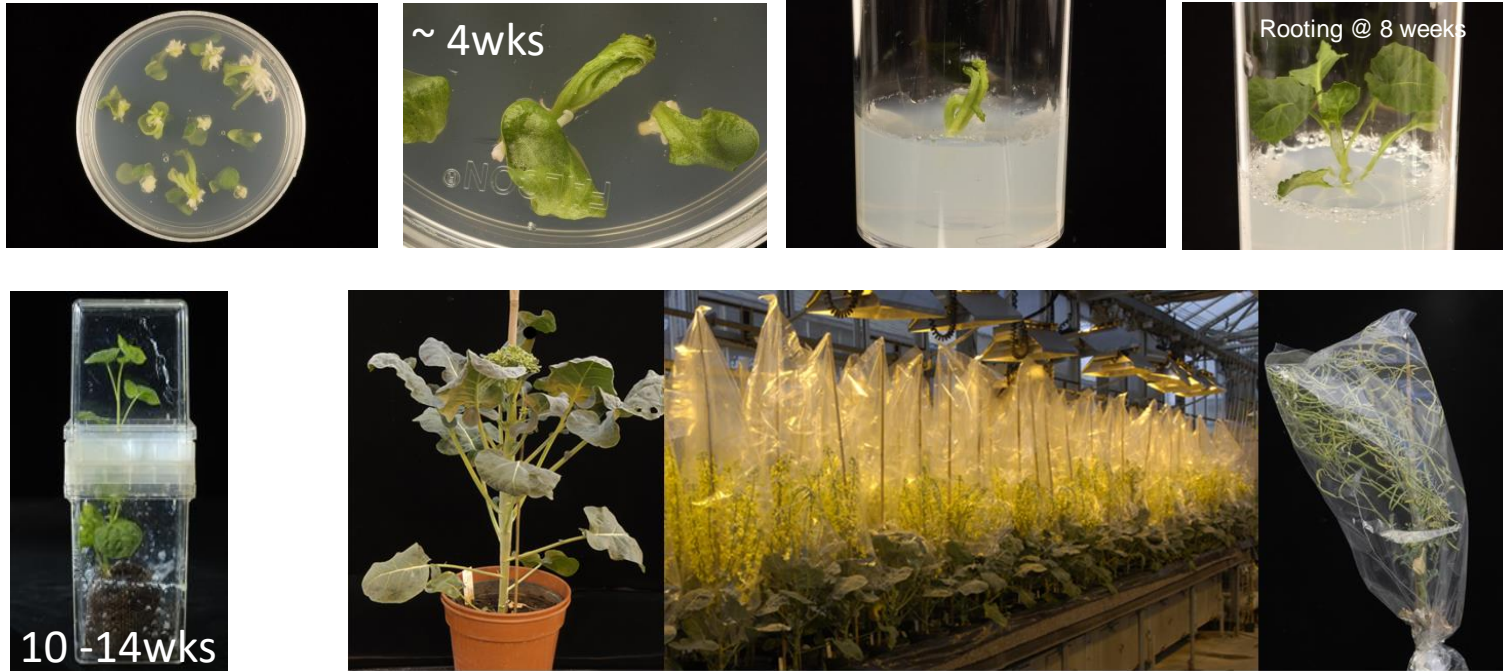
cotyledonary
petiole*



[Brassica oleracea Transformation Protocol](#)
Hundleby and Chhetry 2020

2-7

Full transformation service



21/22 Charges

Basic Construct design/build = £2,500

mark.smedley@jic.ac.uk

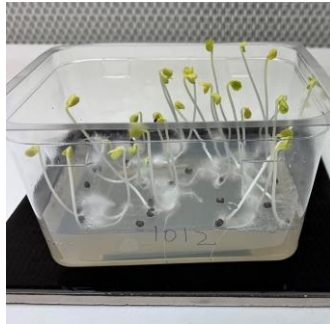


Standard Transformation costs = £3,952 *B. oleracea*

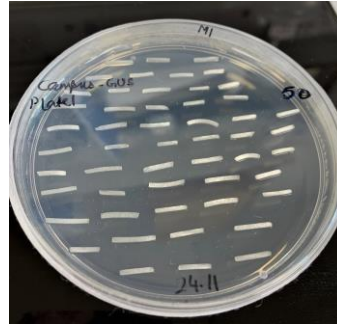
CRISPR Transformation costs = same (excludes genotyping)

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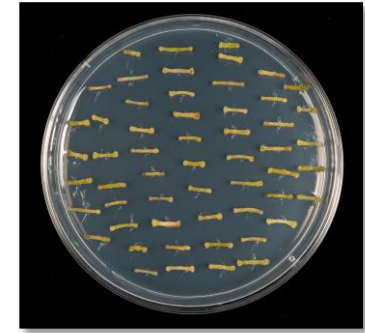
Hypocotyl transformation protocol for *B. napus*



Seed germination
5-6 days



Hypocotyl segments
Co-cultivation – 2 days



Induce callus growth
Selection stage- 3 weeks



Induce roots and transfer to soil



Regenerate shoots



OIL CROPS RESEARCH INSTITUTE
CHINESE ACADEMY OF AGRICULTURAL SCIENCES



CAAS/ JIC collaboration (Steve Penfield/ Judith Irwin)

Hosted Junyan Huang (semi winter ZS6)

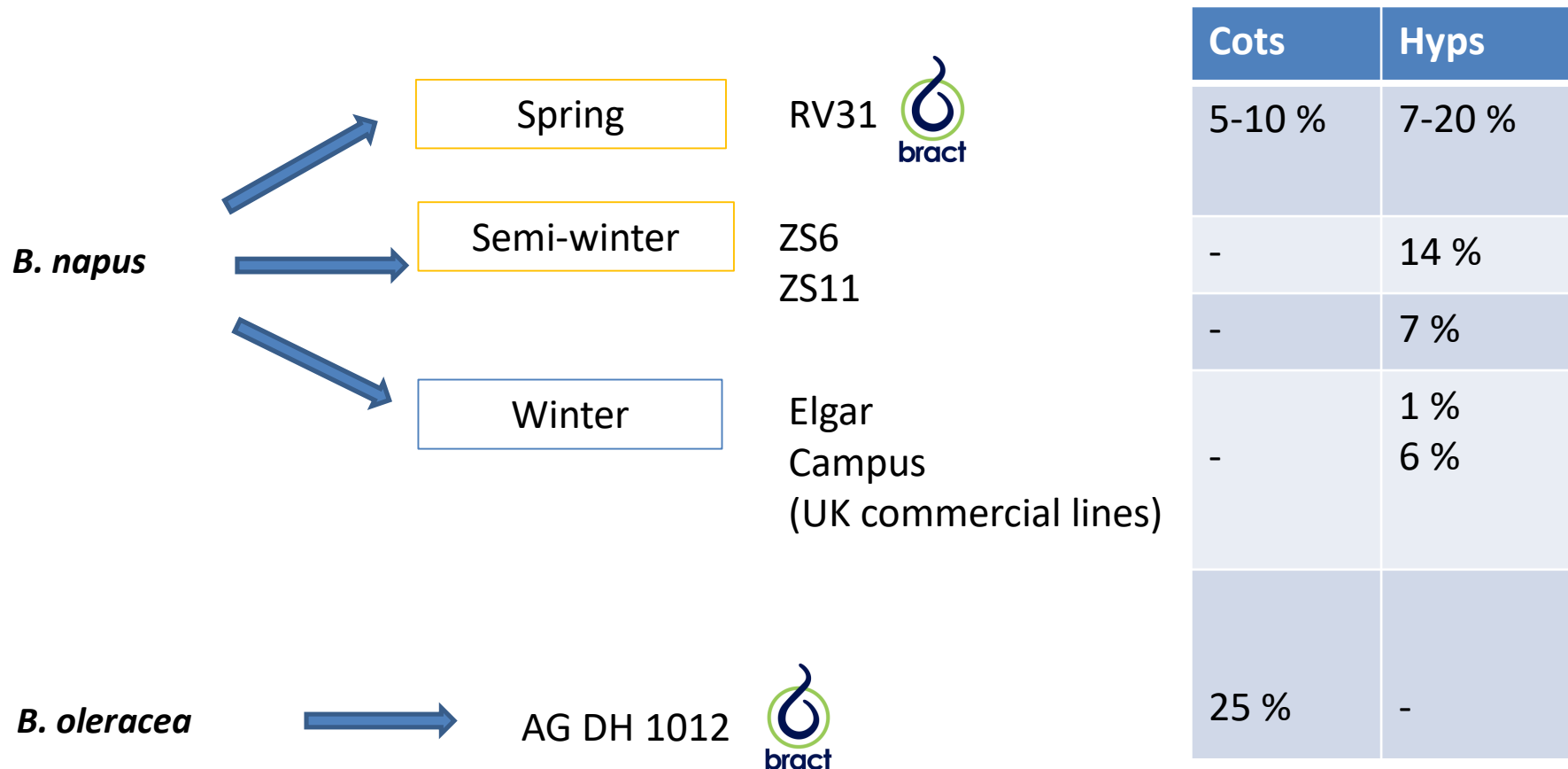
Works for RV31 our spring
genotype > cotyledon approach

But works on genotypes that didn't
perform well using a cotyledon
approach



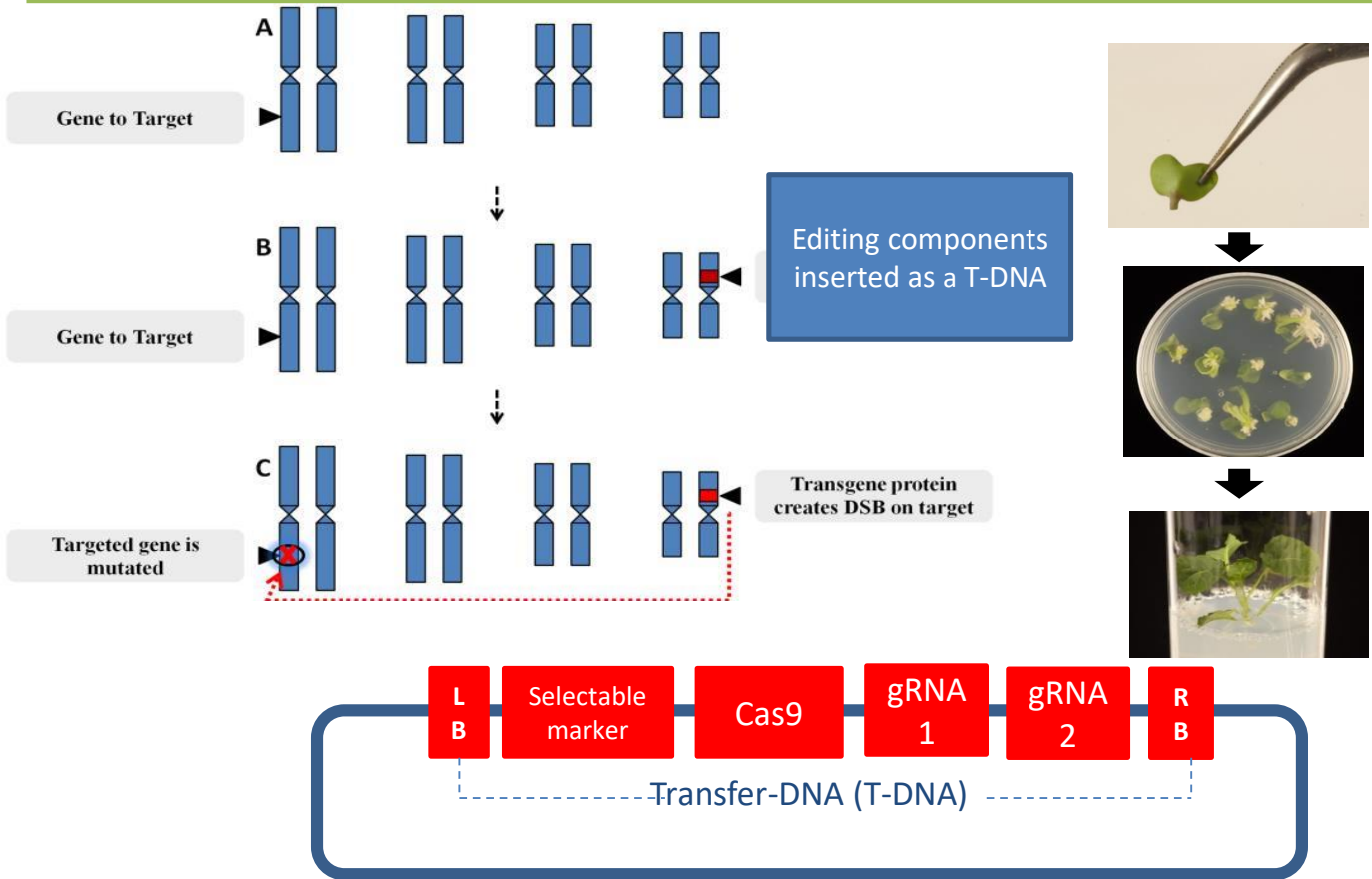
Monika Chhetry

Hypocotyl transformation tested in a range of *B. napus*



Question: what genotypes would be of interest to the community for Bract to support?

Increasing the efficiency of gene editing ... from 10%



By modifying Cas and changes to the guide assembly

...to an editing efficiency of between 60 – 100%

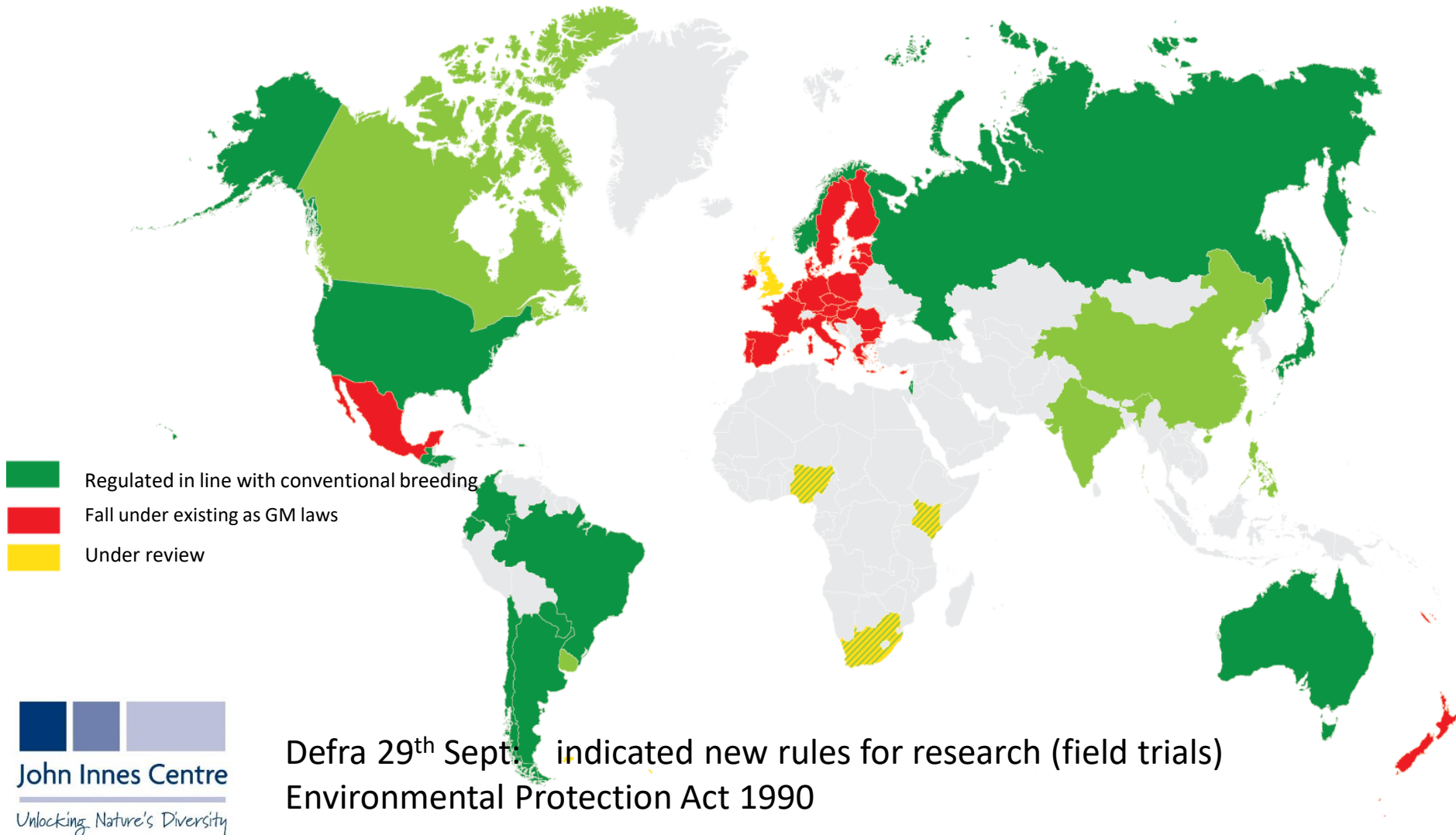


Summary

- GM and CRISPR - routine service for *B. oleracea*
 - Now more confident we can offer this in *B. napus* too
- Editing efficiency has been increased significantly
 - from 10% to 60-100% (tom.lawrenson@jic.ac.uk)
 - In theory we can now introduce multiple guides per construct (to target different genes)
- Hypocotyl system – improved transformation efficiency in *B. napus*
- RV31 genome sequence due 2022 (Rachel Wells)
 - Will make designing guides much easier!
- Some CRISPR field trials no longer require GMO oversight
 - Will still require notification to Defra

Regulatory Climate

When Genome Editing used to produce a product indistinguishable from one developed by conventional breeding approaches...



Genome Editing (CRISPR)

Chromosome

Remove/edit EXISTING DNA (targeted mutagenesis)

Likely to be regulated in line with conventional breeding

OR

Introduce/replace genetic sequence

(update to GM)

Considered to GM by regulatory authorities across the globe

ATGACGGATCAGCCGCAAGCGG

TACTGCGTAGTCGGCGTTGCGCC



John Innes Centre

Unlocking Nature's Diversity