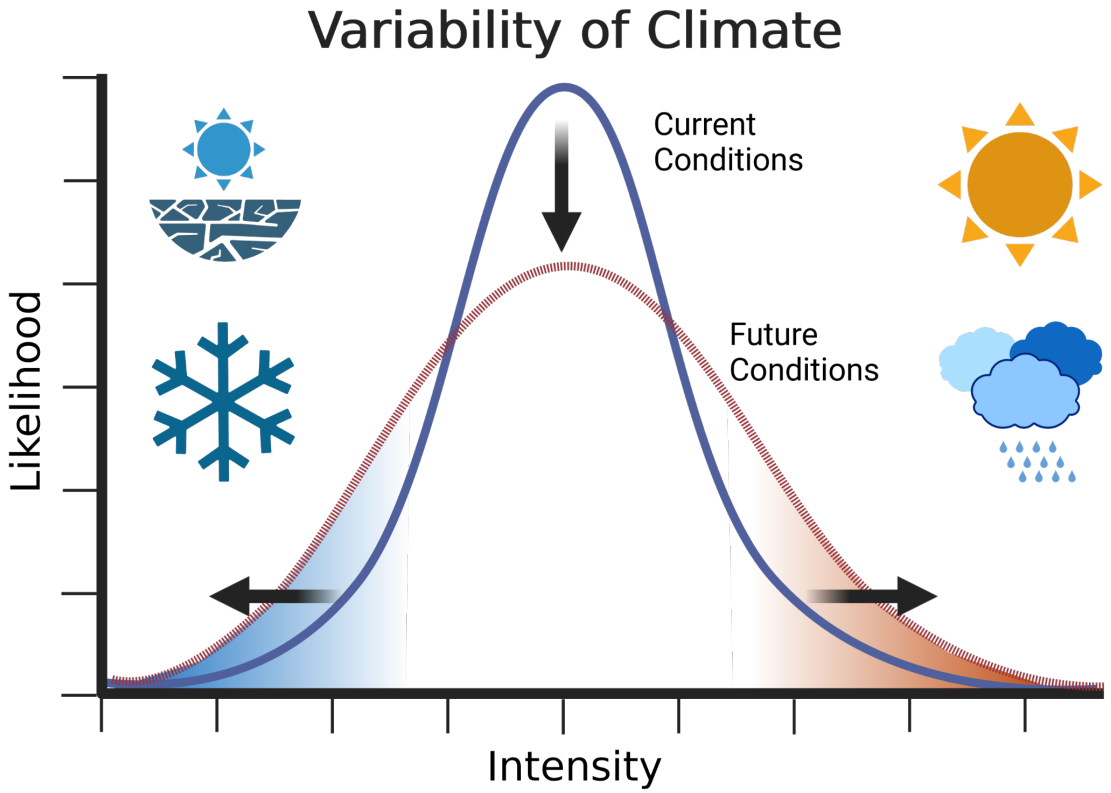
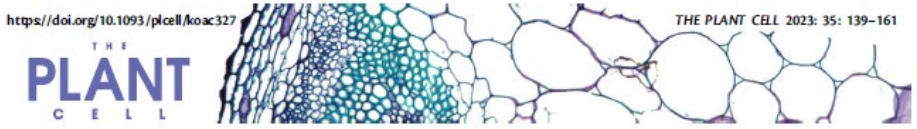
Prospects and progress for gene edited Brassicas

Thanks to all the organisers and especially those behind the scenes of this great event in Wagga Wagga and Sydney

Climate change isn’t just ‘hotter and drier’



- More temperature extremes
  - More frosts and heat events
- More variable Rainfall
  - Longer, harsher droughts
  - Shorter, intense flooding events

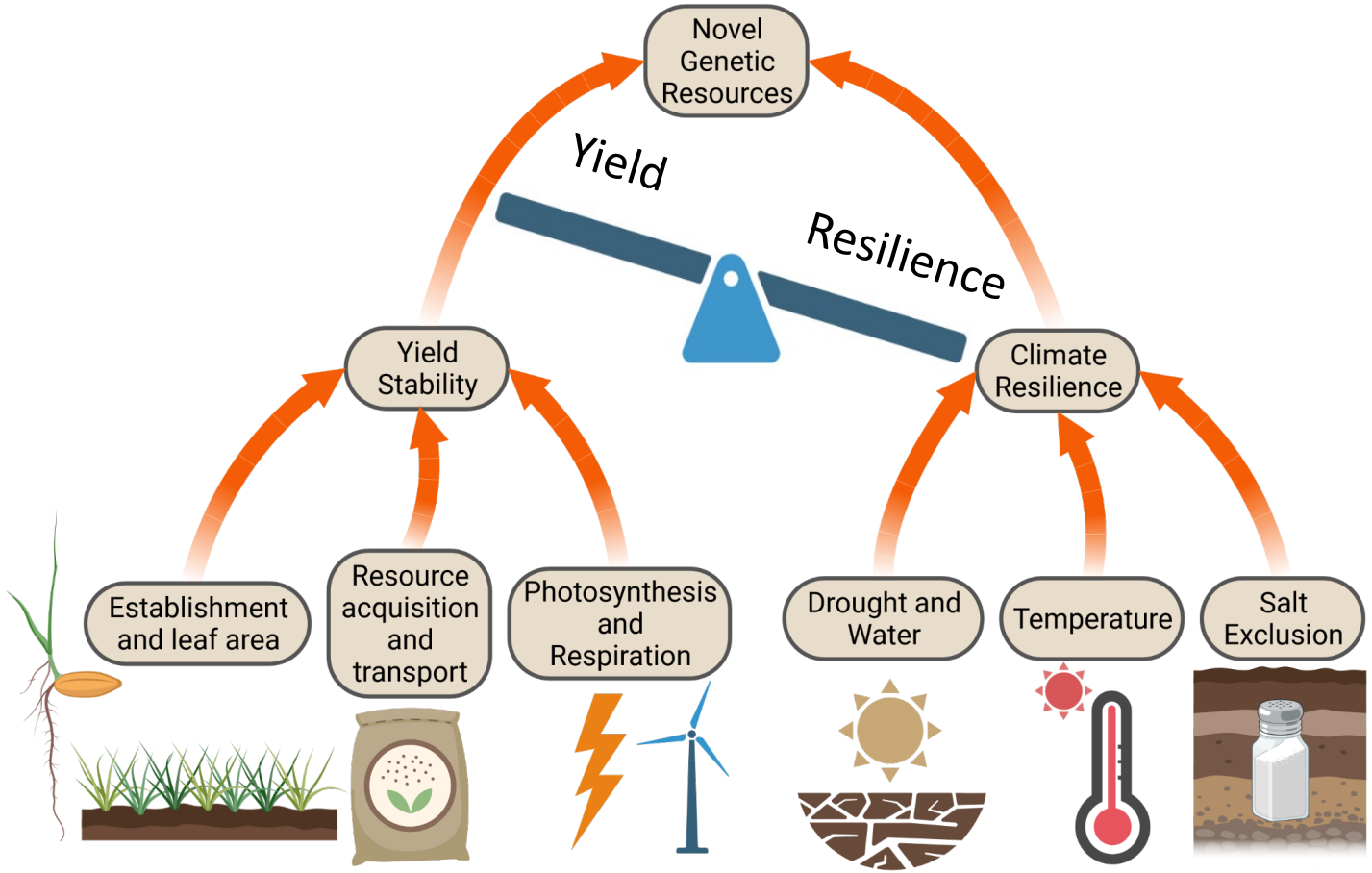


Potential abiotic stress targets for modern genetic manipulation

Bowerman et al Plant Cell 2023

Climate change: higher yielding and more resilient crops

Can we better manage trade-offs and synergistically stack traits?

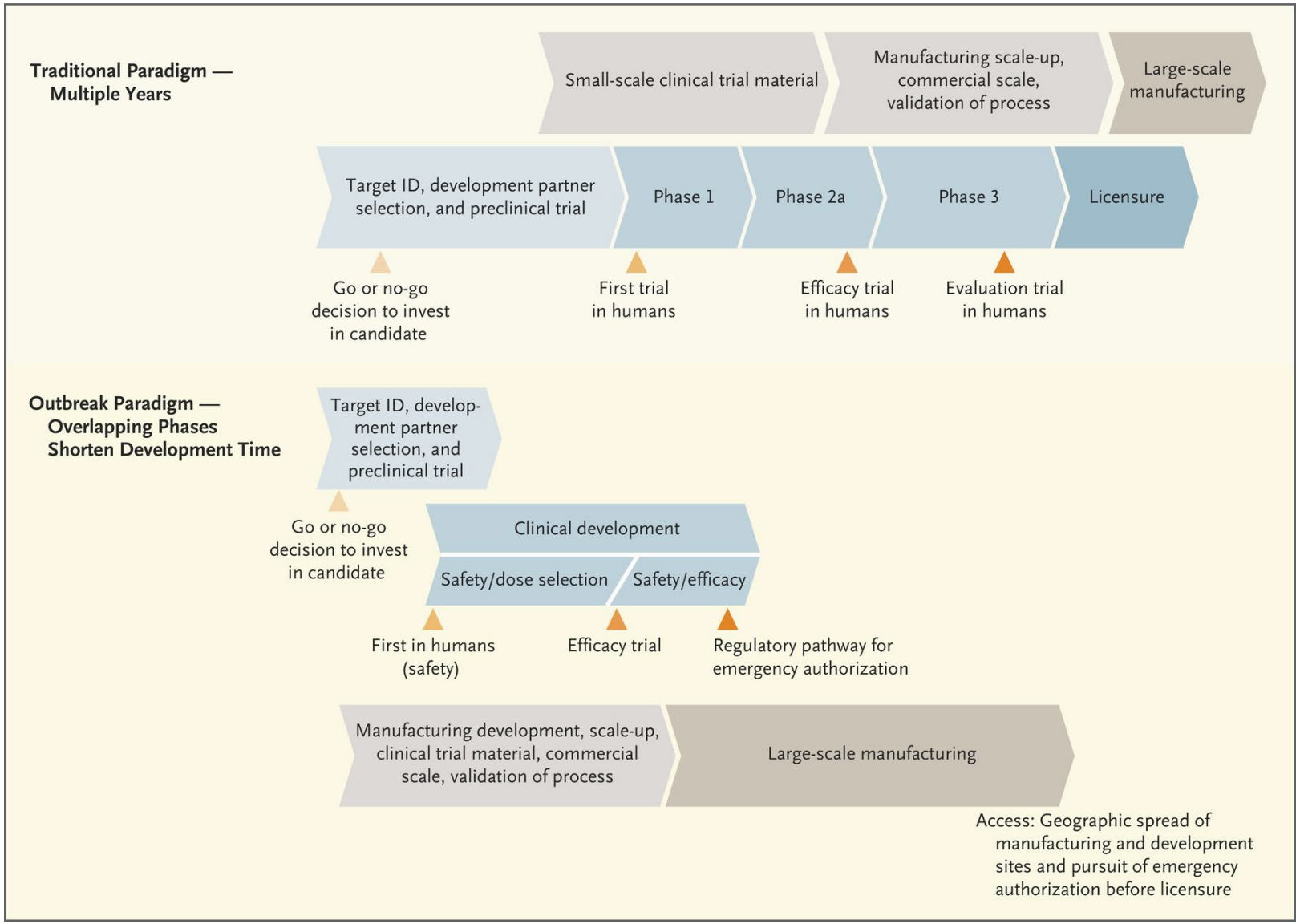


Balancing:

- Yield and Resilience
- Breeding and agronomy with gene editing
- **Technology, training and translation**

Bowerman et al Plant Cell 2023

Fast tracking improvements – learnings from COVID



https://www.nejm.org/doi/full/10.1056/NEJMp2005630

- ❑ Shared data, materials and methods
- ❑ Links across disciplines and countries
- ❑ Novel tools and automation
- ❑ Parallel processes
- ❑ Co-design
- ❑ Close interaction with regulators
  - Early engagement
  - Preliminary-data sharing

Fast tracking crop improvement – a case study

- Goal: Produce leafy brassicas to make nutritious foods taste nicer
- Removed pungency via editing of all copies of type-1 myrosinase gene in *B. juncea**
- Setup a pathway to market through a food distribution system Conscious Foods
- ~5 yrs from founding company to first product on the market

Pairwise introduces first food made with CRISPR technology in the U.S. market

Pairwise says Conscious Greens — part of its Conscious Foods brand — are derived from mustard greens and are part of the same family of vegetables as Brussels sprouts, cauliflower and kale.

https://www.thepacker.com/news/products/pairwise-introduces-first-food-made-crispr-technology-us-market

https://www.pairwise.com/nutrition-sustainability

* **Karslon et al** Plants 2022, 11, 2494.

Australian National University

Australian Government
Australian Research Council

THE UNIVERSITY of ADELAIDE

ARC Training Centre for Accelerated Future Crops Development

Australian Crop Breeders Ltd

nuSEED

AGT

ADVANTA

NSW Government Department of Primary Industries

LongReach PLANT BREEDERS

INTERGRAIN

Australian Seed Federation SOWING SEEDS

CEPLAS

John Innes Centre Unlocking Nature's Diversity

KWS

NIAB

appf Australian Plant Phenomics Facility

BIOPLATFORMS AUSTRALIA

Diversity Arrays Technology Pty Ltd

Grain Trade Australia

CSIRO

GRDC GRAINS RESEARCH & DEVELOPMENT CORPORATION

SARDI SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

❖ Co-design

The Nobel Prize in Chemistry 2020

awarded "for the development of a method of genome editing"

Emmanuelle Charpentier and Jennifer A. Doudna

Why CRISPR? Is it hype?

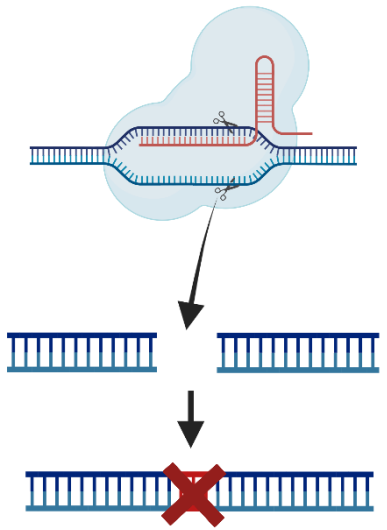
https://www.nytimes.com/2022/06/27/science/crispr-gene-editing-10-years.html

- 5,726 papers in 2023* across all fields
- Gene editing is underway in more than ~40 crops in ~25 countries
- Many reviews and articles in mainstream media
  - Chen et al. 2019 Annual Rev Plant Biology
  - Gao et al 2021 Cell: Gene editing for precision breeding
  - New York Times 2022 First ten years of CRISPR
- First edit in B. napus in 2017
- Multiple edits in oil seeds (see abstracts from yesterday's session)

Defining gene editing - CRISPR

CRISPR CAS can be used to edit genomes, resulting in deletions, insertions or sequence modification at precise locations in almost any species

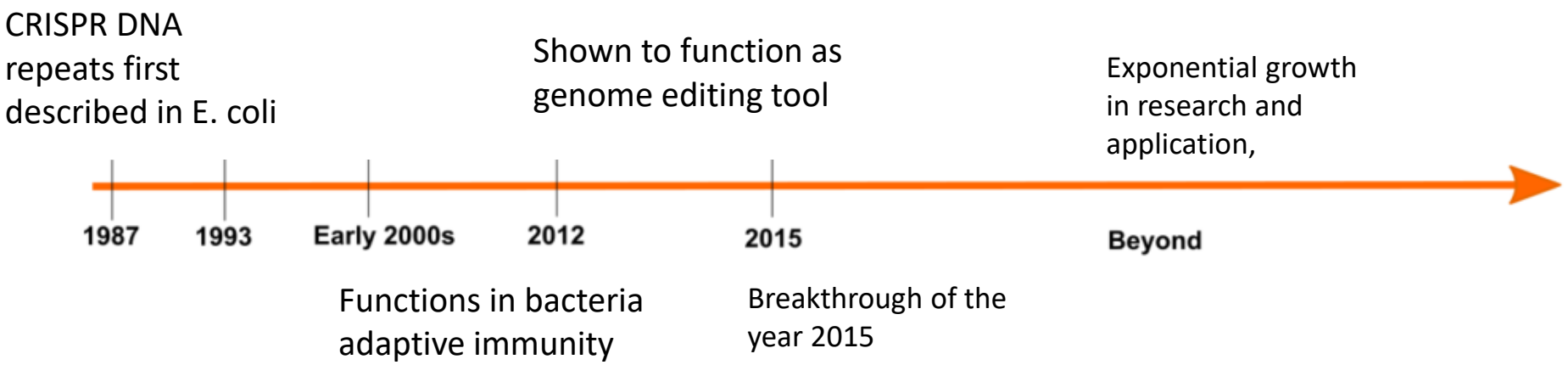
SDN-1



1. **Non-template repair SDN1**

Non-homologous end joining repair

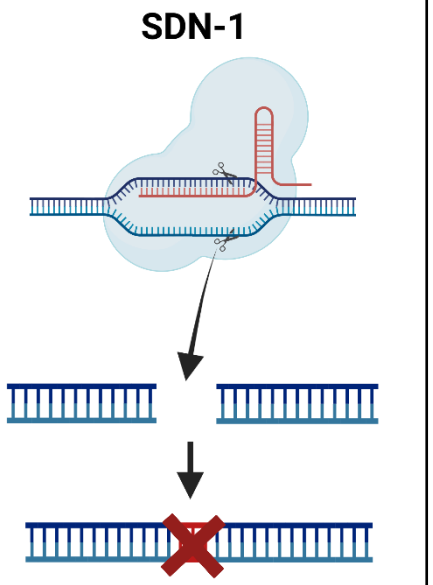
The CRISPR timeline:



Mechanism

- **CRISPR** stands for:
  Clustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats
- The guides target CAS proteins to sequences
- The CAS proteins modify the nucleic acids, for example
  - Cas9 cleaves DNA while Cas13 cleaves RNA.
- Genome edits are stably inherited, but the machinery can be transient

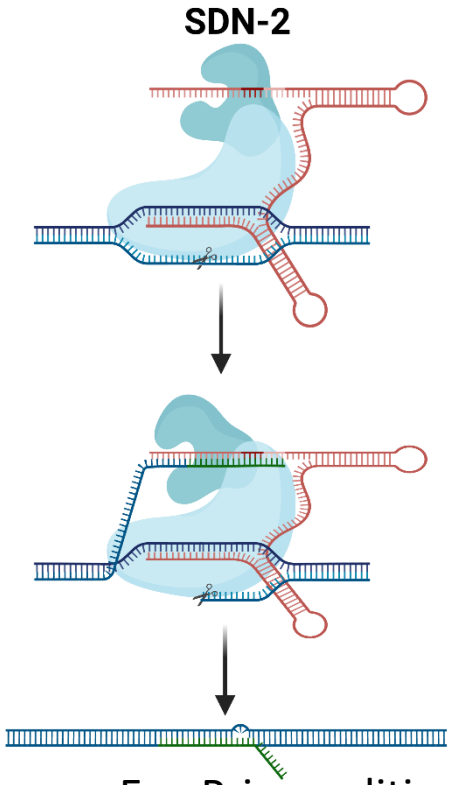
Defining gene editing - two categories (Non-template and template repair)



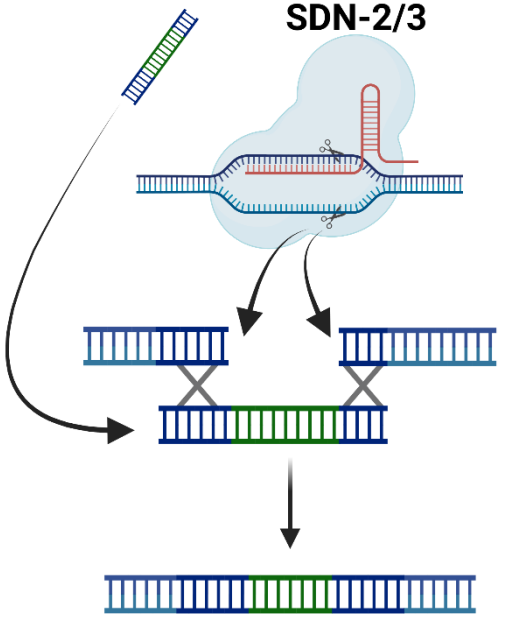
1. **Non-template repair SDN1**

Non-homologous end joining repair

2. Template based repair SDN-2/3



E.g. Prime editing



E.g. Templated homology directed repair

- SDN-1 targeted deletions
  - Routine,
  - Not GMO in Australia

- SDN2 and 3 templated guided modifications
  - Some tech requires optimization in plants
  - GMO in Australia

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Improving gene-editing and transformation toolkits

Julian Greenwood
- Developmental regulators for transformation
- Editing tool kit
Canola and carinata traits

Neelam Gogoi
Deploying nanotechnology for
- novel crop transformation
- transient systems

Hendry Susila
Optimising CRSPR Cas
- Multiplexing
- Minimal Cas for delivery
- Improved HDR
- Prime Editing

Wheat and rice traits

Nay Chi Khin,
- Optimising canola and carinata traits via gene editing

Carrie Shen
- Optimising wheat and barley editing and transgenesis

*Plus 20-30 PhD students

Golden Gate parts library for rapid construct development

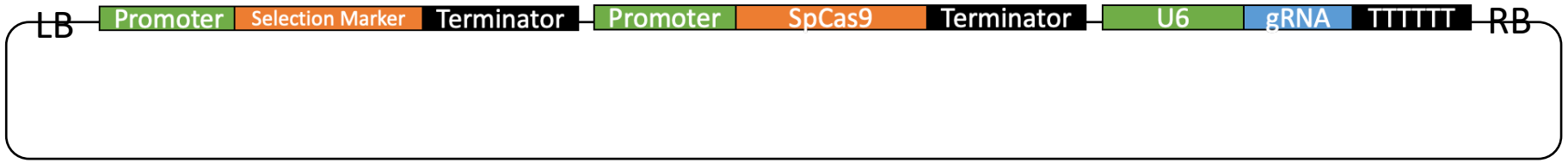
- Assessment of multiple CRISPR systems for SDN-1/2/3 gene editing in plants
- Large-scale MoClo parts for Golden Gate cloning with various Cas and protein accessories

Transgene expression and tagging

Expression of optimized Cas proteins for gene editing

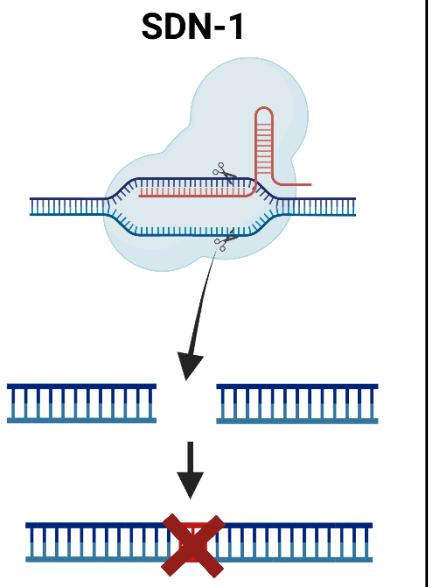
Species specific U6 promotors - for expressing 1-2 guides

Guides expressed as single RNA and processed into individual units by Cys4 - for expressing 2-6 guides



GE or Transgene expression constructs ready for deployment

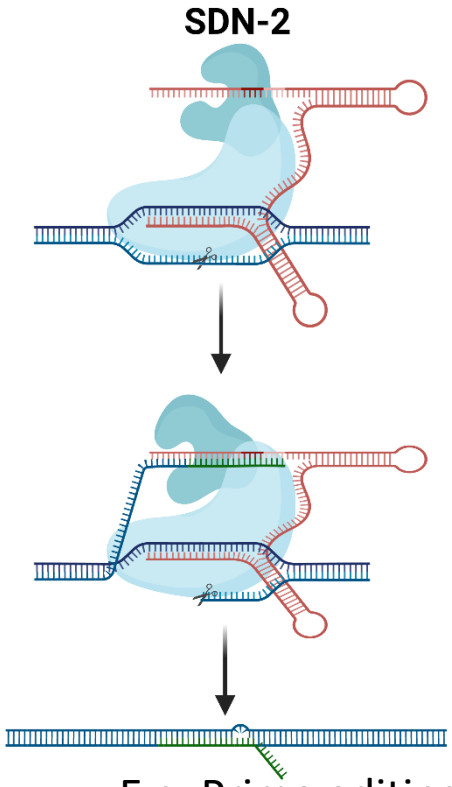
Defining gene editing - two categories (Non-template and template repair)



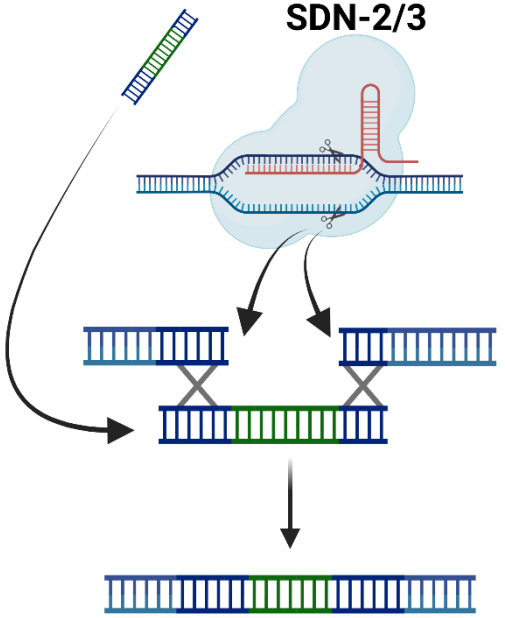
1. **Non-template repair SDN1**

Non-homologous end joining repair

2. Template based repair SDN-2/3



E.g. Prime editing



E.g. Templated homology directed repair

- SDN-1 targeted deletions
  - 8 targets underway in canola
  - 6 targets underway in carinata
- SDN2 and 3 templated guided modifications
  - Some tech requires optimization in plants
  - GMO in Australia

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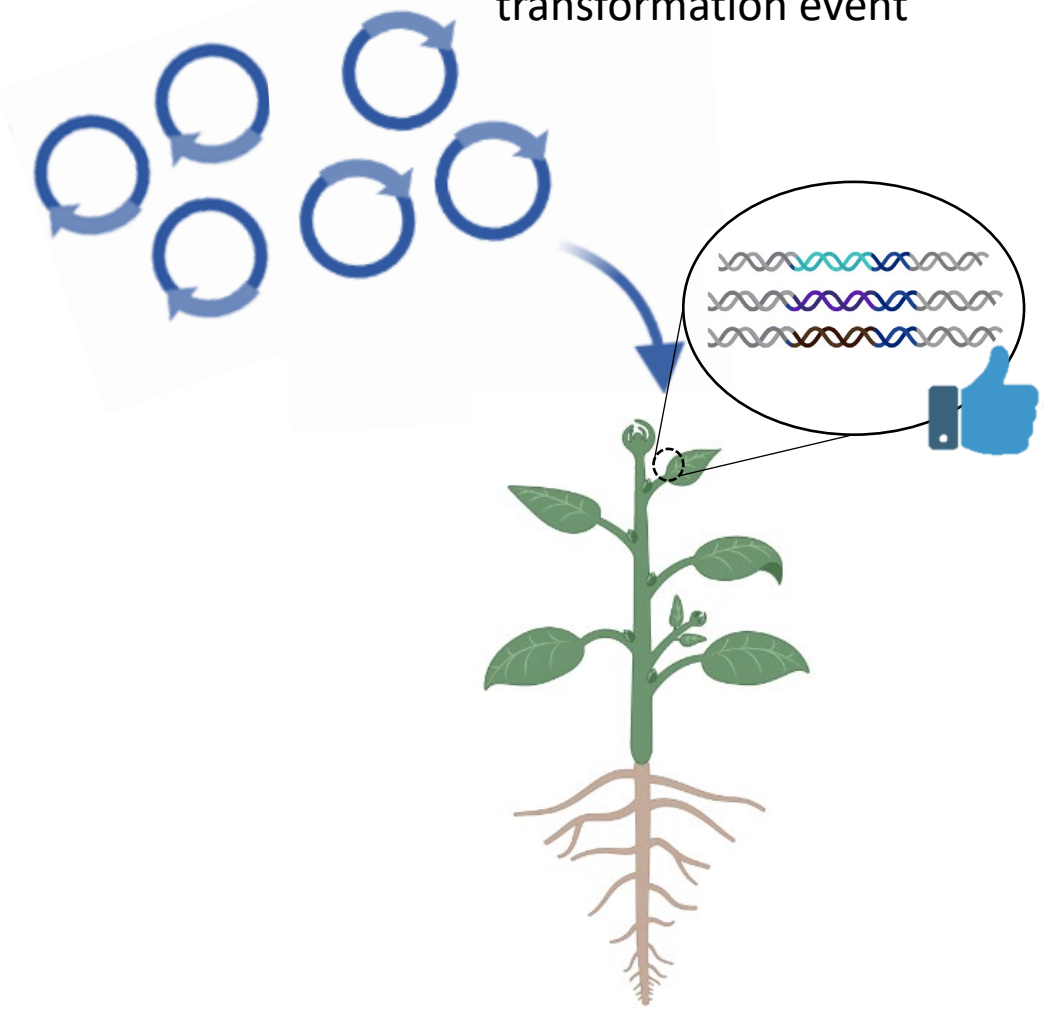
Multiplex gene editing – a simpler alternative for trait stacking

Multiple gene targets in one single transformation event



Direct transformation into elite lines, no introgression

Rapid field evaluation in elite germplasm

Minimal regulatory processes (?)

Removing bottlenecks on transformation

Julian Greenwood
- Developmental regulators for transformation
- Editing tool kit
Canola and carinata traits

Neelam Gogoi
Deploying nanotechnology for
- novel crop transformation
- transient systems

Hendry Susila
Optimising CRSPR Cas
- Multiplexing
- Minimal Cas for delivery
- Improved HDR
- Prime Editing

Wheat and rice traits

Nay Chi Khin,
- Optimising canola and carinata traits via gene editing

Carrie Shen
- Optimising wheat and barley editing and transgenesis

Optimising transformation

- *B. Napus,* wheat and barley transformation up and running.
- Optimising transformation for *B. Carinata, c*hickpea and wheat
- Developing new pipelines for difficult to transform varieties/species

Chickpea

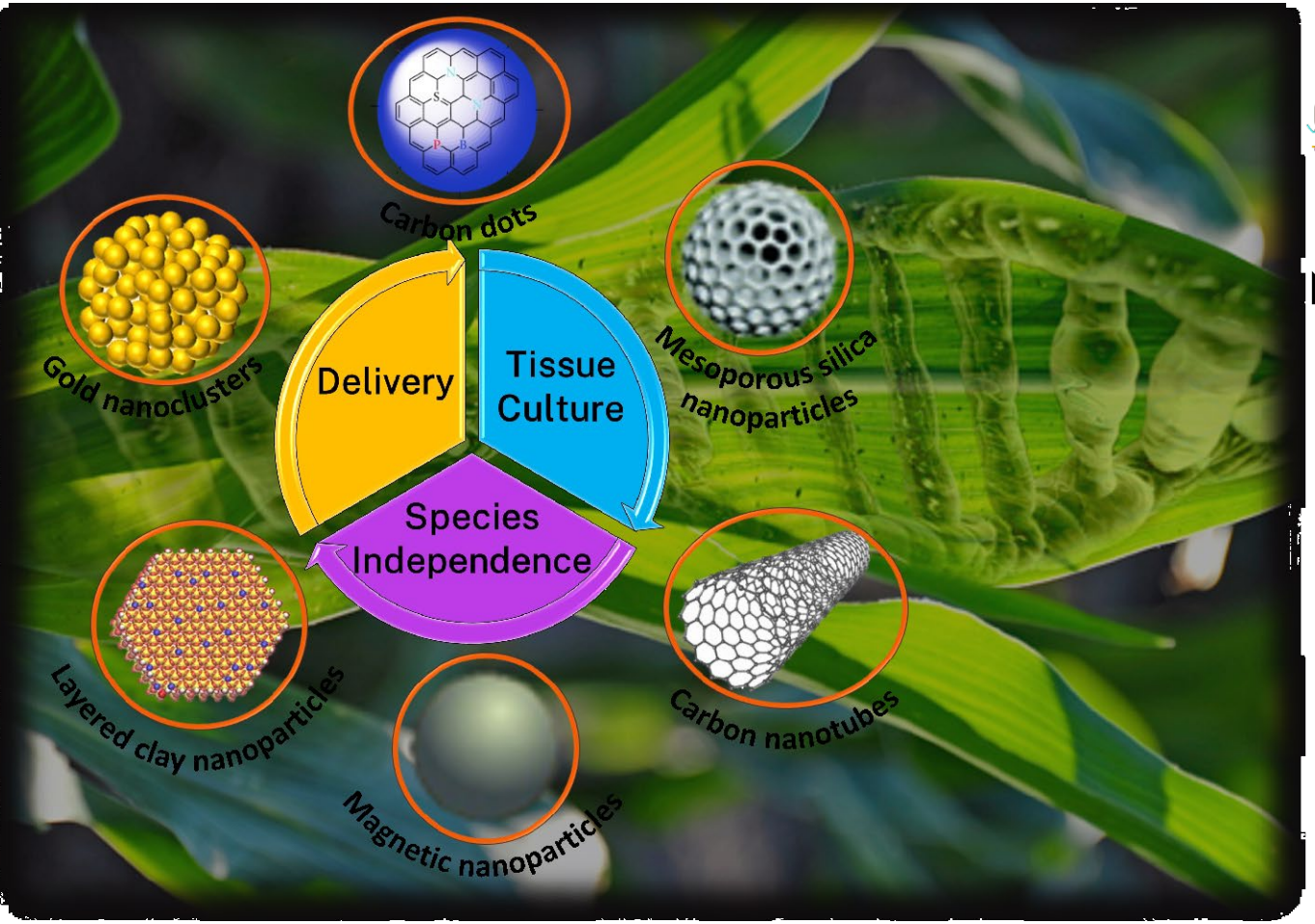
B. carinata

AGG-1

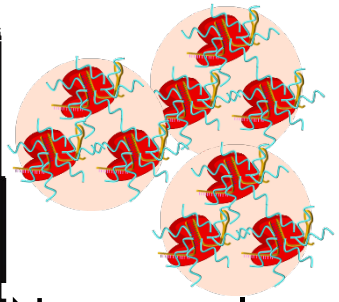
DHN-1

Extending the limits of transformation using Nanotechnology

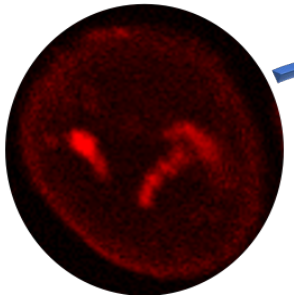
Works for wheat, tomato, mushrooms, now trialing chickpea and brassicas



Working for wheat, tomato and mushrooms

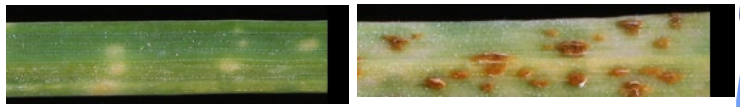


Nanocomplex of CRISPR-RNP



Transformed Pollen

Fertilization using treated pollen

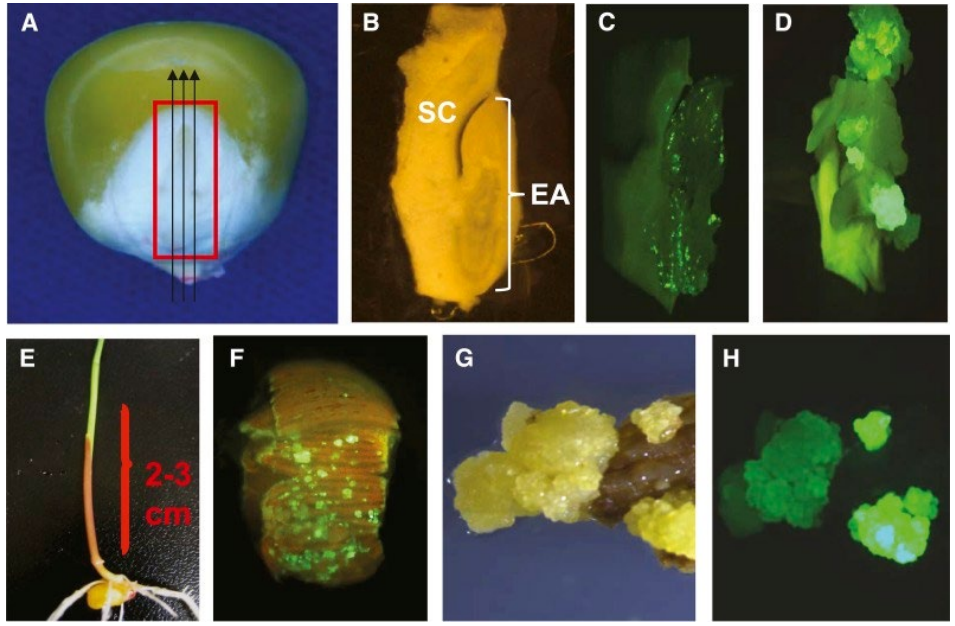


Edited Stem Rust R-genes

Extending the limits of transformation using developmental regulators

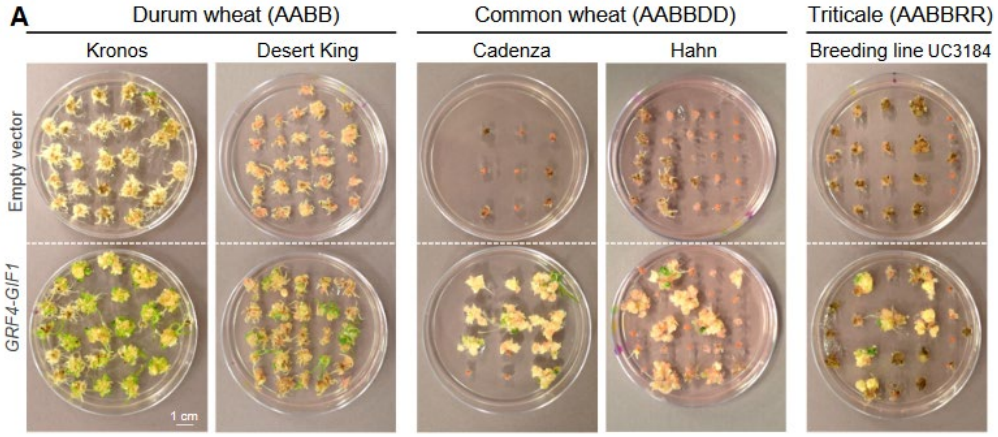
Can potentially transform any tissue and induce shoot formation using developmental regulators!

Overexpression of *BBM, WUS, STM etc.* to induce somatic embryogenesis or improve regeneration of shoots.



(Lowe *et al.,* 2016)

Overexpression of GRF4-GIF



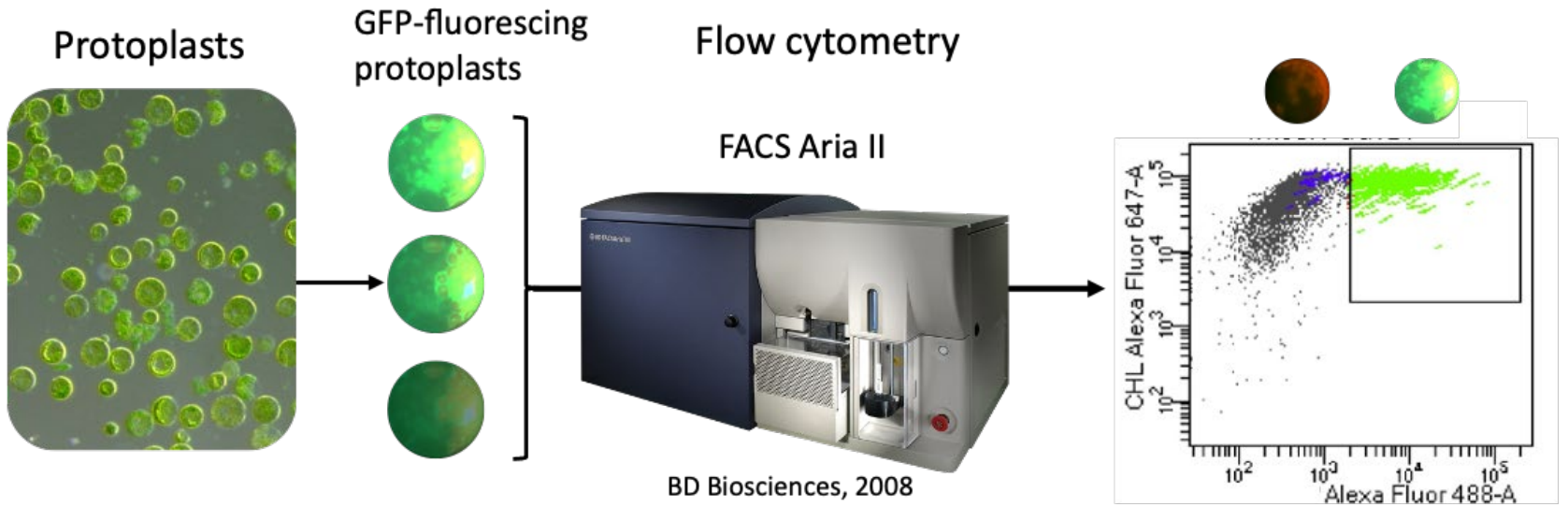
(Debernardi *et al.,* 2020)

Bec Tyrrell,
PhD student

Deploying rapid transient expression systems to optimise editing

Expression System

Using a transiently transformed protoplast system (single cell culture) allowed for high-throughput testing of sequence motifs on protein output.



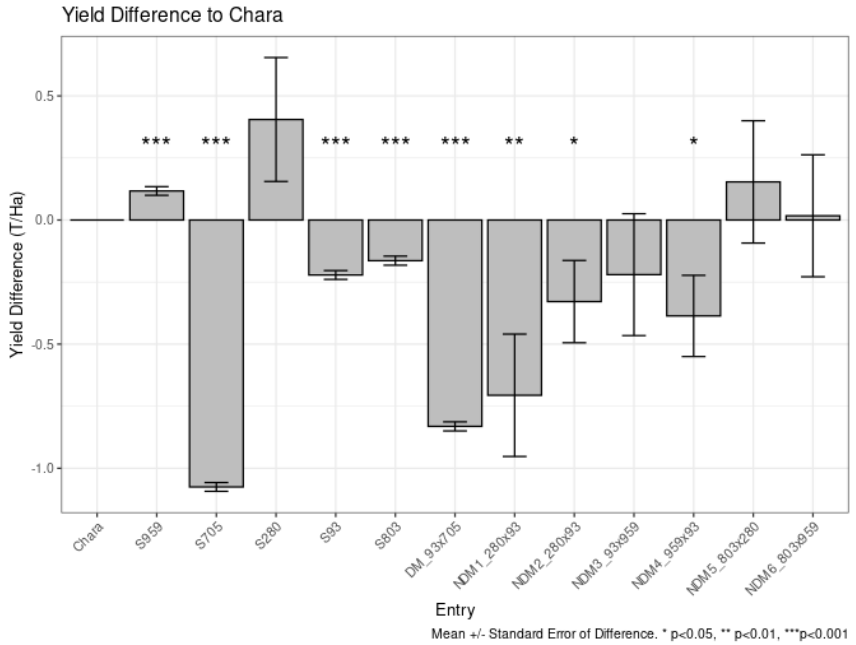
System for the measurement of changes in plant cell protein resulting from 5'UTR edits. The system uses live plant cell expression of protoplasts (Wheat and Arabidopsis) transformed with artificial 5'-UTRs upstream of a *Venus Green Fluorescent Protein* (v*GFP*) reporter and measures protein quantity via flow cytometry.

“A gene edit is only as good as its target”*

Informed by:

- ❖ Climate Models
- ❖ Agronomy and Farm management
- ❖ Phenology
- ❖ Physiology
- ❖ ‘omics
- ❖ (Pan)Genomes / genomic selection

15 genotypes by 11 environments



What gene, which homeologue, what function domain to target

*** Barry Pogson, 2023**

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Crops and target traits for gene editing

Key Crops	**Some Target traits**
• Wheat • Barley	Yield potential, photosynthesis, EUE, drought, salinity, grain quality, and herbicide tolerance
• Canola • *B. carinata*	Pathogen resistance, pod shatter, pod photosynthesis, oil quality and biofuels
• Chickpea	Nitrogen use efficiency, acid soils tolerance

Canola and Carinata Team

Nay Chi Khin

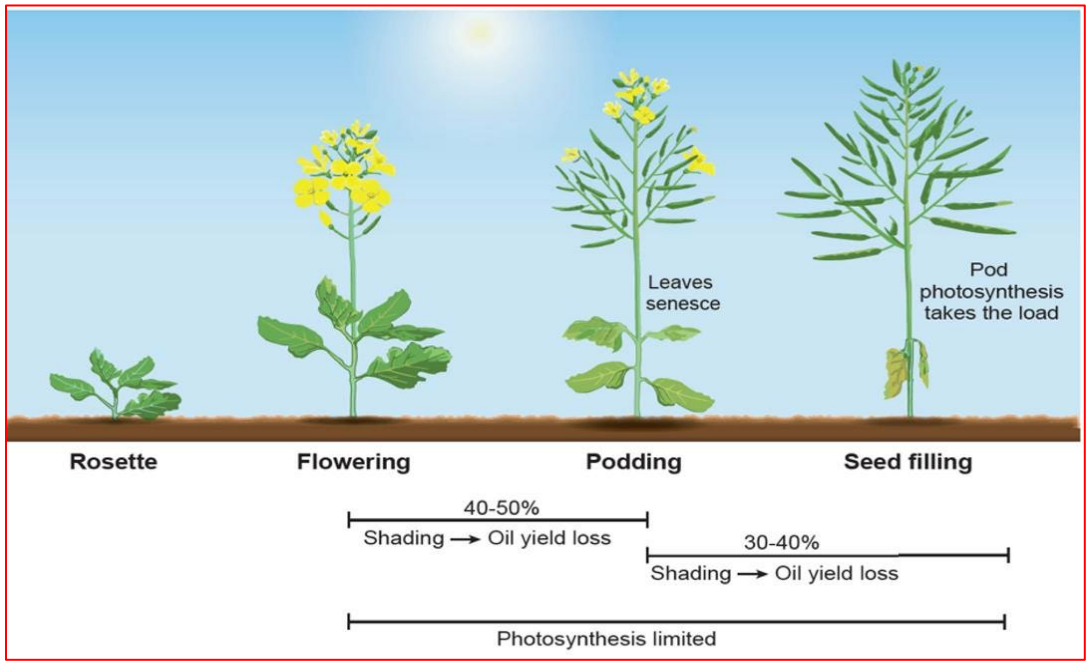
Joint trait focus team with ANU PhD students doing placements at NSW DPI and our partner Breeding Companies and ANU's Dr Nay Chi Khin based at Wagga

- Blackleg resistance
- Pod shatter resistance
- Pod photosynthesis
- Drought tolerance/water use efficiency
- Phenology/architecture (*carinata*)
- Oils for aviation fuel (*carinata*)

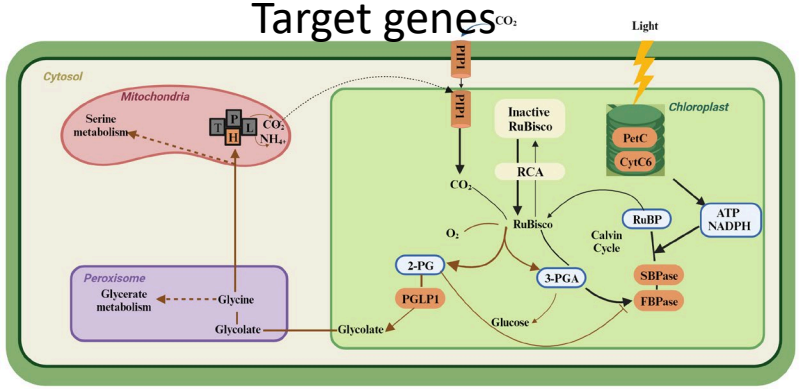
Optimising pod photosynthesis in canola and carinata

Dr Jing Zhang

Sadia Majeed
PhD Student



Target genes



Diversity panel
(18 lines from NSW DPI and Advanta seeds)

Transgenic lines
Overexpressing genes encoding key limiting proteins in photosynthesis

Ground-truth measurement (Licor-6800)

Low to medium throughput phenotyping (Phenospex, hyperspectral spectral imaging, MultispecQ)

Identification of limiting factors for photosynthesis on performance, growth and yield in canola

Selection of lines with improved photosynthetic capacity

Validation under field conditions

Field validation for the GM lines to determine photosynthesis and growth impacts

Impacts of improved photosynthesis on grain yield

Australian National University

CSIRO

NSW GOVERNMENT Department of Primary Industries

ADVANTA

Nay Chi Khin

Gene-editing Tools For Improving Pod-shatter Resistance in Canola and carinata

Julian Greenwood

SDN-1 edits leading to loss-of-function mutations in pod developmental genes Which promote pod shatte

Field deployable, transgene free, pod shatter resistant Canola

Australian National University

GRDC GRAINS RESEARCH & DEVELOPMENT CORPORATION

NSW GOVERNMENT Department of Primary Industries

Created with BioRender.com

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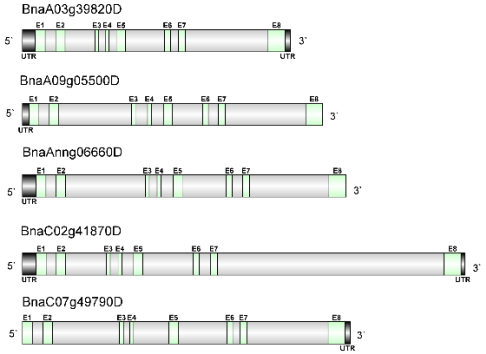
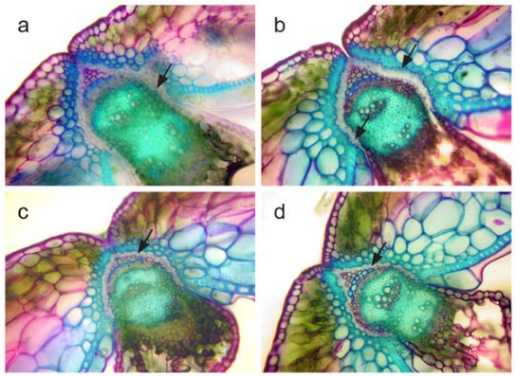
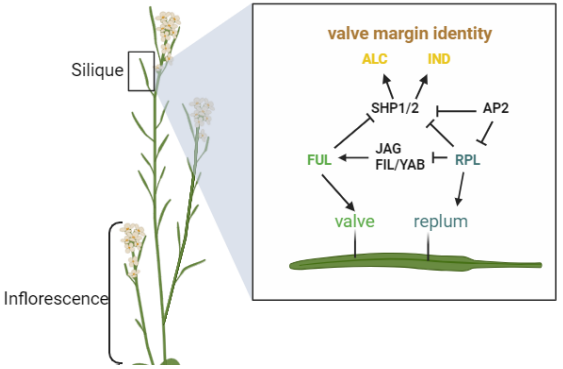
Natural variation and gene targets for pod shatter resistance

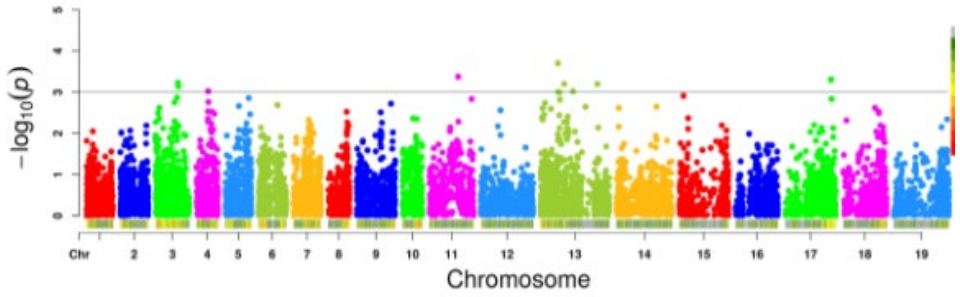
- Target functional variants and expression variation
  - Gene editing
- QTL for epistatic interactions
  - Introgression and backcrosses

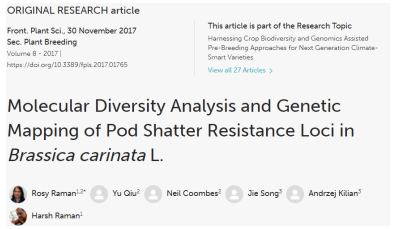






Resynthesis of *B. napus* from *B. rapa/B. carinata* with pod shatter resistant parents




ORIGINAL RESEARCH article

Front. Plant Sci., 30 November 2017
Sec. Plant Breeding

This article is part of the Research Topic

Molecular Diversity Analysis and Genetic Mapping of Pod Shatter Resistance Loci in *Brassica carinata* L.

Raman et al 2014, 2017 2023

Improving Blackleg Disease Resistance in Canola

Arslan Mahmood
PhD Student

Julian Greenwood

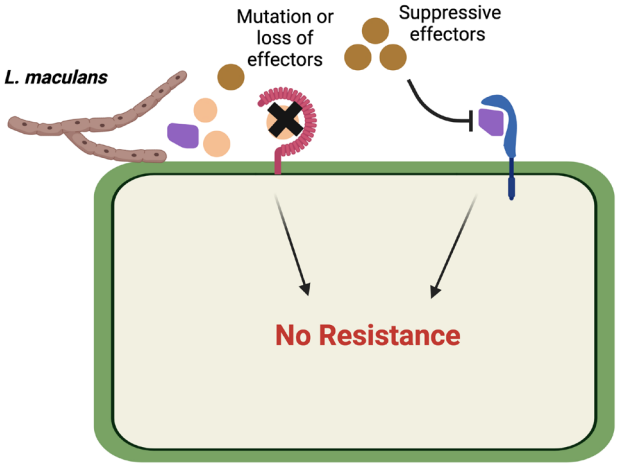
Canola is an important crop but susceptible to blackleg

- **Prominent blackleg symptoms on canola**

Leaf lesion | **Stem infection** | **Pod infection** | **Pseudothecia on canola stubble**

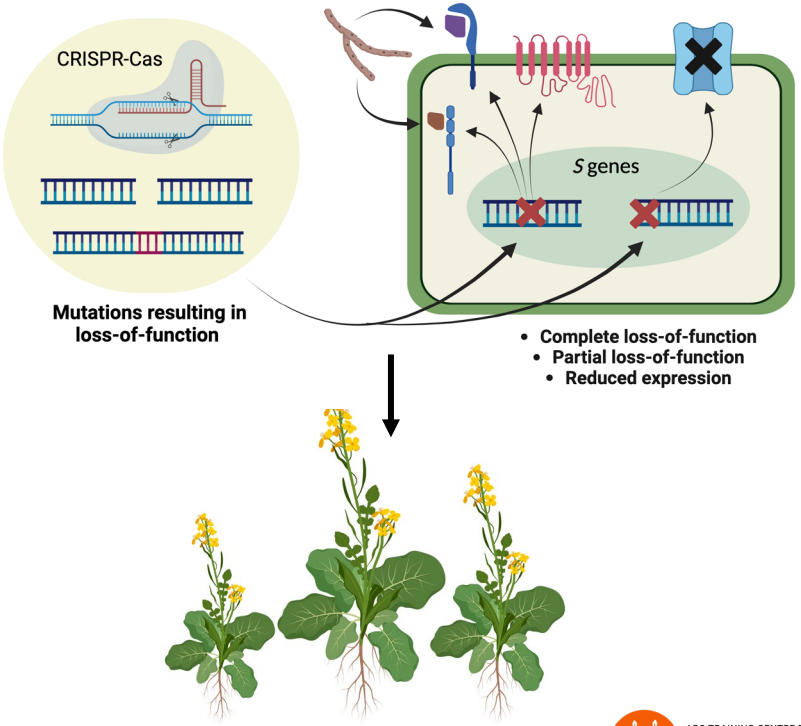
Resistance (*R*) genes do not provide durable resistance

- **Pathogens rapidly evade recognition by major resistance genes**



SDN-1 edits of susceptibility (*S*) genes for provide durable resistance

- **Potential for field deployable nonGM disease resistant plants**



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ADVANTA
ARC TRAINING CENTRE FOR FUTURE CROPS DEVELOPMENT

Commerialization bottlenecks for Carinata

Challenge - apply our technologies to rapidly create Carinata cultivars and demonstrate commercial potential for Australian Agriculture

- 60 plus years of breeding, 100s of Aus Canola varieties
- Advanced traits and performance
- Advanced Gene Technology deployed routinely.

- Populations, diversity panels and double haploids available via Nuseed and NSW DPI
- Potential to rapidly select for better uniformity, yield a and performance
- Gene Tech readily adaptable to carinata.

ARC TRAINING CENTRE FOR FUTURE CROPS DEVELOPMENT

Why Carinata?

HARVESTED COVER CROP
Increased GHG reduction/biomass
Positive impact with no increased land use
Sustainable agriculture practices

NON-FOOD COVER CROP
Additional grower revenue
Certified sustainable contract production
Moisture management
Biodiversity in crop rotations
Reduces weed, disease/pest pressure

DEEP ROOTED BIOMASS
Efficient nutrient uptake
Improves soil quality
Soil carbon sequestration

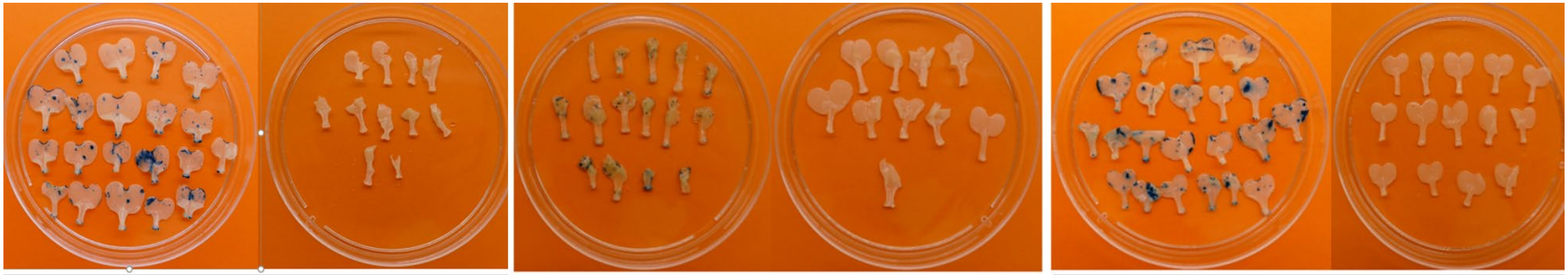
nuSEED® CARINATA

Reshma Roy,
PhD student

Development of efficient transformation in *B. carinata*

- Genotypes being tested
  - AGG-1, AGG-2, DHN-1, DHN-2 and DW3
- Regeneration efficiency ranged from 5-55%
- Transient GUS expression, 100% (pCAMBIA 1305.1)

AGG-1 DHN-1



DHN-1 AGG-2 AGG-1

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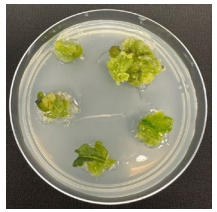
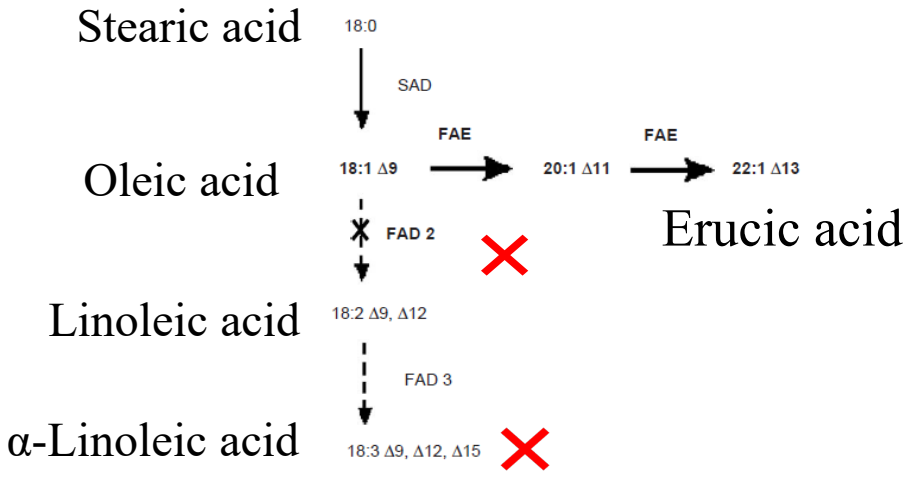
Ebtihal Mohamed
PhD Student

Towards sustainable aviation fuel via *B. carinata*

Targeting erucic acid and accelerating domestication by

- Screening diversity panels and double haploids
- Gene editing traits
  - e.g. fatty acid synthesis





B. carinata double haploids

B. carinata diversity panel

Australian National University

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NSW Government Department of Primary Industries

ARC Training Centre for Future Crops Development

P2 SOCIETY

Program 2 objective

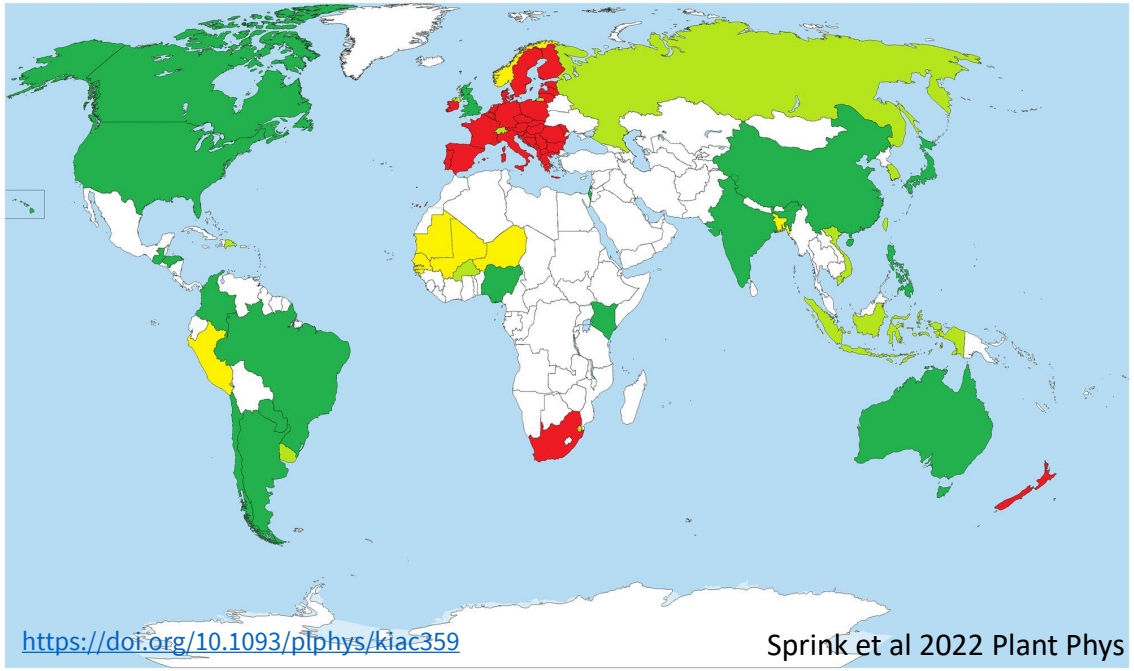
Objective 2: Train researchers and leaders in socio-economic considerations and opportunities for innovation in alignment with community needs.

- *We will equip the next generation of research, industry and policy leaders to make social engagement the norm, through a tailored research/training program.*
- *They will be empowered to develop innovative new technologies aligned with community needs and values. Without engagement of stakeholders and end users, translation is rarely successful.*

ARC TRAINING CENTRE FOR
FUTURE CROPS
DEVELOPMENT

Regulatory Frameworks: Local and Global

Our production is local our markets are global



Sprink et al 2022 Plant Phys

* https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/.

- Globally SDN1 is mostly no or light regulation
- Nationally, SDN1 is not GMO, SDN2 and 3 are classified as GMO

  "SDN1 is half opening the tool box, we only have access to spanners not screwdrivers " -Tress Walmsley, Intergrain

- Global regulation is dynamic and all maps are subject to change*

  e.g. Europe is under review and NZ maybe

- Focus on the product not the process

  "We need policies to be future proofed" -- Osman Mewett BASF

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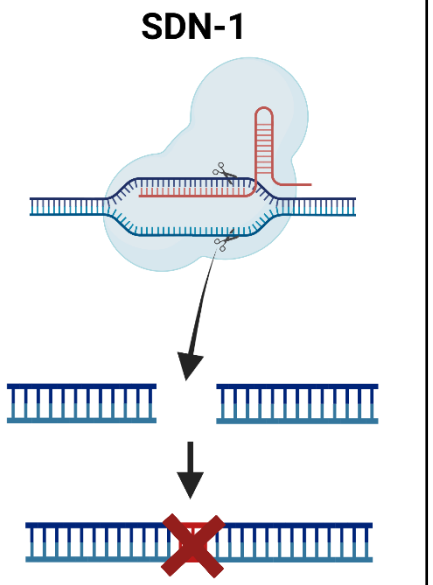
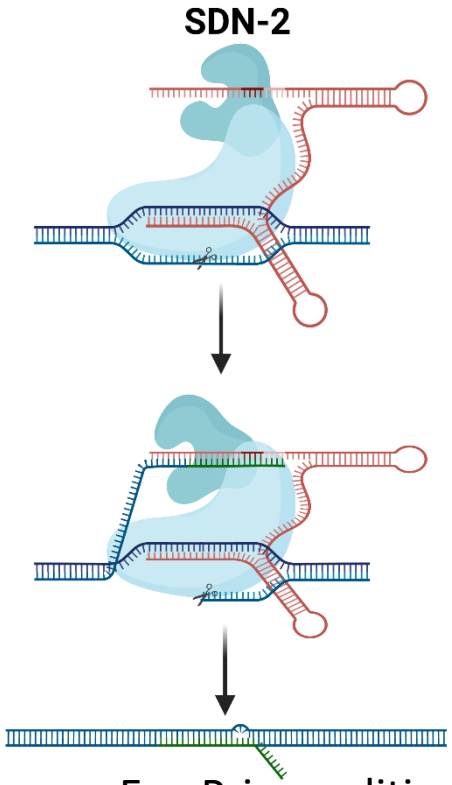
Defining gene editing - two categories (Non-template and template repair)



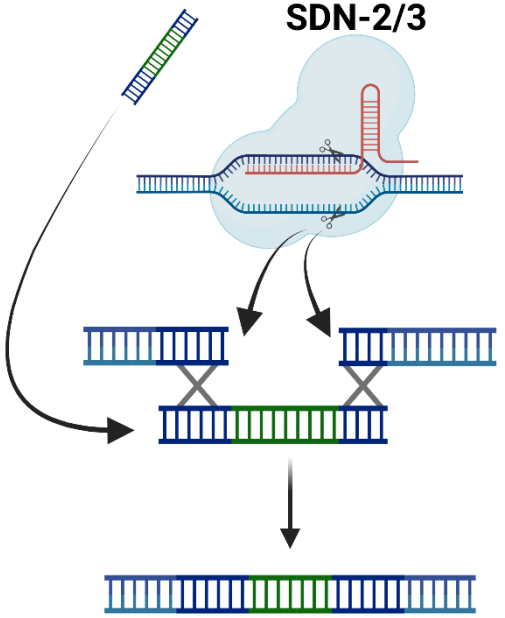
1. **Non-template repair SDN1**

Non-homologous end joining repair

2. Template based repair SDN-2/3



E.g. Prime editing



E.g. Templated homology directed repair

- SDN-1 targeted deletions
  - Routine,
  - Not GMO in Australia

- SDN2 and 3 templated guided modifications
  - Some tech requires optimization in plants
  - GMO in Australia

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Joan Leach

Rachel Ankeny

Innovation needs to be informed

Common societal views and expectations

Benefit needs to be global and local

Technological gains and concerns of food provenance, quality and cost

Technologies that can be incorporated into farming systems

Local informing and inclusion in decisions is essential

Local economics typically outweighs global

Carolin Plewa

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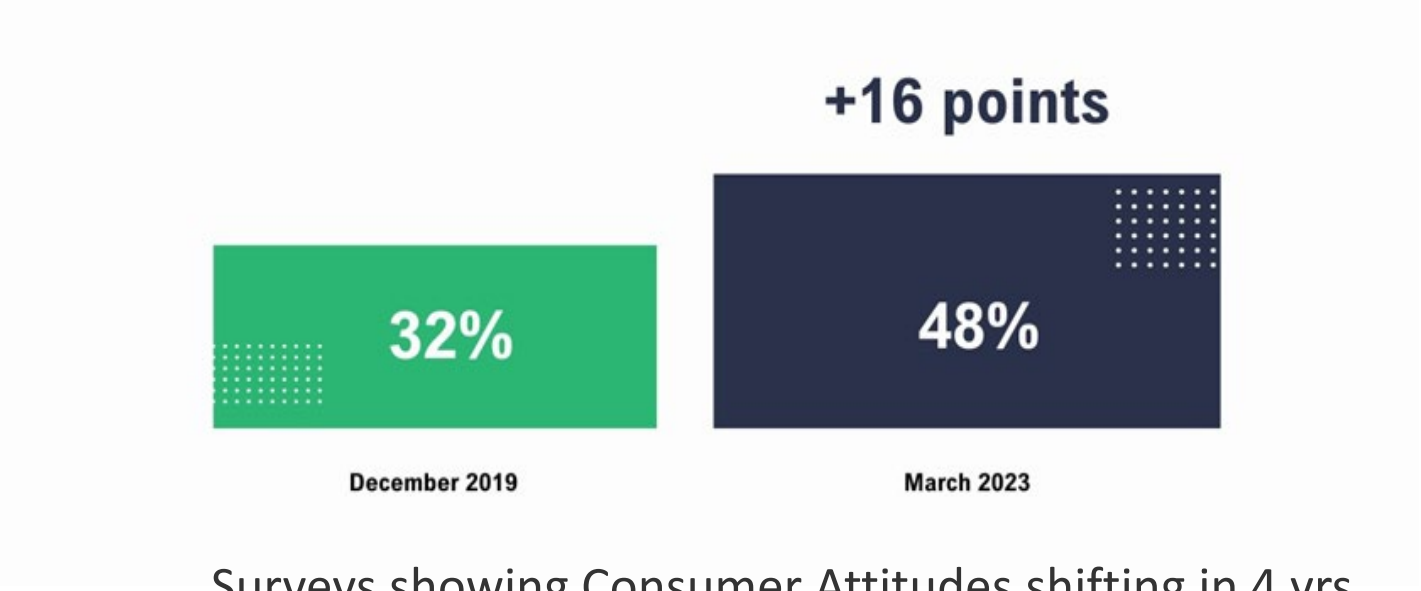
Attitudes to Food Tech are shifting



Surveys showing Consumer Attitudes shifting in 4 yrs

- Growing sentiment supporting new approaches to tackling global challenges
- Younger adults are more open to food technology.

pairwise®

- https://www.pairwise.com/news/new-pairwise-data-shows-that-consumer-attitudes-toward-foodtech-produce-are-changing

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