New Breeding:

* Techniques
* Technologies
* Innovations

David Hudson (SGA Solutions Pty Ltd)

Carl Ramage (Rautaki Solutions Pty Ltd)

Disclaimer

Please note that this presentation is for informational purposes only. Any comments or statements made herein do not necessarily reflect the views of Rautaki Solutions Pty Ltd and SGA Solutions Pty Ltd. The information is intended for the recipient's use only and should not be cited, reproduced or distributed to any third party without the prior consent of the authors. Although great care is taken to ensure accuracy of information neither Rautaki Solutions Pty Ltd nor SGA Solutions Pty Ltd can be held responsible for any decision made on the basis of the information cited.

NBT's – their role in plant breeding

Traditional Plant Breeding

- Plant breeding is a product-oriented, science-based discipline for improving plants. Plant breeding is rooted in population development, selection theory, quantitative genetics, statistical analysis and an increasing number of support technologies. It involves generating genetic variation, selecting desirable plants, stabilizing inheritance of desirable characteristics, testing in multi-locations over many years, and multiplying the best performing plants.
- Plant breeders have always used the creation of new variations of plant characteristics to provide solutions for resistance to plant diseases and pests, to increase tolerance to environmental stress, to improve quality and yields, and to meet consumer expectations. Plant breeding depends upon genetic variability within and across related species as a basis for developing new plant varieties with improved characteristics.
- To create a new plant variety, plant breeders have generally relied on two sources of genetic variation as a basis for new characteristics: the inherent diversity in a plant's gene pool and new, naturally occurring variants of existing genes.

Traditional Plant Breeding:
Diversity is the key to successful development.

Traditional Plant Breeding

- Breeders often make crosses between plants of diverse genetic makeup to produce new combinations of genetic characteristics which result in diverse morphological or quality characteristics in the progeny plants. The natural diversity of different sources of germplasm within a species or its close relatives is a primary source of genetic variation.
- Genetic variation can also be increased by using mutations – changes in the DNA sequences of the plants. Natural and induced mutagenesis can introduce different classes of mutations, one of which is the point mutation.
- Since the 1950s, well over 3200 crop varieties have been directly developed by induced mutations. Some examples of the successes of mutation breeding include:
  - High-yielding and short barley for brewing industry
  - Heat tolerance and early maturity in cotton
  - Seedless watermelon
  - Multiple disease resistances in tomato.
  - Ruby Red grapefruit
  - Gold Nijisseiki Japanese pear which is disease resistant
  - Peanuts with tougher hulls
  - Semi-dwarf rice with higher yields
  - Virus resistant cocoa plants
  - Canola, sunflower and soybeans with healthy fatty acid composition (e.g. Monola)
  - Canola with herbicide tolerance (Triazine Tolerant and IMI Tolerant canola)

Key driver for New Plant Breeding Techniques

- Innovation in plant breeding is necessary to meet the challenges of global changes such as population growth and climate change.
  - By 2050 there will be 9.7 billion people on the planet that will require food to eat, feed for animal production and energy.
- Agriculture has been able to cope with these challenges until now.
  - In 1960: 1 hectare was enough to feed 2 people
  - By 2025: 1 hectare will be needed to feed 5 people
  - By 2050: farmers will need to produce 70% more food than today to sustain the world's population. (Source:UN)
- However, further efforts are needed and therefore plant breeders search for new plant breeding techniques.

Why NBT's?

- The main driver for the adoption of new plant breeding techniques is the great technical potential of these techniques. Most of the techniques can be used for producing genetic variation, the first step in plant breeding.
- This is achieved through:
  - targeted mutagenesis
  - targeted introduction of new genes or gene silencing.
  - selection of plants with specific traits, the second step in plant breeding.
- The new plant breeding techniques show technical advantages when compared to 'older' techniques: some (ODM and ZFN technique) allow site-specific and targeted changes in the genome. For many of the techniques the genetic information coding for the desired trait is only transiently present in the plants or stably integrated only in intermediate plants. Therefore, the commercialised crop will not contain an inserted transgene.
- The second main driver for the adoption of new plant breeding techniques is its economic advantages. The use of new plant breeding techniques makes the breeding process faster which lowers the production costs. For example, cisgenesis uses the same gene pool as conventional cross breeding, but is much faster as it avoids many steps of back-crossing.

Examples of NBT's

- Zinc finger nuclease (ZFN) technology (ZFN-1,ZFN-2 and ZFN-3)
- Crispr-CAS
- TALENS
- Oligonucleotide directed mutagenesis (ODM)
- Cisgenesis and intragenesis
- RNA-dependent DNA methylation (RdDM)
- Grafting (on GM rootstock)
- Reverse breeding
- Agro-infiltration (agro-infiltration "sensu stricto", agro-inoculation, floral dip)
- Synthetic genomics

- All techniques have been adopted by commercial breeders and the most advanced crops could reach the stage of commercialisation in the short to medium term (2-3 years) in the event of these techniques not being classified as resulting in GMOs.
- The main constraints for the adoption of the techniques are the regulatory uncertainty and the potentially high costs for risk assessment and registration (if the crops derived by these techniques are classified as GMOs). Crops resulting from most of the techniques cannot be distinguished from conventionally bred crops and detection is therefore not possible.

What is Gene Editing?

Gene editing refers to biotechnologies that allow small and precise changes to the genome (DNA) of an organism (e.g. animal, plant and microbe).

What is Gene Editing?

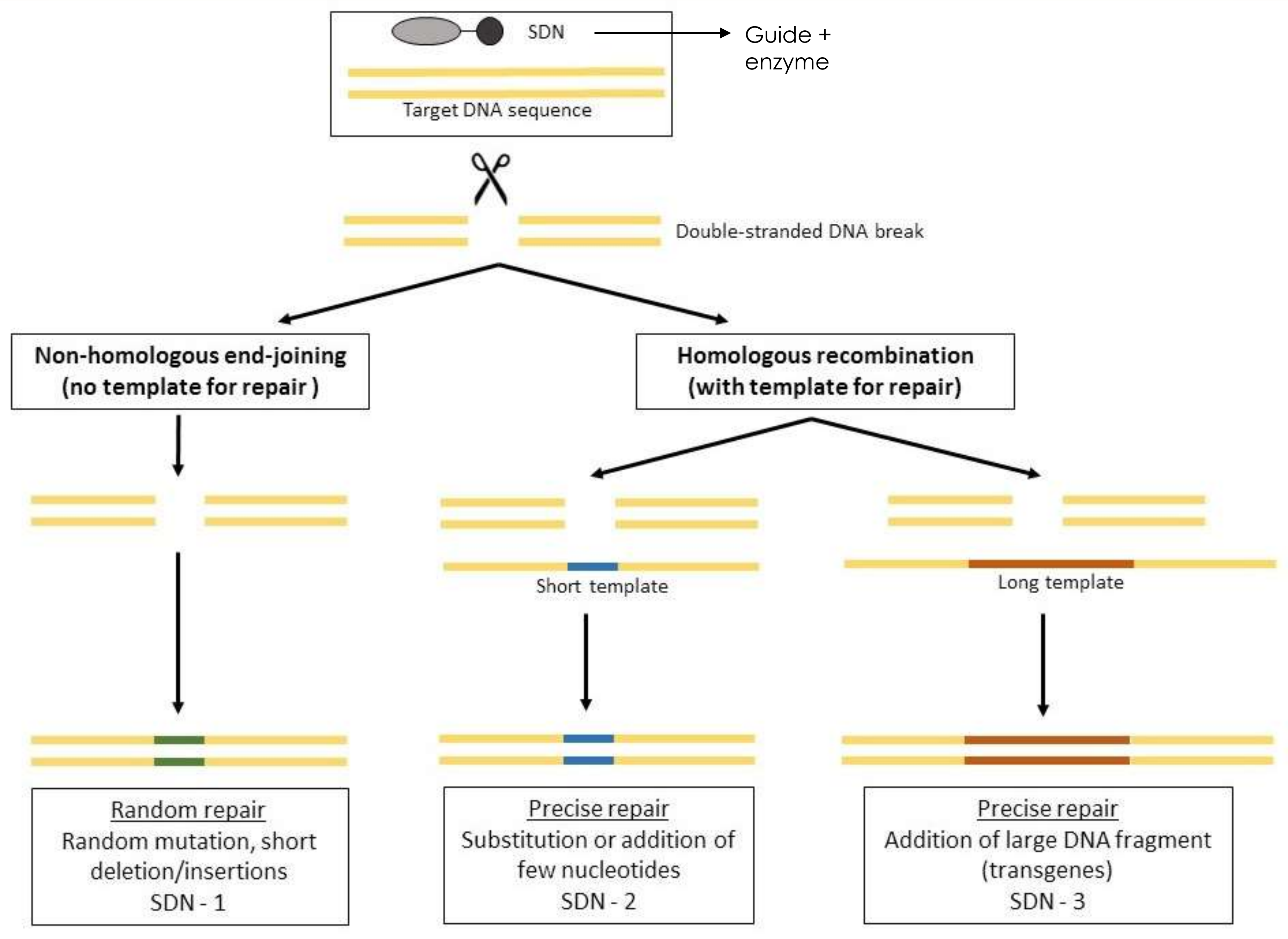
There are at least **three generations of technologies** that allows gene-editing in plant and mammalian species (ZNF, TALENS, and CRISPR).

All of them use a "guide" to find a specific genomic region, at that point scientists then use an enzyme cut/break the DNA at that location.

The DNA break is then **repaired** by the cells at **random** or **using a template** that is provided, generating different classes of gene-editing.

The gene-editing class follows the type of DNA repair used by the cells

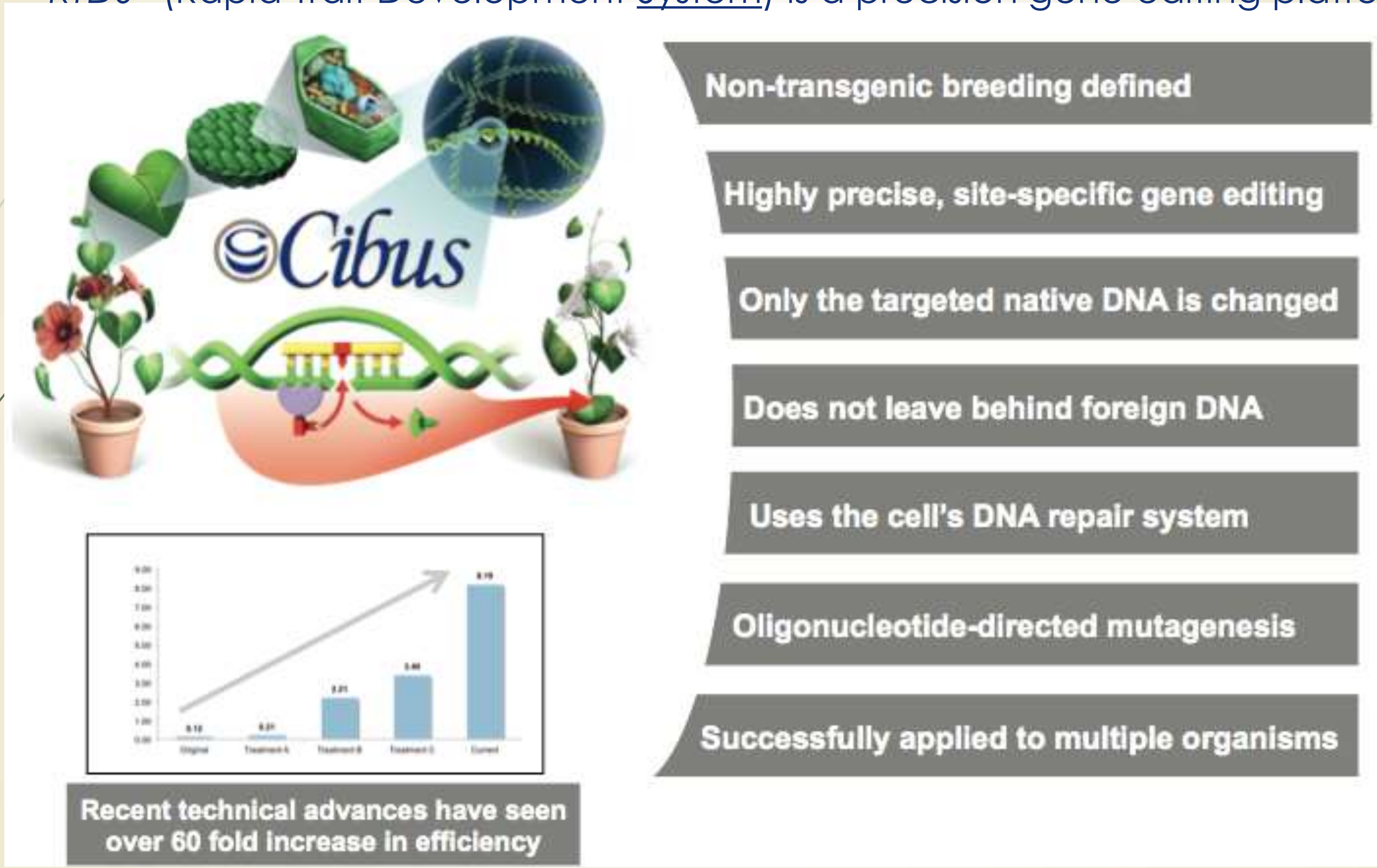
- **SDN-1:** The DNA break is repaired without a template. This is a error prone systems routinely used by the cells. Usage: mainly used to **knock-down a gene**.
- **SDN-2:** The DNA break is repaired using a short template that is used to induce specific changes. Usage: precise **DNA base substitution or small insertions or deletions**.
- **SDN-3:** Similar to SDN-2, but uses longer repair template. Usage: can be used to **introduce a whole gene**. Most likely will lead to an organism that will be considered genetically modified.


SDN
Guide +
enzyme
Target DNA sequence
Double-stranded DNA break
Non-homologous end-joining
(no template for repair)
Homologous recombination
(with template for repair)
Short template
Long template
Random repair
Random mutation, short
deletion/insertions
SDN - 1
Precise repair
Substitution or addition of
few nucleotides
SDN - 2
Precise repair
Addition of large DNA fragment
(transgenes)
SDN - 3

What is Cibus's Technology Platform? *RTDS*

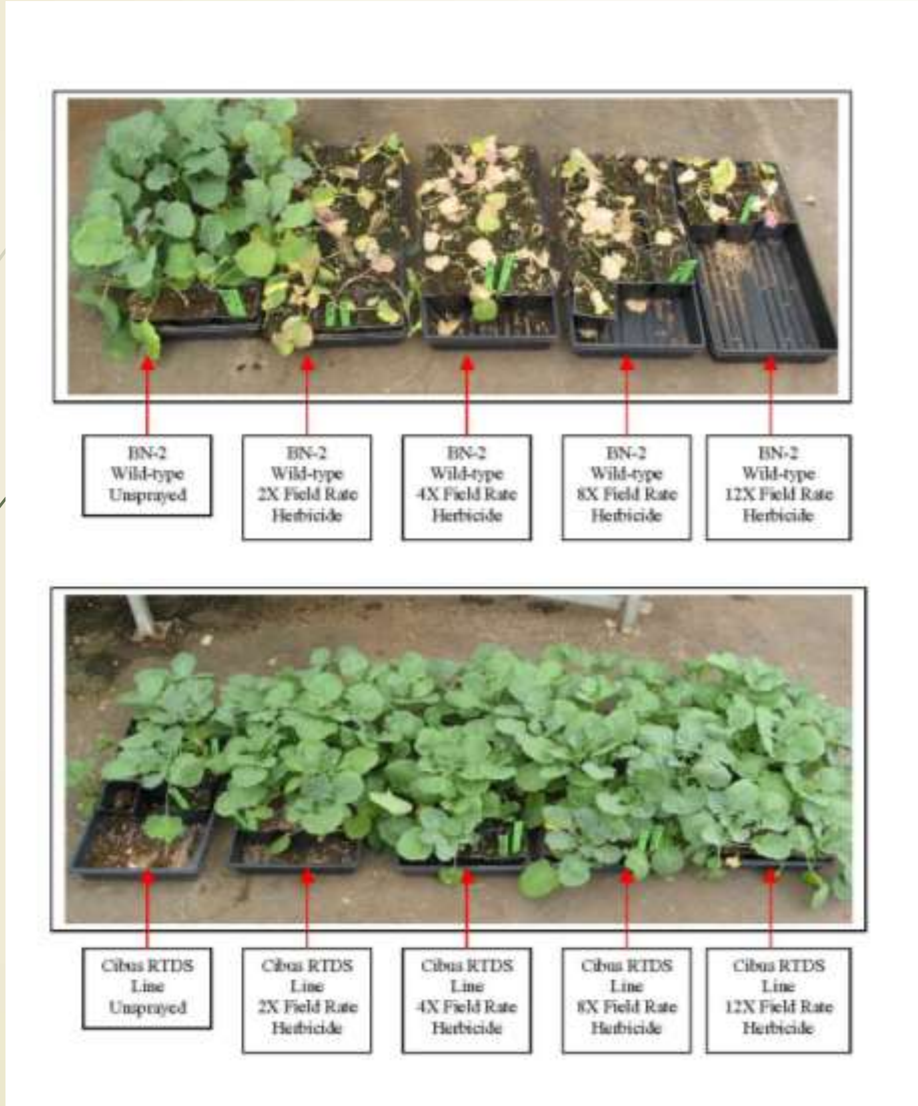
(*RTDS* – includes many technology processes leading to non-transgenic breeding)

» "*RTDS*" (Rapid Trait Development System) is a precision gene editing platform.



First Product to Market

Sulfonylurea (SU) tolerant canola



- Picture on left shows canola resistant to 12X field rate of targeted herbicide using ***RTDS*** technology

SU CANOLA

Applications of NBT in plant breeding

- ***Farmer Benefits***
  - Through improved seed, plant breeding innovation provides yield stability, despite a changing climate.
  - Plant breeding innovation creates plants that can resist pests and diseases, enabling more choice and flexibility for farmers, and potentially fewer crop inputs.
  - Plant breeding innovation provides quality seed which meets the challenges and needs of farmers.
- ***Farmer Products***
  - Improved seed adapted to farmer's needs leads to more reliable harvests and stable incomes.
  - Thanks to innovations in plant breeding we can:
    - reduce the cost and time required to bring improved seed to farmers
    - rapidly adapt crops and plant varieties to changing climate
    - increase effective options for weed, disease and pest management
    - increase food production under environmental stress factors caused by climate change and extreme weather conditions.

New products based on NBT

Cibus' new ***SU Canola***™ is a non-transgenic (non-GMO) sulfonylurea (SU) herbicide tolerant canola

Cibus' new ***Flax*** will be the first non-transgenic (non-GMO) glyphosate tolerant crop. It is expected to be launched in the United States in 2019 and in Canada a year later.

Applications of NBT in plant breeding

- ***Environmental Benefits***
  - Plant breeding innovation results in improved seed that can increase yields while decreasing greenhouse gas emissions and reducing environmental impact.
  - By developing new seed varieties that are better able to withstand attacks from pests and diseases we can reduce and optimize the use of crop inputs.
  - Improved seed varieties that increase yield result in more crop per acre. This means that more forest, flora and fauna can remain untouched by agricultural production, preserving natural habitats.
- ***Environmental Products***
  - Plant breeding innovation can result in plants that survive and even thrive in extreme weather conditions.
  - Increasing yields on less land using conservation tillage supports soil health and optimizes the use of farmland, fuel, labour and water while more efficiently using crop inputs.

Applications of NBT in plant breeding

- ***Consumer Benefits:***
  - Plant breeding innovation enables us to meet consumer expectations with improved plants that provide longer-lasting, fresh, nutritious and affordable food, as well as fuel and fiber.
  - Plant breeding innovation contributes to the health and well-being of consumers and has the potential to improve quality of life.
- ***Consumer products***
  - vegetables with a higher resilience to transport and storage
  - cereal varieties suitable for gluten-intolerant/celiac
  - Disease
  - crops with increased nutrients
  - optimized bio-fuels as an alternative to fossil fuels
  - hypoallergenic plants for clothing and furnishings
  - flowers, trees and turf for sustainable green spaces

Current Regulatory Status

"UNCERTAIN"

Key Challenges

- Should we regulate these traits?
- How would we classify them?
  - Many traits for livestock are Generally Regarded As Safe (GRAS)
  - Traits already exist
  - Opportunities to “fix” broken genetics
- Current R&D small animal–Schd 3 Part 1 1.1(a) PC1 NLRD
- Are there any off-target effects from editing?
- Any regulatory principles need to apply broadly
  - Plants, animals, bacteria, fungi, viruses, invertebrates, aquatic organisms etc.
- Process vs Product based regulation
- Trade implications
  - Multiple agencies–who would have jurisdiction?
  - How would we know?
  - AP or LLP?

Australian regulatory environment for new breeding technologies – Current Status.

- Key Regulators
  - Office of Gene Technology Regulator - Human health safety & the environment
  - Food Standards Australia & New Zealand - Food Safety & labelling
- Regulations established in 2001, with a 4-5 year cycle of review.
- Current Gene Technology Act & Regulations are based on "process" vs "end product" – products derived from NBT process currently require deregulation.
- OGTR and FSANZ completing reviews of respective Act's and Regulations governing biotechnology with a particular interest in making changes that reflect innovations in biotechnology.
- Likely outcomes:
  - No change to the Gene Technology Act & Regulations being based on "process".
  - Some changes expected to align definitions between regulatory agencies.
  - Some efficiency gains expected in the regulatory process.
  - Potential for introduction of risk based 'tiers' of regulation.
  - Potential exists for increased flexibility to respond to changes in scientific understanding and understandings of risk
  - **Draft amendments to the Regulations indicate that:**
    - **organisms modified using site-directed nucleases without templates to guide genome repair (i.e. an SND-1 approach) would not be regulated. Oligo-directed mutagenesis and SDN-2 and SDN-3 would, however, be regulated.**
    - **some RNAi use is to be exempt from regulation as well as requiring a licence to undertake any dealings that use gene drives.**

Summary
Plant Breeding – Tool Box

- NBT's
- Genome Wide Selection
- Advanced Scientific Computing – Big Data
- Phenomics / Genomics / Metabolomics etc
- Genomic Estimated Breeding Values (GEBV's)
- Double haploids
- ……..

Winning from Digital & Technological Disruption

Advanced Scientific Computing – Big Data

More Data Storage **400X**

More Memory **280X**

AgTech and Big Data for Impact Delivery

Advanced Air- & Ground-Based Phenomics Platforms

AgTech and Big Data for Impact Delivery

LMS400 Sick LiDAR

Navcom RTK GNSS receiver

CR3000 datalogger

FMX Display

Getac F110 Tablet

Trimble GreenSeeker

Apogee SI-111 IRT sensor

Baumer sonic sensor

EM38 MK2

⇒ New sensors for new phenotypes enabling phenomics at scale

‘AgTech and Big Data for Impact Delivery’

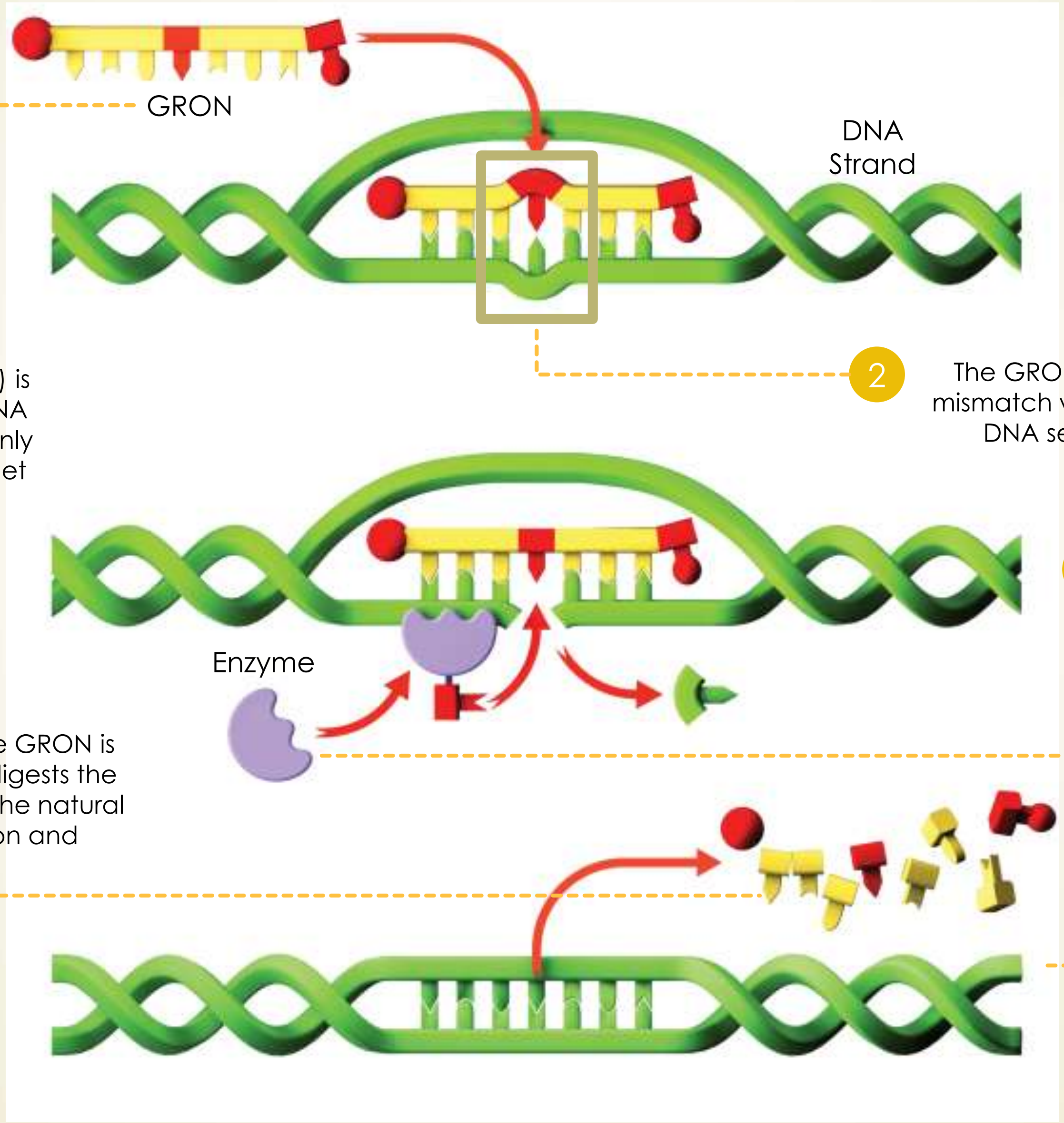
AgTech and Big Data for Impact Delivery

Summary

"Into the future, the skill and success of plant breeders will be determined by their ability to use their resources efficiently, retaining proven methodologies and augmenting them with novel approaches to meet their breeding objectives. It may be that with the plethora of new tools, efficient plant breeders will have to be equally ruthless with their access to and use of technology."

Thank You

RTDS™ GRON Explained – Creating a Change



1. A Gene Repair Oligonucleotide (GRON) is paired with the plant DNA sequence. The pairing only occurs at the gene target region.

2. The GRON creates a mismatch with the plant DNA sequence.

3. The plant's native DNA repair enzymes recognize the mismatch and repair the plant's DNA using the GRON as a template.

4. Following the repair, the GRON is removed and the cell digests the GRON. This is all part of the natural process of cell division and multiplication.

5. *RTDS* is complete and the targeted gene has been repaired.