

Rapid Trait Development System: Non-Transgenic Approach to Providing Plant Improvements in Canola

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**ASA Meeting
3 November, 2010
Long Beach, CA**



Introduction

- ❖ Cibus Background
- ❖ Discussion of Cibus Technology
 - Rapid Trait Development System (*RTDS*)
- ❖ Commercial Progress
 - Canola development
- ❖ *RTDS* Future Potential

Cibus

Developmental stage company with offices in:

- San Diego, CA (25,000 sq. ft. lab, greenhouses)
- St Paul, MN
- Wemeldinge, Netherlands

Over 50 full time employees (> 40 in R&D).

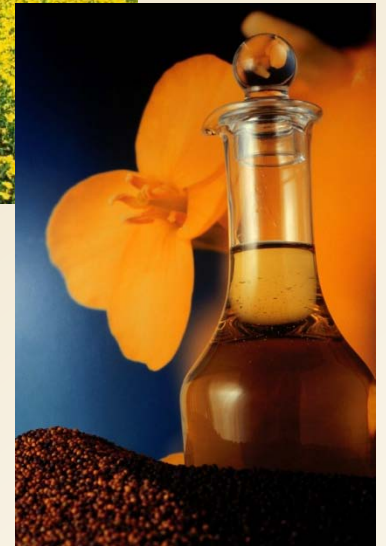
Core capabilities in Cell Biology, Molecular Biology and Biochemistry



Our Strategic Intent

To be a leading developer of plants with novel traits used in:

- Crop production
- Industrial markets
- Food industry



Cibus Partners

- **Makhteshim Agan**

Crop protection traits in 5 crops.

- **BASF**

Europe and N.America IMI tolerant traits

- **National Sorghum Producers**

Clethodim tolerant grain sorghum

- **Farmer Group developing non-GM traits in rice**

Herbicide tolerant rice

- **Major European Oil Seed Rape company**

Herbicide tolerant winter oil seed rape / canola

- **Farmer Group developing non-GM traits in potato**

Herbicide and quality improvements

- **Flax Council of Canada**

Herbicide tolerance in flax

- **Brett Young Seeds**

North American Distribution Partner



What is *RTDS*?

- ❖ Chimeraplasty
- ❖ Genoplasty
- ❖ Targeted Gene Repair (TGR)
- ❖ Oligonucleotide-directed mutagenesis
- ❖ Targeted mutagenesis

Economic Relevance of Mutagenesis in Plant Breeding

- ❖ FAO (2000) list over 2.250 cultivars derived directly from mutagenesis programs in more than 175 plant species
- ❖ Mutagenesis has led to the development of many different agronomic advances, including:
 - Dwarf plants (or plants bred for a specific height)
 - Higher-yielding, disease-resistant and earlier-maturing crops that improve the quality and availability of food for consumers
 - Herbicide tolerant crops for more efficient weed control
 - Enhanced food quality through better grain composition
 - Seedless grapes and seedless oranges
 - Bigger flowers in a wide array of colors.

Regulatory Status of RTDS in the US

- ❖ Cibus **RTDS**[™] technology is classified as mutagenesis technique by US Department of Agriculture



- USDA-APHIS has reviewed **RTDS** & provided Cibus a letter stating that **RTDS** is a mutagenesis technique
 - Products developed using mutagenesis are approved to be developed, tested, marketed and sold in the United States without regulatory constraints
- ❖ Since USDA-APHIS classified **RTDS** as non-GMO there are no issues with FDA

Plant Breeding Progression

Traditional Breeding

Problem: Slow



Traditional Mutagenesis

Problem:

- While accepted by consumers, the technology is random and not reliably predictable

Transgenic (GMO)

Problem:

- High regulatory cost
- Poor consumer acceptance
- Long breeding cycle to stabilize trait
- Multi-nationals own most patent rights

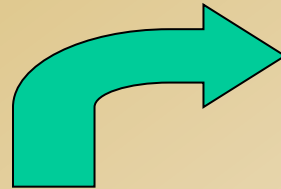
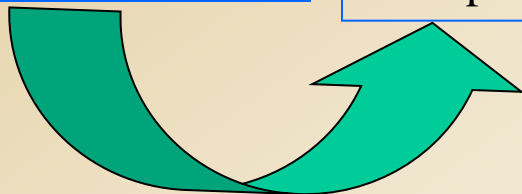
Genomic Revolution

Problem: Road map - but no tool to make specific DNA sequence changes



RTDS :the key link

- Mutagenesis tool used for site-specific DNA changes
- Minimally invasive
- Plant makes own endogenous changes
- A native process for genetic control

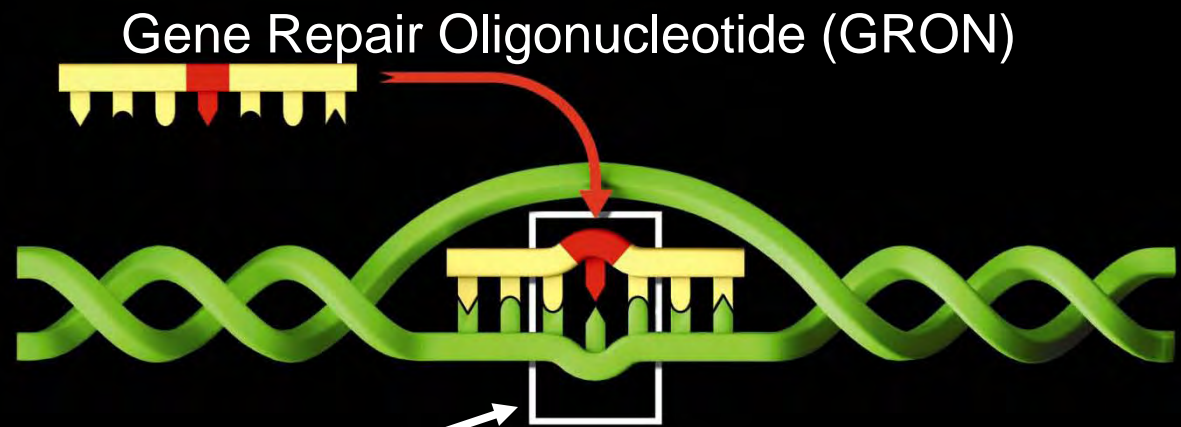


We Call *RTDS* “Gene Conversion”

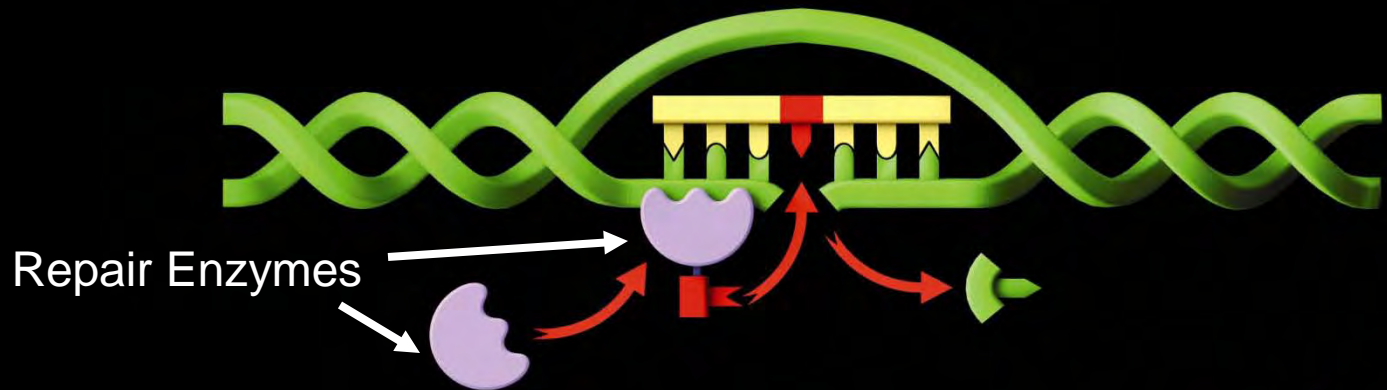
How gene conversion works.....

- ❖ Every time a cell copies its' DNA, it makes “scrivener errors” or spelling mistakes.
 - These spelling mistakes occur all the time and are a source of natural variation.
 - Every cell has its' own DNA repair machinery responsible for correcting spelling mistakes.
- ❖ *RTDS* makes targeted DNA changes by harnessing the cell's own natural DNA mismatch-repair machinery to guide a desired change in the plant gene.
- ❖ An DNA oligonucleotide “template” is introduced to the cell to guide the change

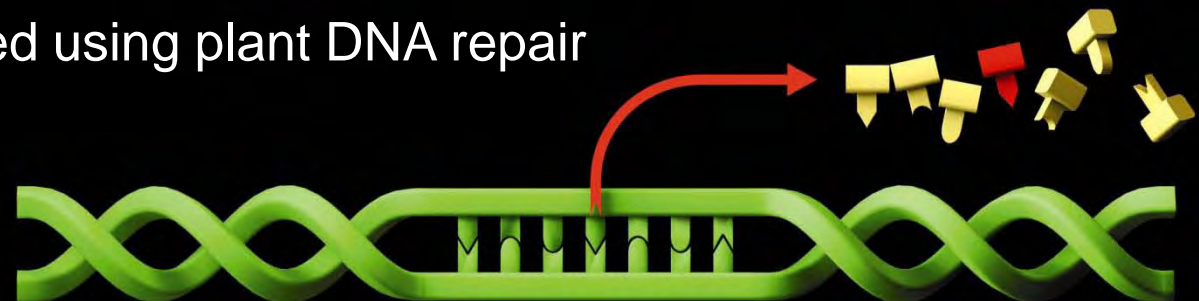
RTDS **Mechanism**



Pairing – creates a Mismatch

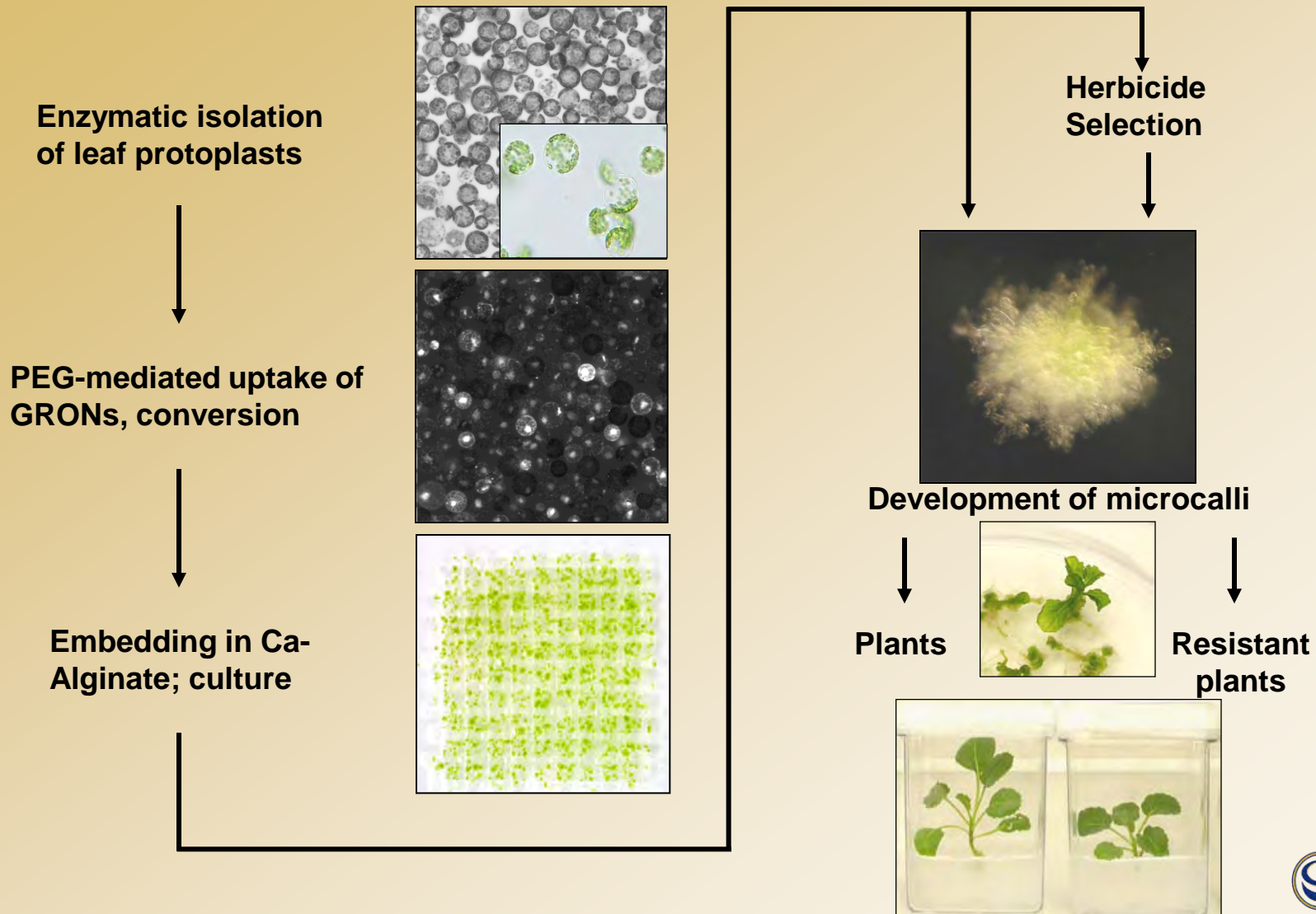


Mismatch is repaired using plant DNA repair



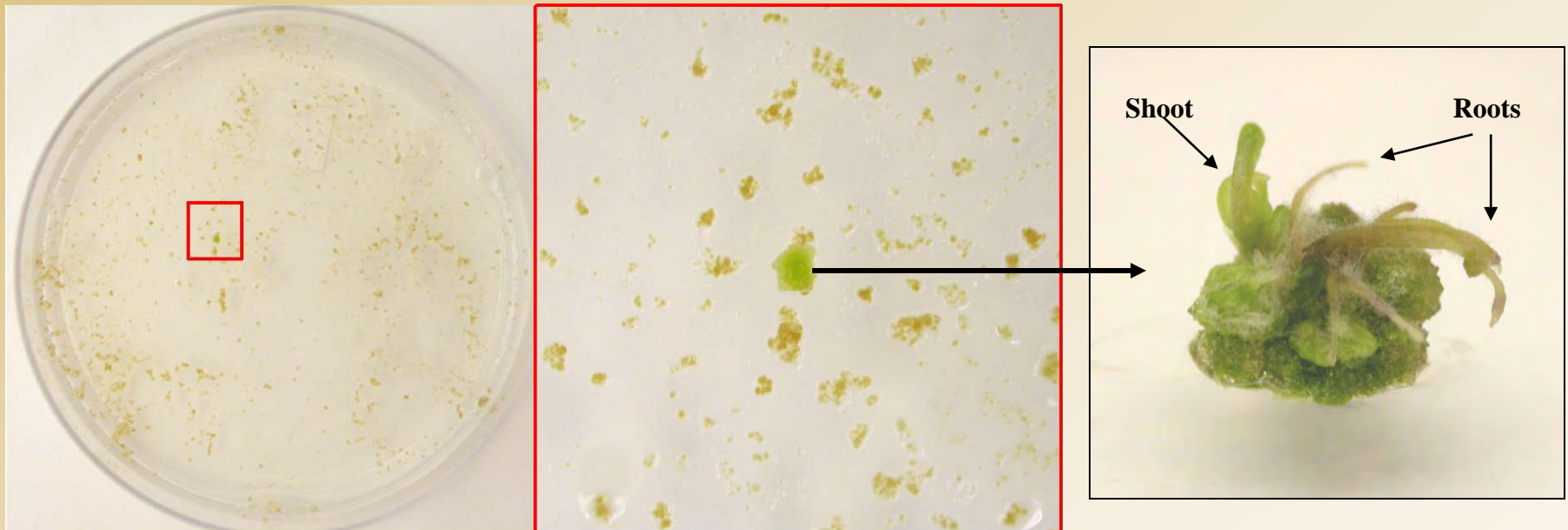
Plant gene repaired – directed using **RTDS**

RTDS in Canola

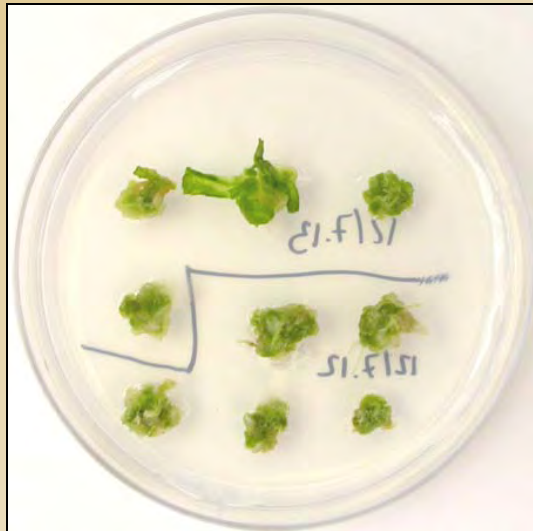


Brassica *RTDS* of AHAS gene

RTDS of a *Brassica* germplasm for SU tolerance
-material is regenerating from calli formed from
protoplasts



Regeneration from protoplast-derived calli



RTDS Canola – 2008 Greenhouse Results



BN-2
Wild-type
Unsprayed

BN-2
Wild-type
2X Field Rate
Herbicide

BN-2
Wild-type
4X Field Rate
Herbicide

BN-2
Wild-type
8X Field Rate
Herbicide

BN-2
Wild-type
12X Field Rate
Herbicide



Cibus RTDS
Line
Unsprayed

Cibus RTDS
Line
2X Field Rate
Herbicide

Cibus RTDS
Line
4X Field Rate
Herbicide

Cibus RTDS
Line
8X Field Rate
Herbicide

Cibus RTDS
Line
12X Field Rate
Herbicide

• *RTDS* has been used successfully in a number of crops:

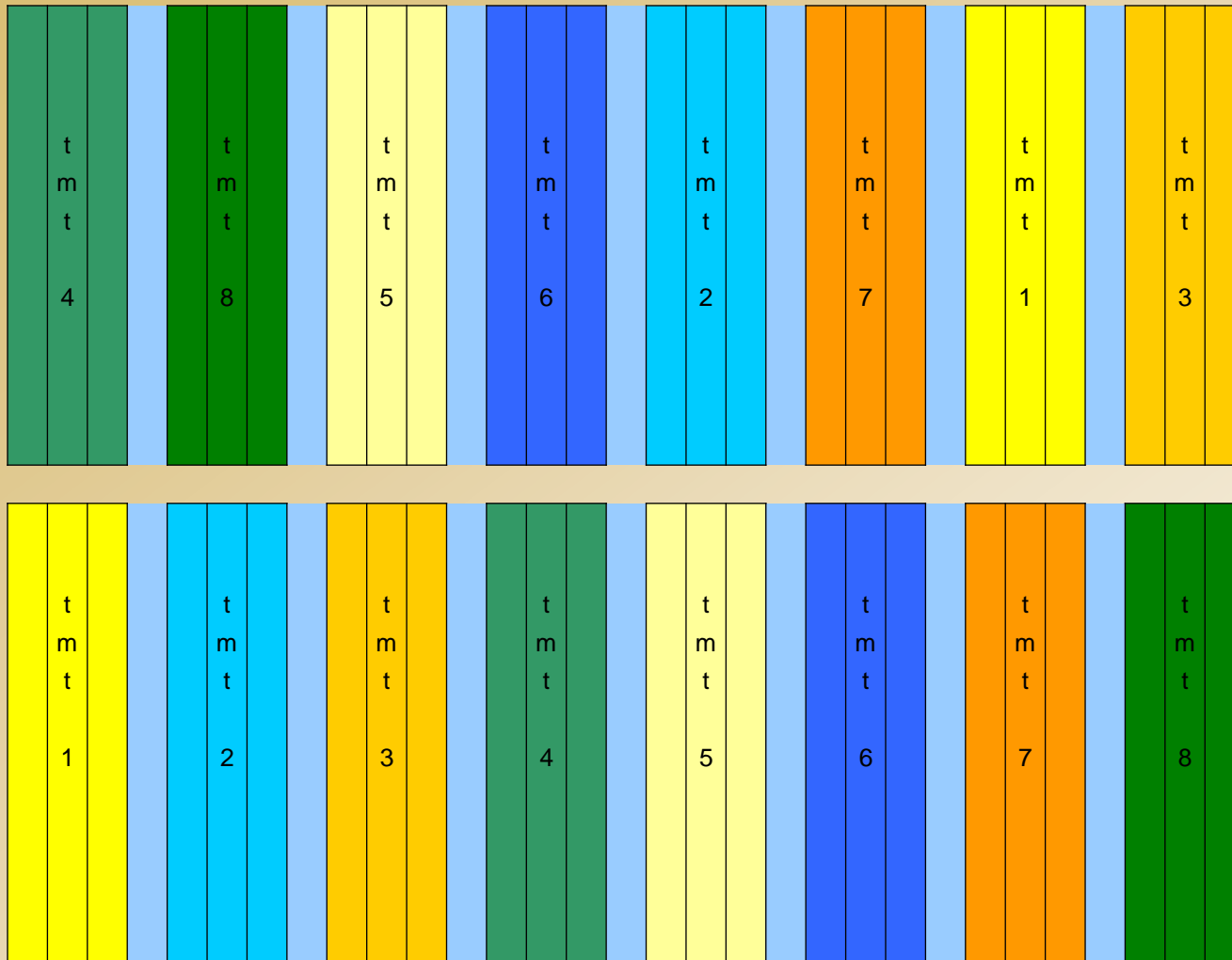
- Corn
- Tobacco
- Canola / Winter Oil Seed Rape
- Rice
- Wheat

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

2008/9 Brawley Testing

- ❖ Field testing to confirm greenhouse results
- ❖ Planted 0.75A
- ❖ Divided into equal sized plots
- ❖ Each plot was sprayed with sulfonylurea herbicides
- ❖ Plots were rated for herbicide damage and taken to yield

Field Layout



Plot size:
6 rows wide
210 feet long

- Control - no herbicide
- Harmony Extra + Select
- Harmony Extra + Accent
- Harmony Extra + 2X Accent
- Primisulfuron
- 2X Harmony Extra + Select
- 2X Harmony Extra + Accent
- 2X Harmony Extra + 2X Accent

Cibus SU Herbicide Tolerant Canola



Visual ratings: no difference between control and 1X levels. Slight yellowing seen at 2X rates

Brawley Yield Data

Treatment		Plot	Mean	
Number		Size (a)	Yield (kg/ha)	
1	Control - no herbicide	0.05	2518	ab
2	Harmony Extra + Select	0.05	3319	a
3	Harmony Extra + Accent	0.05	2705	ab
4	Harmony Extra + 2X Accent	0.05	2743	ab
5	Primisulfuron	0.05	2506	ab
6	2X Harmony Extra + Select	0.05	3231	a
7	2X Harmony Extra + Accent	0.05	3213	a
8	2X Harmony Extra + 2X Accent	0.05	3448	a

Conclusions

- ❖ RTDS successfully targeted and changed AHAS locus from wild type to resistant
- ❖ Resistance was stable and inherited normally
- ❖ Greenhouse data was validated in field test
- ❖ 2010 field testing done with hybrid version in North Dakota (summary not yet complete)

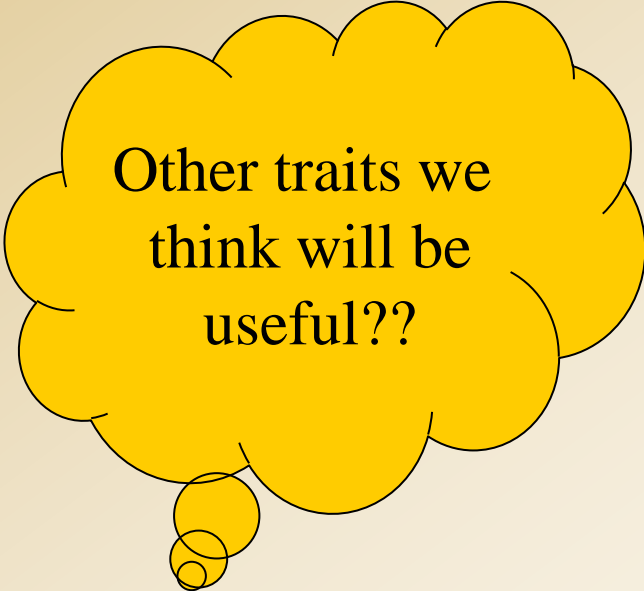
Cibus' *RTDS* Technology Summary

❖ Cibus' *RTDS*:

- Uses **natural** mismatch repair enzyme **systems** inside the plant to change DNA
- Single gene site specific mutations - **very precise**
- Conversion occurs in the endogenous gene therefore **native** endogenous **expression patterns** are conserved.
- **Stable**, heritable genomic **changes** that modify native plant genes.
- **Quick to market** since no regulatory approval is needed
- **Less cost compared to transgenics**
 - 1/10 of cost compared to transgenic methods since huge regulatory costs follow development costs
- Once “**operating system**” is developed we can develop many different types of traits
- ***RTDS*** technology works in all crops (all organisms containing DNA)

Future Trait Targets

- ❖ Herbicide Tolerance
 - Glyphosate, SU, PPO, Auxins, other??
- ❖ Disease Resistance
 - Blackleg
 - Clubroot
 - Sclerotinia
- ❖ Insect Resistance
- ❖ Oil Modification
 - Healthier Oils, like low saturated fats
 - Nutraceutical Oils
 - Industrial Oils – including biofuels
- ❖ Stress Tolerance
 - Heat and drought



Other traits we
think will be
useful??

Limited only that the DNA coding for the trait must be in the plant

RTDS

A New Non-Transgenic
Tool for Trait
Development