Cibus

Harnessing the Power of Bio-Diversity

Cibus’ “Rapid Trait Development system” (RTDS™) is an environmentally friendly smart breeding tool.
Cibus

- Development stage company with offices located in San Diego, CA, Minneapolis, MN, and the Kapelle, Netherlands.
  - 25,000 sq. ft. laboratory and office space (SD).
  - Greenhouses and field trial grounds (SD).
- Over 75 full time employees (~55 in R&D).
Cibus Introduction

• Cibus develops commercial products from its novel technology platform **RTDS**.

• **RTDS** offers targeted genetic improvements without being transgenic (GMO) – **RTDS** is considered a mutagenesis technology by the USDA.

• Cibus’ technology and proprietary traits are protected by 16 patents and 39 pending applications.

• Applications are broad:
  – agricultural such as for value-added trait development for crop protection improvements;
  – industrial and nutritional such as for valuable oils
  – includes microorganisms like yeast and bacteria
What is RTDS™?

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

Directed, unspecific

RTDS - Directed, specific DNA Repair

Directed, specific and insertion

Synthesized oligo linked to mutagen

Synthesized oligo as Triple Helix forming structure – may also be produced in bacteria

Synthesized oligo part of Gene Repair OligoNucleotide (GRON) molecule
5. **Gene Repair Oligonucleotide (GRON)***

(A) Stylized GRON showing regions of DNA and RNA (2’-O-methyl modified), a nick and hairpin (total GRON is approx. 68 nucleotides in length).

(B) Stylized ssDNA GRON showing a Cy3 dye at the 5’ end and a reverse base (idC) at the 3’ end (total GRON is approx. 40 nucleotides). The red box is the location of the targeted nucleotide in both GRONs.
Rapid Trait Development System (RTDS™) in Plants

Herbicide Treated
Wild-type
RTDS - plant
Creating a change in the letters of DNA code using RTDS™

1. A Gene Repair Oligonucleotide (GRON) is paired with the plant DNA sequence. The pairing only occurs at the designed 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 repairs the plant 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. The RTDS is complete and the targeted gene has been repaired.
Advantages of RTDS

- Modifications are site specific and result in single gene conversions.
- **RTDS** Conversion occurs in the endogenous gene, therefore, native endogenous expression patterns are conserved.
- Stable, heritable genomic changes are created that modify native genes.
- **RTDS** is functional in ALL organisms tested – these include – human and animal cell lines, bacteria, yeast and plants.
- Multiple sequence specific GRONs can be synthesized.
In summary - Cibus RTDS Technology

It is a site-specific “smart” mutation technology that can be used to alter crop plants non-transgenically.

- Single amino acid or base pair change or deletion.
- Conversion rate is one event in $10^3$ to $10^4$ in plants and as high as $10^2$ in micro-organisms.
- Uses the plant’s natural DNA repair system.
Cibus Goals – Include …

- Weed Control – the farmer’s new "crop operating system" – for example….
  - Glyphosate, Imidazolinone, Sulfonylurea, Clethodim, Protoporphyrinogen Oxidase (PPO) and Glutamate Synthase (GS).
- Disease resistance
- Environmental Stress tolerance
- Oil Pathway Modification
  - Healthier Oils, like low saturated fats
  - Nutraceutical Oils
  - Industrial Oils – including biofuels
RTDS Technology in Plants

-Oilseed Rape (OSR) example
RTDS for Herbicide Tolerant Crops

Introduction

Regeneration w/o Selection

Regeneration with Selection

Mutation Selection for Herbicide Resistance

Year 1

- Target cells (e.g., microspores, protoplasts, suspensions)
- Herbicide selection
- GRON delivery
- Regeneration

Year 2

Molecular Characterization and GRON Design (QC/QA)

GRON Introduction

Regeneration w/o Selection

Molecular/ Biochemical Assay

Phenotypic Screening

Further evaluations (GH, field, etc.)

Generalized scheme
Acetolactate Synthase (ALS) and Herbicide Tolerance

- ALS is a key enzyme in the biosynthesis of branched-chain amino acids.

- Herbicide-resistant plants have been identified which have mutations in ALS that prevent IMI (imidazolinone) or SU (sulfonylurea) herbicide binding.

- Introduce GRONs that target a codon change that results in herbicide resistance

- 3 independent amino acid changes targeted in Oilseed Rape using RTDS.
RTDS in Oilseed Rape

- Enzymatic isolation of leaf protoplasts
  - Excellent uptake of GRONs
  - Development of microcalli in alginate beads

- Herbicide Selection
  - Development of microcalli
  - Plants
  - Resistant plants
RTDS of AHAS gene

RTDS of a OSR germplasm for IMI tolerance

- material is regenerating from calli formed from protoplasts
**FAM-labeled AS-PCR (S653N)**

**positive sample**

**negative (WT) sample**
RTDS OSR– 2008 Greenhouse Results

RTDS has been used successfully in a number of crops (below) and multiple genes:

– Corn
– Tobacco
– Canola / Winter Oil Seed Rape
– Potato
– Rice
– Sorghum
– Wheat

Picture on left shows canola resistant to 12X field rate of targeted herbicide using RTDS technology
### Cibus SU Herbicide Tolerant Canola

<table>
<thead>
<tr>
<th>Rep 2 - 20d post spray</th>
<th>1x Field Rate Combination A</th>
<th>1x Field Rate Combination B</th>
<th>1x Field Rate Combination C</th>
<th>2x Field Rate Combination A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsprayed – hand weeded</td>
<td></td>
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</tbody>
</table>

**FIRST FIELD TEST in Brawley California – non-hybrid line**
2010 Field Testing - Langdon ND Controls

Unsprayed Control 1  Unsprayed Control 2
Cibus Hybrid – Commercial SU Canadian Rates

SU - No mix
Mix of SU 4:1
Mix of SU 3:1
Mix of SU 2:1
Strengths and Challenges of RTDS

STRENGTHS

• Smart, precise and targeted only to gene of interest
• No unintended effects
• Gene expression occurs at the right time (native genes and promotors)
• Non-transgenic
• Lower cost
• Breeding with the trait is facilitated using markers.
• Genetically stable – the same as natural mutations

CHALLENGES

• Today, some genes are potentially more difficult to target
• Non-selectable traits continue to be a challenge
• Limited to what we know about the genome
• Cell culture in certain plant species can be recalcitrant.
Thank you
more information about RTDS
and the Cibus Team are presented
at -- www.cibus.com