Development of Cibus' Trait Machine™ to Efficiently Apply Non-Transgenic Gene Editing

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Abstract

An increasing number of traits are being discovered and developed using gene editing. Simple traits conditioned by a small number of genes can be managed through traditional backcross breeding, but more complex traits are being discovered that cannot easily be backcrossed into new, elite breeding lines. The concept of a Trait Machine™, which is defined as the optimization of several procedures used in sequence to efficiently develop complex traits in multiple elite lines, is essential to optimize the use of the editing technologies to accelerate plant breeding. Components of a Trait Machine might include robust tissue culture protocols, efficient gene editing and molecular sampling, robotics, robust plant regeneration, growth and phenotyping. Cibus has developed the Trait Machine for canola and is applying it to develop complex and combined traits into diverse elite canola lines.

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The Problem: Population Growth and Climate Change Require Innovation in Agriculture

- **80+ million** new mouths to feed every year
- Estimated that food production **must double by 2050**
- The availability of **arable land** per capita has been reduced by nearly 50% since 1960

Source: Population Reference Bureau, FAO, United Nations Environment Programme (UNEP)

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Tools To Improve Innovation in Agriculture

The Rapid Trait Development System™ (***RTDS***™) is a family of technologies for gene-editing that precisely and efficiently produces non-transgenic plant traits

SINGLE PLANT	ISOLATE A SINGLE PLANT CELL	CHOOSE DESIRED TRAITS / STACK MULTIPLE TRAITS	REGENERATE PLANT WITH DESIRED TRAITS FROM SINGLE CELL
Flexibility to develop traits in elite genetics	*Significant cell culture know-how*	*Patented technology allows for very precise edits*	*Less than 5 years and less than $10 million for entire trait development process*

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3

How to Edit? (GRON-Mediated Process)

Cibus *Rapid Trait Development System*

CRISPR/TALEN

1	2	3	4	5	6
Use directed nucleases (such as CRISPR / TALEN) to make a targeted cut in DNA sequence	GRON is paired with the plant DNA sequence. The pairing only occurs at the gene target region	The GRON induces or makes a mismatch with the plant DNA sequence	The plant's native DNA repair enzymes recognize the mismatch and repair the plant's DNA using the GRON as a template	Gene-editing is complete and the targeted gene has been repaired	Following the repair, the GRON is degraded by the cell's natural processes

- ***RTDS*** can make directed spelling changes beyond a simple knockout, which allows us to develop traits such as herbicide tolerance and to stack them
- Herbicide tolerance traits cannot be produced non-transgenically by only using CRISPR/TALEN

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Plant Cell Culture is a Key Capability

- The gene edited single cells are cultured and nurtured back into whole plants step by step through a highly controlled, complex, and proprietary process, built over the last 17 years
- Our technology enables us to design and transfer multiple plants with desirable traits from the lab to the greenhouse within months

 CIBUS 4

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Proprietary *RTDS* Family of Technologies and Know-How

What to Edit	How to Edit	Cell Culture Expertise		Robust IP Portfolio	
Genomics Expertise	GRON	Cell Culture	Automation	Proprietary Know-How	Patent Portfolio
• Deep fundamental understanding of crop genetics	• Make desired genetic edits in single cells using our proprietary GRON	• Regenerate single cells into entire plant with desired trait in months	• Use advanced robotics to accelerate trait development	• *RTDS* processes protected as trade secret, including how to nurture single cells into whole plants	• 300+ patents and applications for gene editing methods, GRONs, herbicide tolerance traits, and other traits

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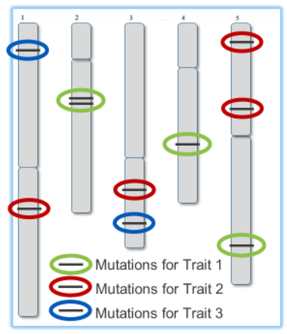
The Challenge: Developing Multiple Complex Traits

Precision mutagenesis introduces the exact change(s) of interest without unwanted background mutations

- Any nucleotide change at essentially any genomic location can be made
- One or a few nucleotides changes can be made
- Multiple (even tightly linked) loci can be edited simultaneously
- With **NO** linkage drag

- **Multiple traits add complexity which require the development of tools to improve efficiency of trait conversion**
- **SOLUTION: A Trait Machine™**



Mutagenesis illustration in *Arabidopsis sp.*

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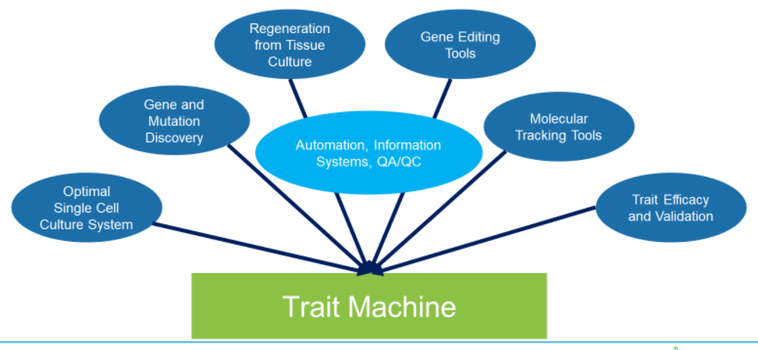
7

Capabilities Enabling Cibus' Trait Machine™



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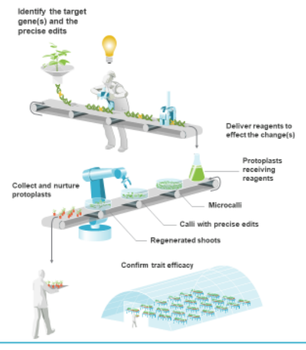
Technology to Support a Trait Machine™



Cibus' Trait Machine enables customized plant products with tailored traits meeting our customers' needs and reduces the time to commercialization.

Technology Advancements Supporting Development of a Trait Machine in Canola

- Ability to culture material with diverse genetic backgrounds (i.e., SOSR and WOSR)
- Increase in gene editing frequency by orders of magnitude
- Plant regeneration improved over 1200% in most lines
- Time from seed to seed of edited plants reduced to 9-12 months
- Proven ability to edit multiple loci simultaneously

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Increasing Efficiency is Key to Developing an Efficient Trait Machine

- Custom robotics added at several stages of the trait development process to meet the needs of cell and molecular biologists
- Increase in efficiency, accuracy and speed compared to manual work
- Improves ability to make multiple changes simultaneously
- Scalable platform for multiplexed gene editing: multiple edits, multiple loci

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Cibus Trait Machine™ Features

1. Truly non-transgenic; able to edit plant genes without integrating foreign genetic material
2. Uses elite genetic parental lines as the starting material for the gene-editing process
3. Moves from single cell to regenerated whole plant possessing desired traits more efficiently than comparable technologies
4. Standardized, precise, reproducible and automated, making trait development customizable enabling rapid trait stacking
5. Highly scalable using newly acquired robotics, to largely automate the process

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Platform for Accelerated Product Development

- The Trait Machine is being optimized in various crops including canola, rice, and flax.
- The principles apply to all plants.
- Accessible traits include:
  - Tolerance to environmental stress factors, including heat and drought
  - Crop yield improvement
  - Quality characteristics including oils and starch
  - Herbicide tolerance – multiple modes of action
  - Enhancing the durable natural defense systems of plants to fungal and bacterial diseases

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Proven Ability to Stack Non-Transgenic Traits and Develop Customized Seed Products that Benefit…

Growers				Processors	Consumers
Parental or Elite Genetics	Herbicide Tolerances	Other Biotic Stress Tolerances	Abiotic Stress Tolerances	Other Agronomic Traits	Consumer Traits
Choose Starting Germplasm / Variety ☐ Elite Germplasm 1 ☐ Elite Germplasm 2 ☐ Elite Germplasm 3	Select all that apply ☐ None ☐ Herbicide Tolerance 1 ☐ Herbicide Tolerance 2 ☐ Herbicide Tolerance 3	Select all that apply ☐ Pest Control 1 ☐ Resistance to Disease 1 ☐ Resistance to Disease 2	Select all that apply ☐ Drought Resistance ☐ Heat Tolerance	Select all that apply ☐ Pod Shatter Reduction ☐ Maturity - Point to Harvest Time (early, mid, or late)	Select all that apply ☐ Healthier Oils ☐ Allergen Free ☐ Healthier Starches

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Inspired by Nature™

CIBUS™