yOIL

An All-Encompassing Solution to the Green Seed Problem

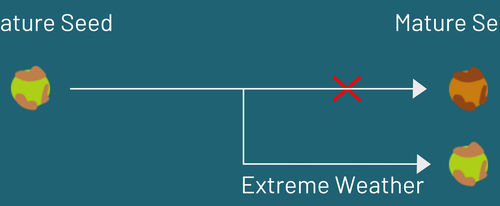
iGEM 2019 Calgary

UNIVERSITY OF CALGARY

Christian Emond, Sarah Walker, Cassandra Sillner, Andrew Symes, Juan Sebastian Alvarez, Michaella Atienza, Sara Far, Sravya Kakumanu, Nimaya De Silva, Jean-David Rousseau, Jake Liu, Michael Wahba, Rylan Marianchuk, Prabhkeerat Dhatt, Jacob Grainger, Marija Drikic, Elke Lohmeier-Vogel, Mayi Arcellana-Panlilio

"Green is the difference between profit and loss"
- Craig Shand, Farmer

Exposure to extreme weather conditions causes canola seeds to stay green at maturity.



The oil pressed from green canola seeds contails chlorophyll, which lowers the quality of the oil

- Faster Spoilage
- Bitter Taste
- Lower Smoke Point

"The green seed problem is the largest problem in the canola industry"
- Dr. Marcus Samuel, Professor

FARMER

SUNNY DAYS

Weather prediction is an important tool for farmers to inform their planting decisions.

Currently, weather models are often inaccurate and short-term.

Using neural networks and principle component analysis, our team created Sunny Days, a novel weather prediction tool.

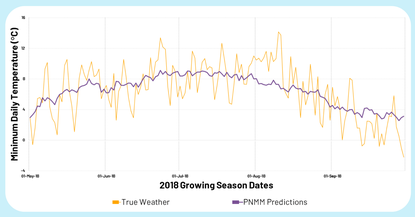


Figure 1. Comparison of Sunny Days weather predictions to real weather trends

Sunny days has been shown to be able to predict the weather within 2.5℃ up to 6 months in advance.

Currently, we are in the process of expanding the region of impact of Sunny Days to help more farmers.

(ACIS, 2019)

"The success of a farmer's growing season is largely defined by green seed content"
- Ward Toma, Alberta Canola Producer's Commssion

GRADER

MEAN GREEN MACHINE

The percentage of green seeds in a seed batch determines its grade. As the proportion of green seed increases, its selling price decreases.

In the industry, seed grading is done manually, resulting in inaccurate grading.

We created Mean Green Machine, a seed grading machine alongside a software called the GreatGrader that uses computer vision to grade seeds.

After testing with the Canadian Grain Commission, Mean Green Machine and GreatGrader were shown to have an overall error rate of 0.843%.

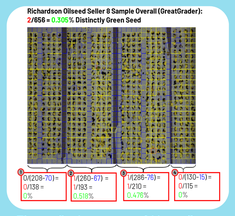


Figure 2. Accuracy of Mean Green Machine and GreatGrader in grading seeds

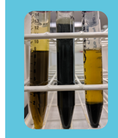
Currently, we are in the process of improving our design with the Canadian Grain Commission for use in the industry.

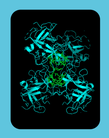
OIL PRODUCER

CHLOROPHYLL EXTRACTION

"Clay is the most expensive input cost"
- Dallas Gade, Richardson Oilseed

Acid Activated Clays	6GIX Protein
Current Industry Standard	**Our Solution**
20% oil loss	Specific binding
Non-specific binding	Environmentally-friendly
Environmental concerns	Water-soluble
	(Bednarczyk et al., 2015)





Construct Design

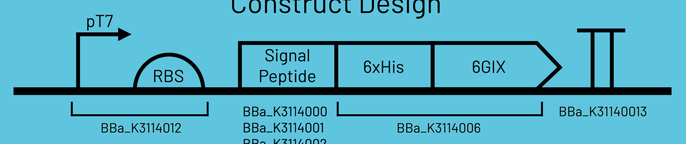


Figure 3. Genetic construct design for inducible 6GIX protein production. Constructs were generated using one of six signal peptides for secretion, or without a signal peptide.

Emulsion System

We emulsified our 6GIX protein to keep it stable and to maximize the oil-water interface where it is active.

Using dilution line experiments and Support Vector Classification (SVC), we created phase diagram models to inform our emulsion design.

Our emulsion system was designed to minimize oil loss and maximize chlorophyll removal.

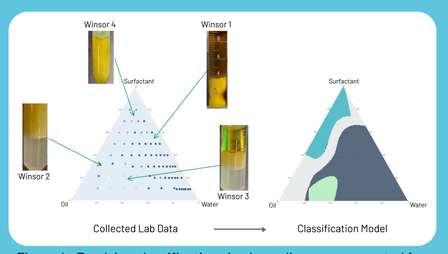


Figure 4. Emulsion classifications in phase diagrams generated from experimental data.

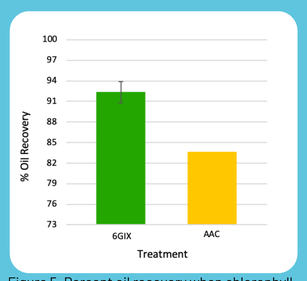


Figure 5. Percent oil recovery when chlorophyll recovery is 80% for AAC and 6GIX emulsion treatments.

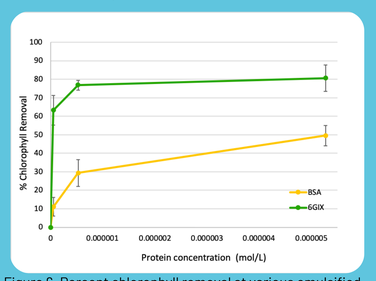


Figure 6. Percent chlorophyll removal at various emulsified protein concentrations for 6GIX and BSA control at room temperature.

There are diminishing returns in 6GIX efficiency at higher concentrations due to its instability at the oil-water interface.

Using iGAM, we created ModGIX, an improved 6GIX protein with 12 amino acid changes. Protein dynamics modelling of ModGIX showed improved stability in emulsion.

iGAM

The international Genetic Algorithm Machine is a Genetic Algorithm that we generated to allow for informed protein modification.

"Anybody growing canola in Alberta will have to deal with [fungus]"
- John Mayko, Farmer

MARKET

CHLOROPHYLL REPURPOSING

Repurposing the extracted chlorophyll waste into **pheophorbide a** can help cover industrial investment costs by introducing a new revenue stream.

Process

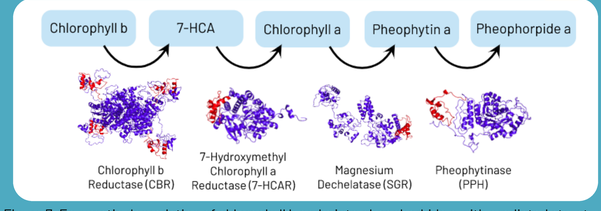


Figure 7. Enzymatic degradation of chlorophyll b and a into pheophorbide a with predicted structural modelling for enzymes in the pathway using Rosetta comparative and *ab initio* methods. ICARUS is in red.

Construct Design

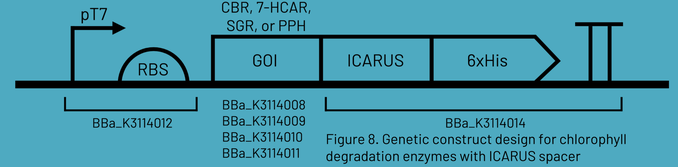


Figure 8. Genetic construct design for chlorophyll degradation enzymes with ICARUS spacer

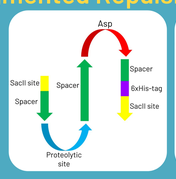
BOTS

After struggling with repetitive GC-rich sequences in construct design, we created BOTS, a tool for codon optimization and simplified gene synthesis.

ICARUS

iGEM Calgary's Augmented Repulsion Universal Spacer

Designed to allow purification of large electronegative proteins



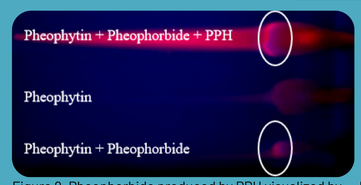


Figure 9. Pheophorbide produced by PPH visualized by thin layer chromatography under UV light.

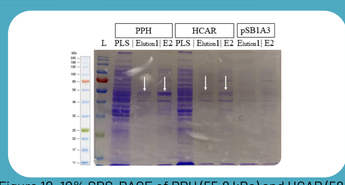


Figure 10. 10% SDS-PAGE of PPH (55.8 kDa) and HCAR (58 kDa) post-loading fractions (PLS) and elution fractions .

Pheophorbide a as an Antifungal

Sclerotinia sclerotiorum – one of the most prevalent fungal pathogens among canola crops, resulting in major losses to farmers (Bolton et al., 2006)

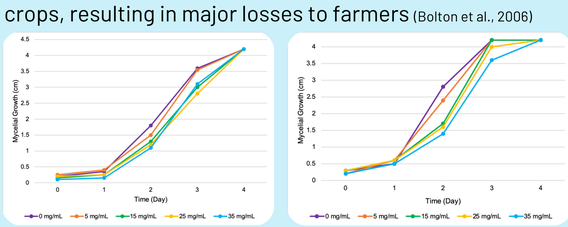


Figure 11. Mycelial growth of *S. sclerotiorum* with pheophorbide a in dark (left) and in light (right) conditions.

ACHIEVEMENTS

- Analysed the market by meeting with over 30 stakeholders from every part of the canola industry
- Submitted 28 BioBricks to the iGEM registry as part of a novel chlorophyll capture and degradation collection
- Successfully produced, purified, and emulsified 6GIX protein using our generated phase diagrams, allowing us to remove chlorophyll from green oil with a higher oil recovery than acid activated clay at the same processing conditions
- Successfully utilized iGAM to create ModGIX, an improved version of 6GIX with increased stability
- 6xHis-tag purified 7-HCAR and PPH using ICARUS, a spacer designed to overcome problematic electrostatic interactions between the protein and His-tag (Meguro et al., 2011)
- Created BOTS, a codon and synthesis optimization tool for parts design
- Successfully converted pheophytin into pheophorbide a using PPH
- Characterized pheophorbide a's ability to inhibit the growth of canola pathogen *S. sclerotiorum*
- Generated weather predictions 6 months in advance with a Mean Absolute Error within 2.5 degrees Celsius by utilizing the Principal Component Neural Network Mean Model
- Achieved low error in grade in Canadian Grain Commission-conducted tests of Mean Green Machine with a grading time of less than 5 minutes
- Developed, pilot tested, and distributed a university level synthetic biology course to 5 other iGEM teams

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ATTRIBUTIONS

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