Soybean: Way more than just nice yellow seeds

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NAPB Webinar Series 2015: The Science of Selections

Plant Agriculture

Education and Work Experience

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- B.Sc. (Agr). - University of Novi Sad, Serbia - 1988
  - Thesis topic: Sugar beet physiology

- Ph.D. - University of Guelph - 1996
  - Dissertation topic: Genetics of modified fatty acid composition in canola (*Brassica napus* L.) seed

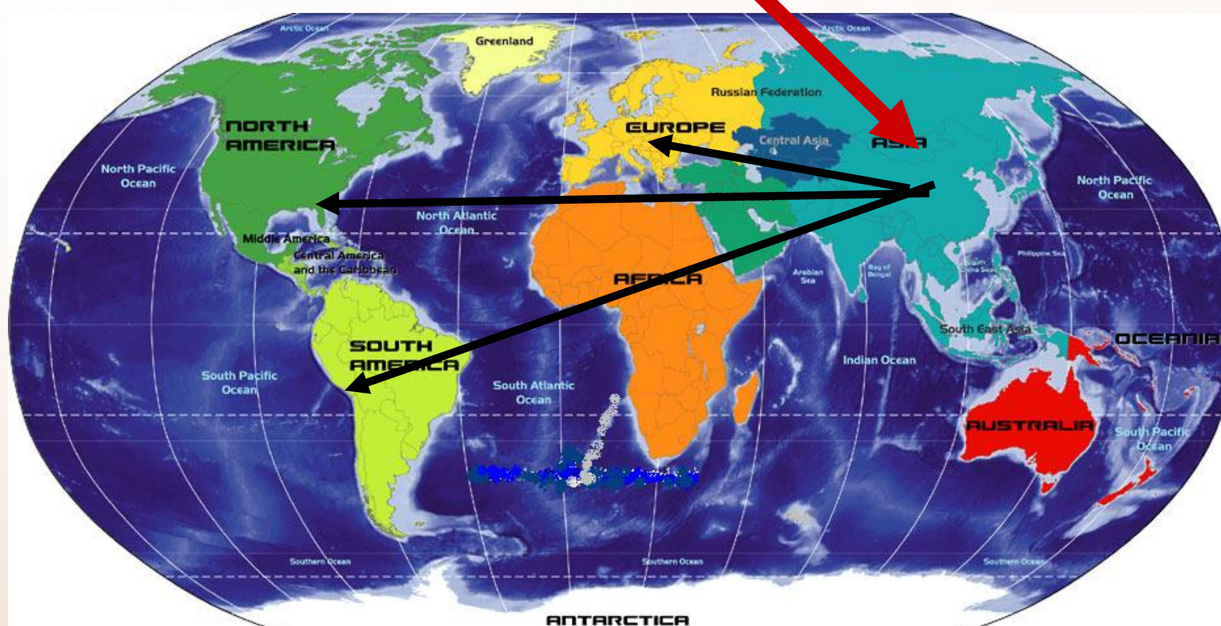
- Sunflower breeder, Institute of Field and Vegetable Crops, Novi Sad, Serbia: 1989-1991
- Specialty Oil Canola Breeder – Viterra, Saskatoon, Saskatchewan, Canada – 1996-1998
- Soybean Breeder at University of Guelph: 1998 to date

University of Guelph

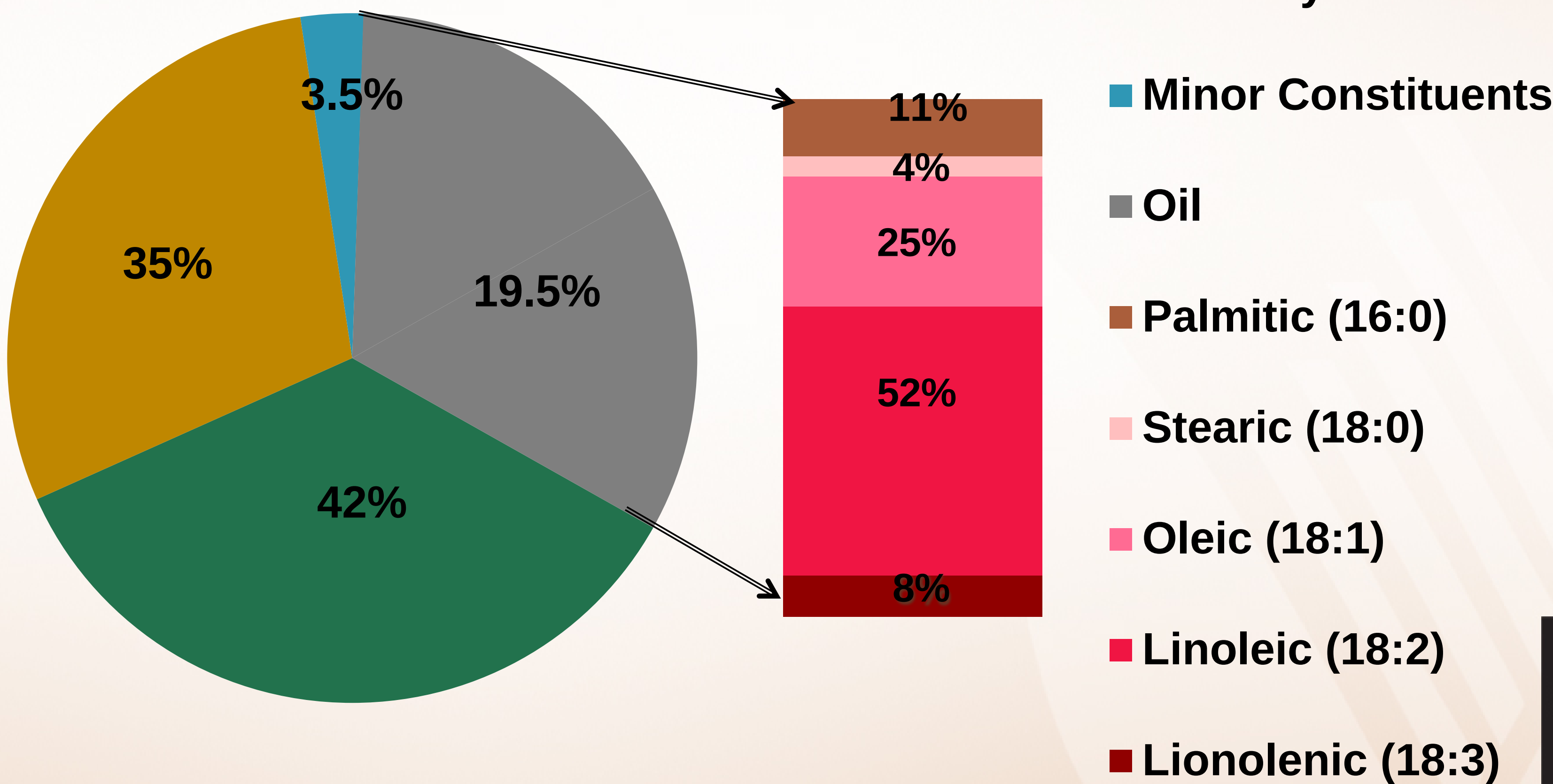
- 1874 - Ontario Agricultural College (OAC) established as the oldest agricultural school in Canada
- 1964 – OAC became a founding college of the newly created Univ. of Guelph
- > 27,000 students including 2560 graduate students

Soybean [Glycine max (L.) Merr]

- **2n = 40**
- Family *Fabaceae* or *Leguminosae*
- Domesticated from wild relative ***G. soja***, 6000-9000 yrs ago
- World's largest protein and oilseed crop; ***20% oil, 40% protein***
- Introduced to North America in 1765 (Savannah, Georgia)



Soybean Seed Constituents



(Rajcan et al., 2005)

Soybean

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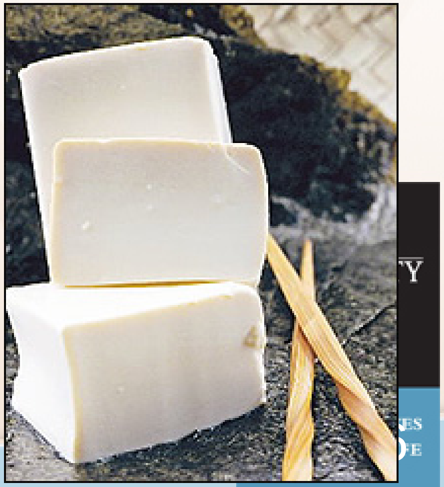
- Many products (>300)
- Seed rich in nutraceutical compounds with health effects
- High quality oil - can be altered genetically
- Protein contains all essential amino acids



Soybean uses

- Used in the production of food for human consumption

Tempeh

Soymilk

Soynuts

Miso

Tofu

Soynut butter

GUELPH'S CONTRIBUTION TO CANADIAN SOYBEAN

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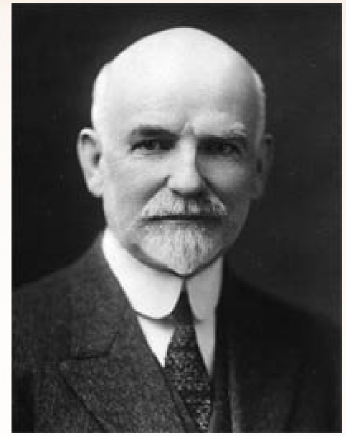
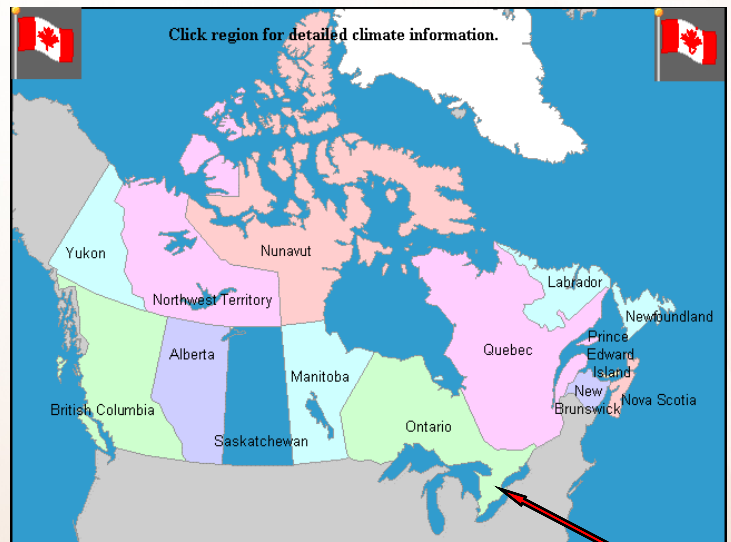
- In 1893, Professor ***Charles Zavitz*** introduced soybean to agriculture in Canada
- Prof. Zavitz developed first Canadian soybean variety **"OAC 211" in the 1920's as forage soybean**
- 2014 Canadian production area exceeded 2 million ha (over 4 million ac)
- ~70 % grown in Ontario





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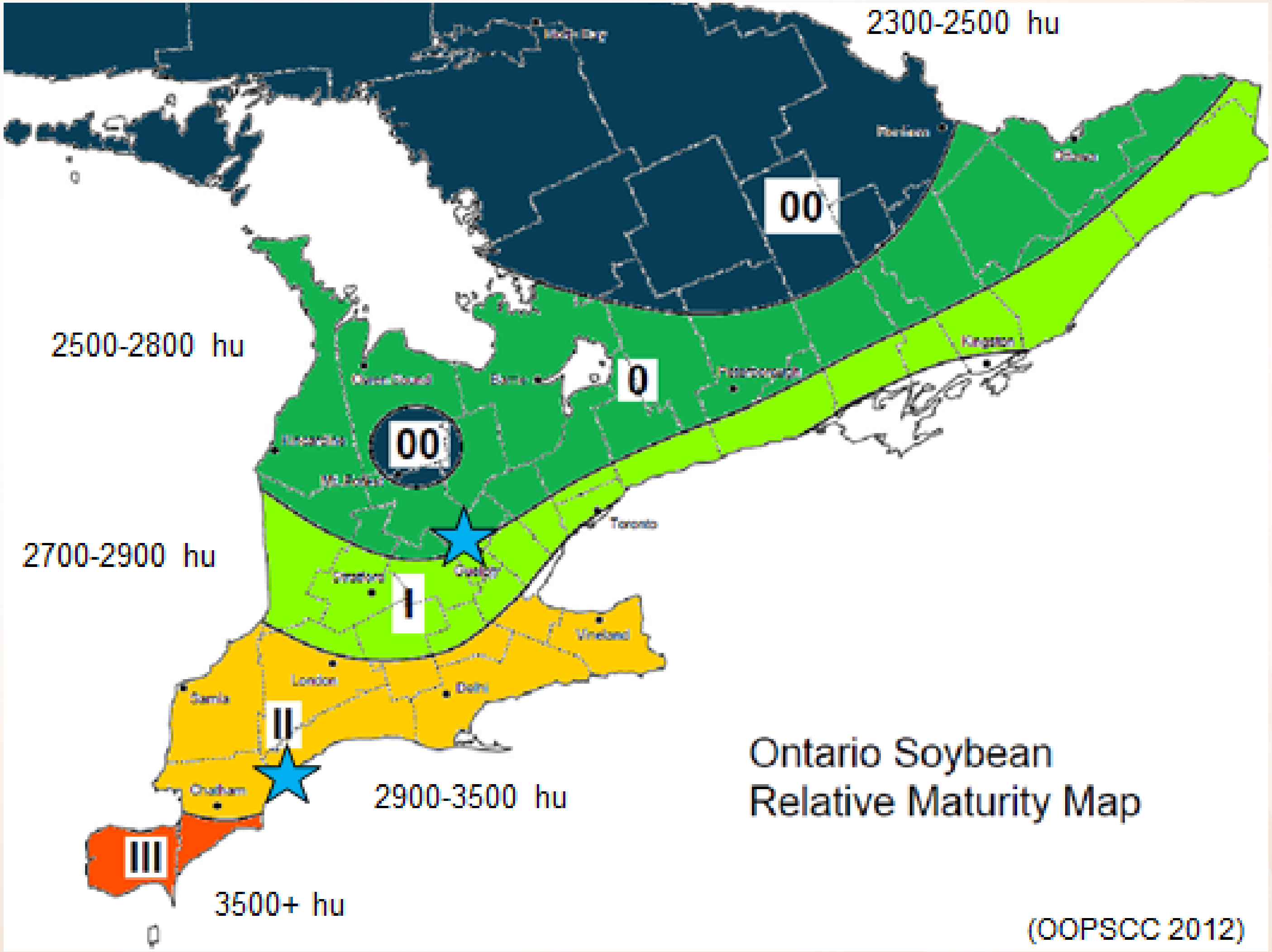
Soybeans in Ontario



Soybean Breeding @ Guelph

- Since 1970's over ***115 soybean*** varieties released by the University of Guelph
- Some have been around more than 20 years - ***OAC Bayfield***
- Cultivars released every year
- **OAC Wallace, OAC Kent**
- ***OAC Drayton, OAC Avatar, OAC Prescott, OAC Strive, OAC Eve***

Artificial Crossing in Soybean

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Prepared by Lin Liao

➢The structure (morphology) of the soybean flower

1 pistil (stigma)
10 stamens
1 calyx with 5 sepals
1 corolla with 5 petals

$\left\{ \begin{array}{l} \textbf{1 Standard} \\ \textbf{2 Keels} \\ \textbf{2 Wings} \end{array} \right.$

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Artificial Crossing in Soybean

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- Preparation of the female flower
  - **Choose a flower bud**

The bud of the female plant is swollen and the corolla can be seen through the calyx

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Artificial Crossing in Soybean

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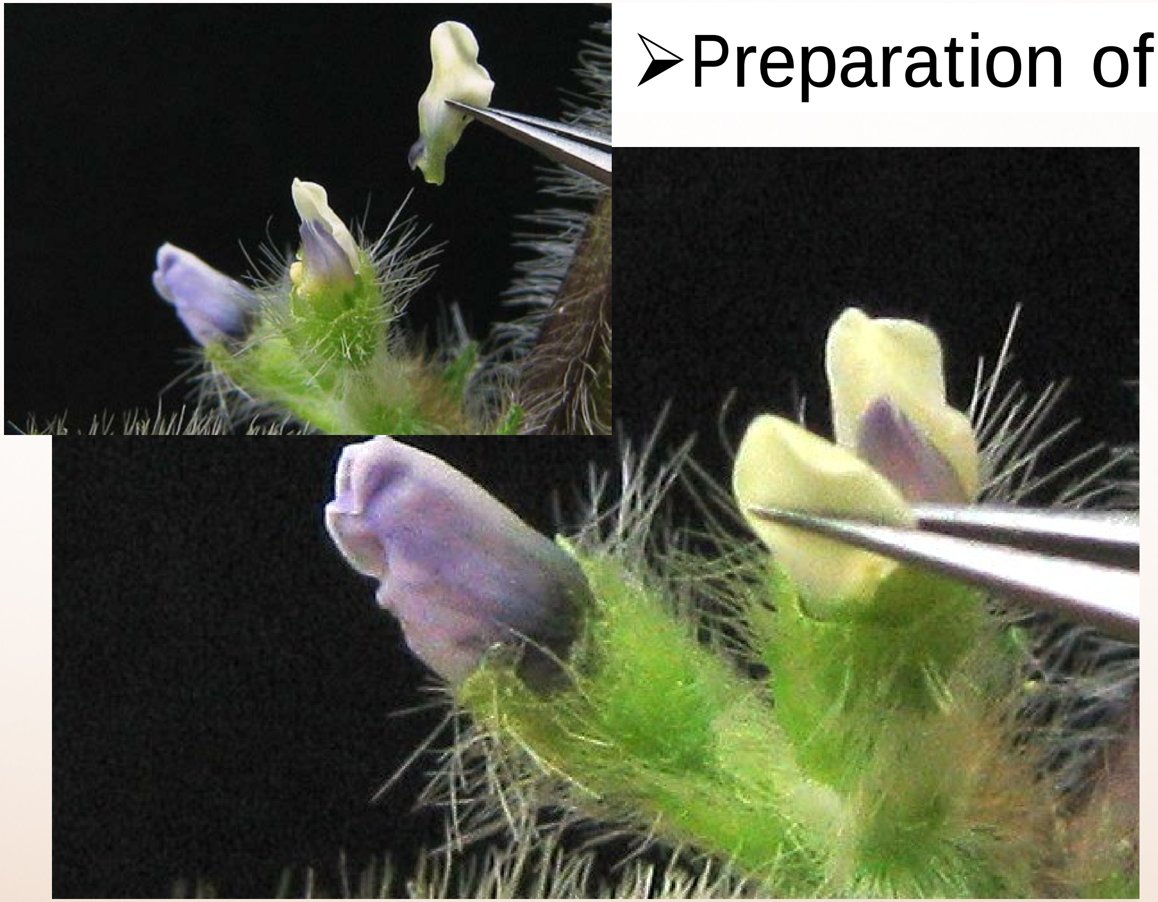

➢Preparation of the female flower

- **Remove the calyx and corolla**

  The two wing petals are removed next with tweezers.

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Artificial Crossing in Soybean

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➢ Preparation of female flower

- **Remove the anthers**
  = emasculation

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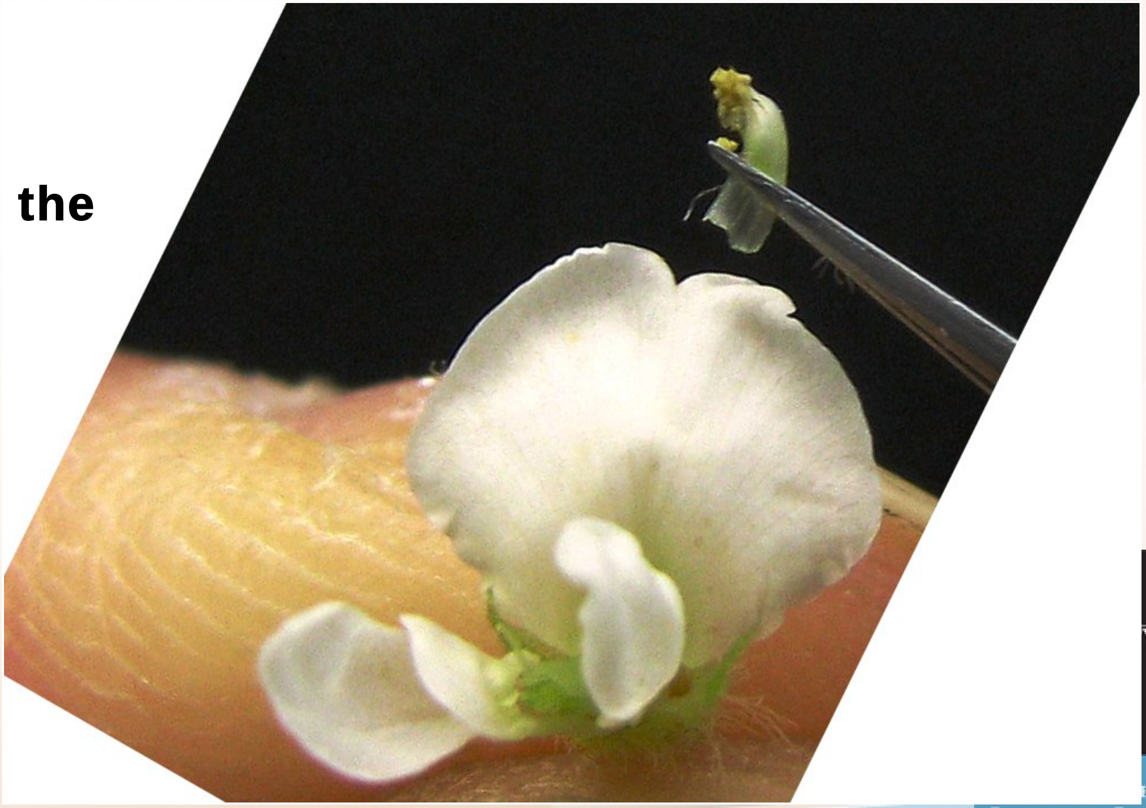
Artificial Crossing in Soybean

- Male flowers selection

- **Take the anthers**

To get the anthers, the stamens and the pistil have to be removed together.



Artificial Crossing in Soybean

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- Pollination

Gently brush the anthers against the stigma of the female flower.

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Artificial Crossing in Soybean

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The crossing is finished...

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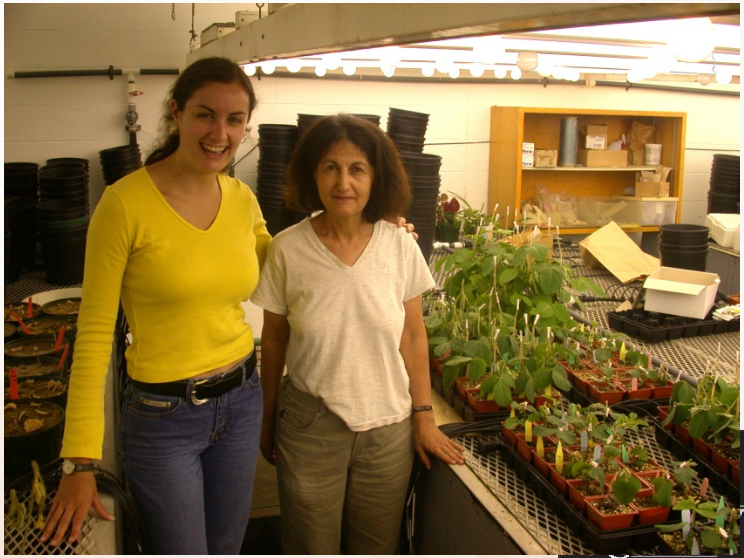
Soybean Breeding @

- ***<u>Typical Bi-parental cross:</u>***
- *Parent A x B = F1, single seed descent to F4 or F5, selection, release variety at F9 or F10*
- *Single seed descent (SSD)*

Soybean Breeding @

- Make about 200 crosses per year
- All crosses made in the growth rooms (GR)
- F_1 seed harvested in April
- Planted in GR, seedlings transplanted in Ridgetown
- F_2 seeds produced in October
- Sent to Costa Rica for 2 generation advance by SSD



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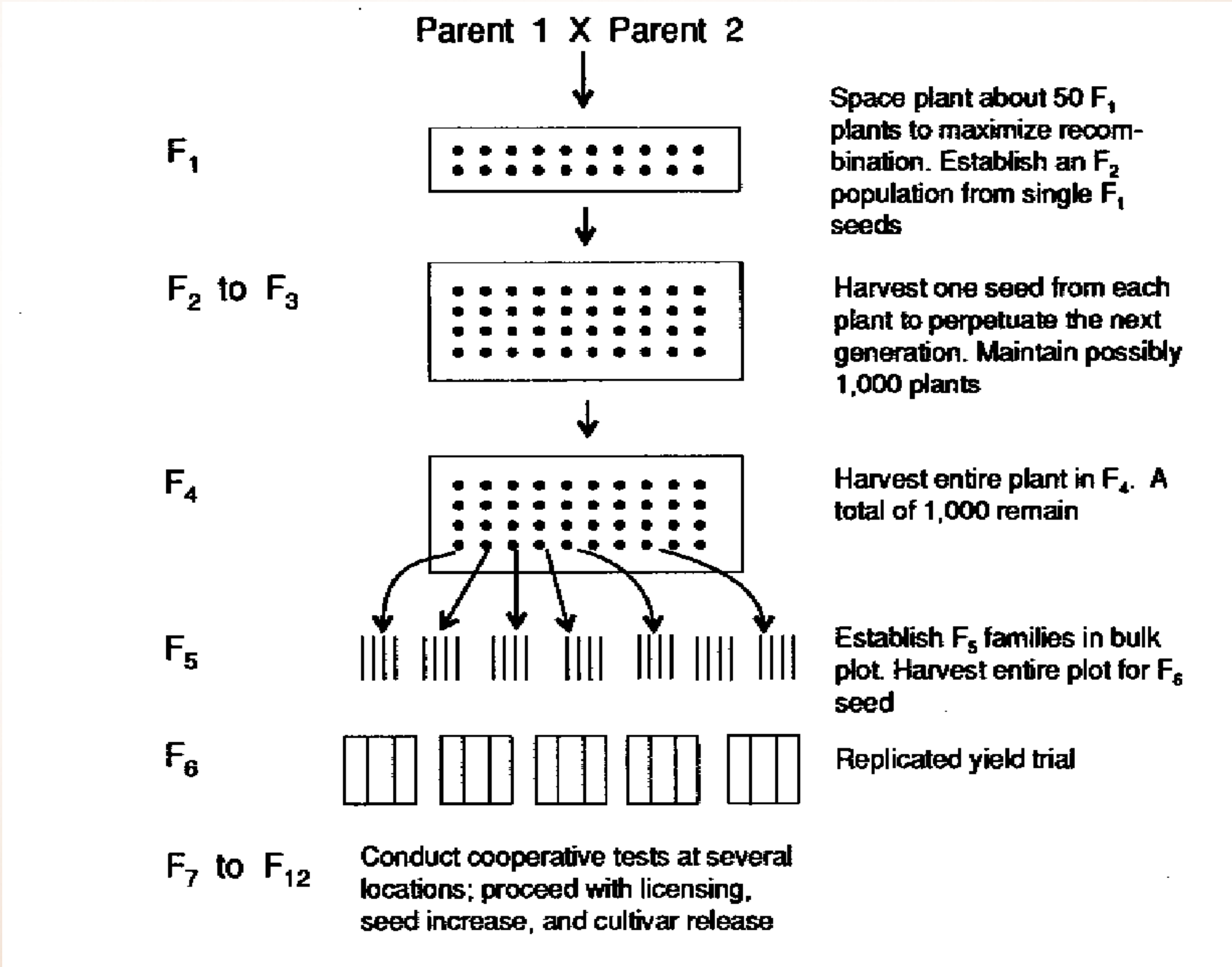
Soybean Breeding @



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‘The Soybean Breeding Path’

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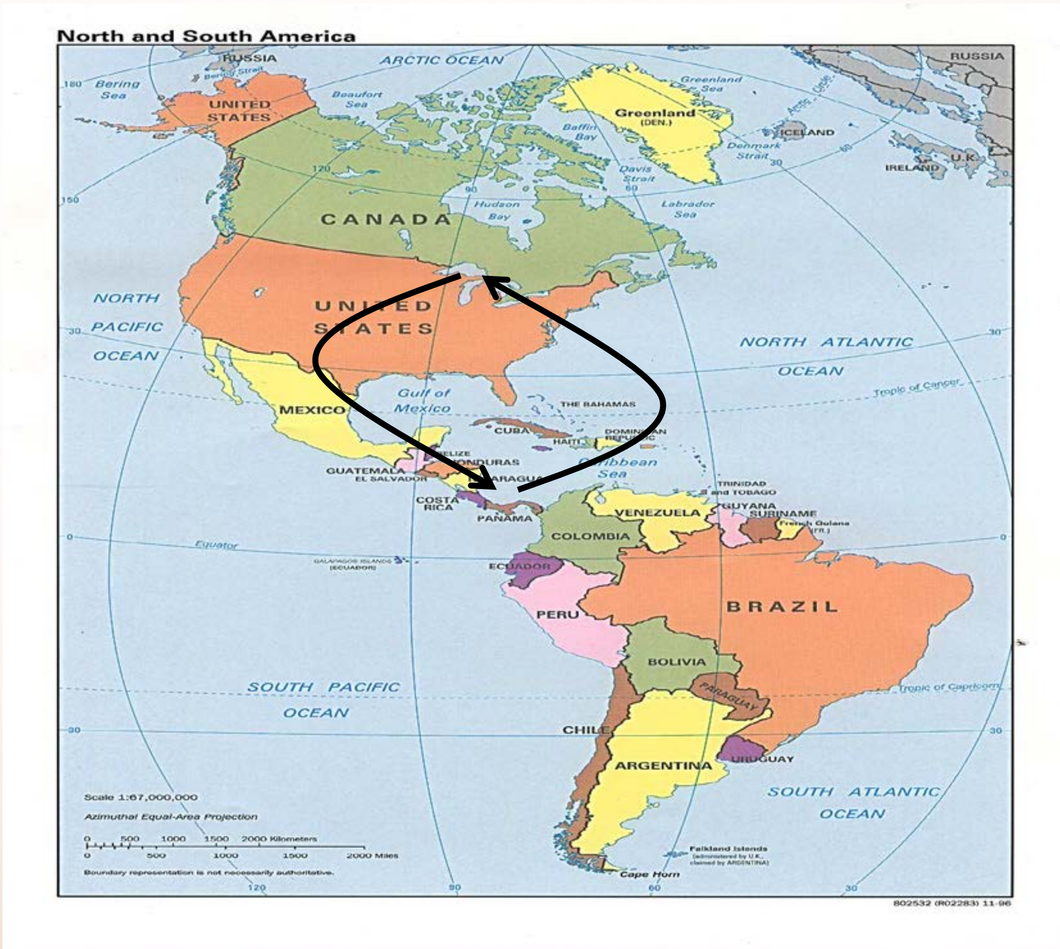

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Soybean Breeding @

- F_4 seed comes back from ***Costa Rica*** the following spring

- F_4 seed planted in Woodstock, ON, for single plant selection

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Numbers game….

- F4 seed is planted in the F4 - "Costa Rican" nursery in Woodstock, ON
- There could be **up to 40,000 F4 plants**
- Between **4000 to 6000 single F4 plants** based visual agronomic appearance
- The single plant selections the following year are planted as F5 'headrows', single row plots, 4 m long
- Selection based on maturity and plot appearance

Numbers game…

- 500-700 plots selected from the headrows to go into the F4:6 preliminary yield trials (PYT) at one location based on maturity

- The PYT with F4:6 lines are planted as unreplicated CRD with repeated 5 to 6 checks covering a range of maturity

- 150 to 200 F4:7 grown in the advanced yield trials in two locations with 3 reps each

Woodstock Research Station Plots (Google Earth)

“The home stretch”

- 30 to 50 lines selected for testing in multi-location yield trials over 2 years
- Breeder seed production initiated after the first or second year of Ontario yield trials
- ***3-5 cultivars released to the seed industry every year***

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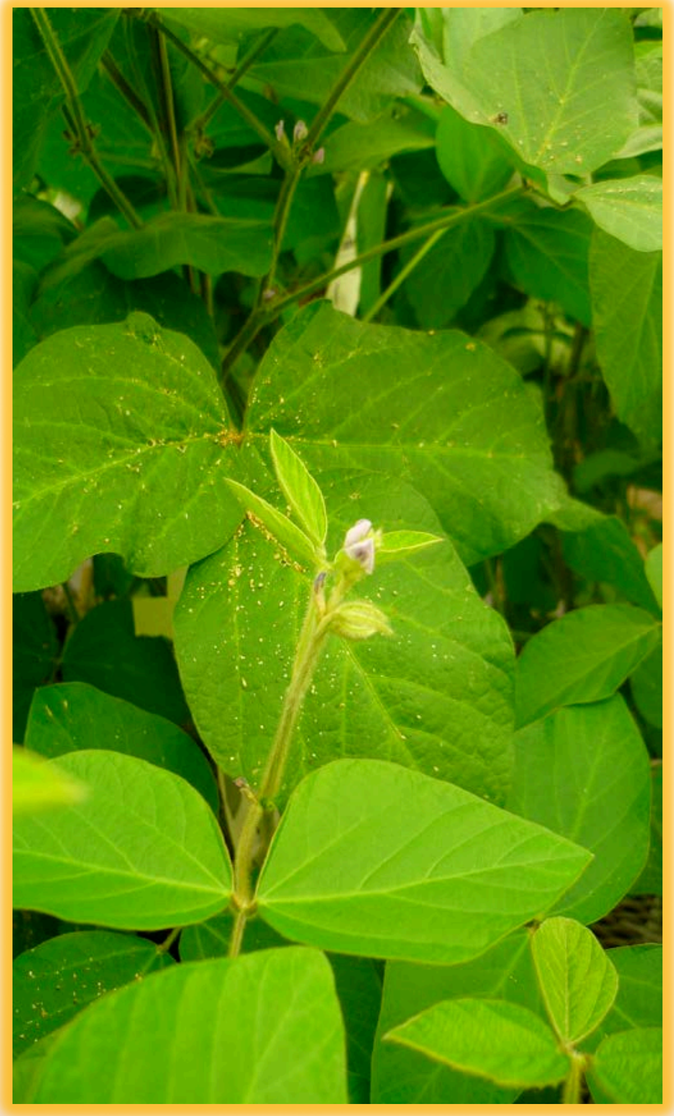

Soybean Seed Traits – Breeder's Opportunity:

- Fatty Acids
- Isoflavones
- Tocopherols (Vitamin E)
- Saponins

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Soybean Opportunities, i.e, *Value Added Traits*

- Adding to profit margin
- Opportunity to differentiate in the market place
- Paradigm shift from commodity to '***niche markets'*** and specialty value-added products

Many seed traits to work on – examples planted on Peter Hannam's farm near Guelph

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Example: Soybean Isoflavones

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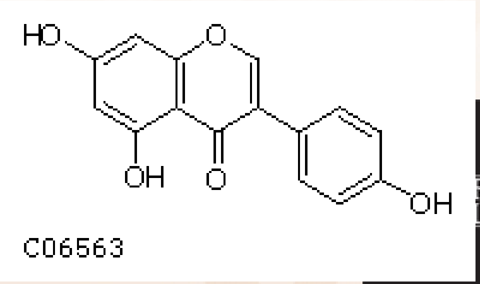
- Mostly recognized for their **health benefits** – prevention and/or suppression of cancer, osteoporosis, heart disease, menopause symptoms

- Produced via the ***Phenylpropanoid** Pathway*

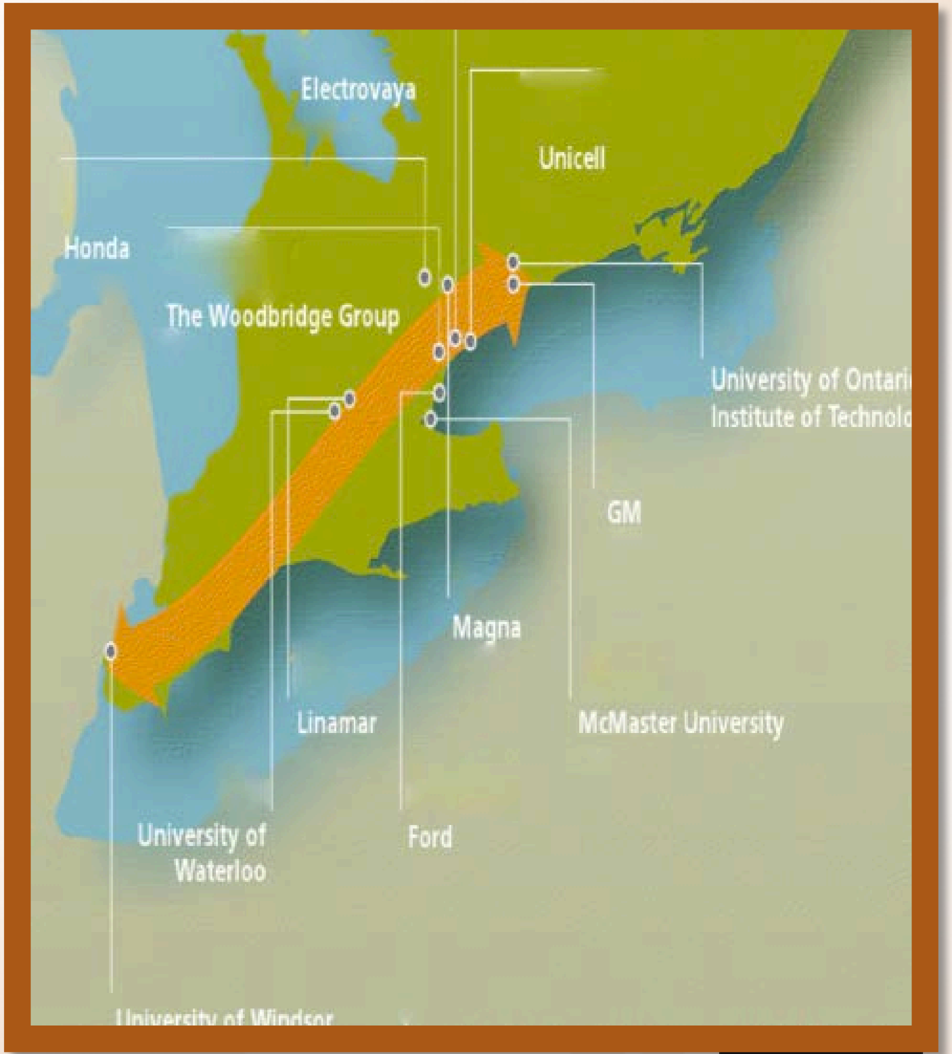
- Three main isoflavones: Daidzein, Genistein, and Glycitein



Genistein

Using Soybean Oil in Automotive Industry

- **Ontario produced close to 2.34 million vehicles in 2012**
- **Soy-based polyurethane foam found in seat cushions, head restraints and arm rests of vehicles manufactured by Toyota, Ford, Honda and GM**
- **High linoleic (18:2) oil preferred**



(BDDCl., 2012; Bioplastic Innovation, 2012, OMAFRA, 2010)

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FIELD PERFORMANCE OF HIGH LINOLEIC SOYBEAN

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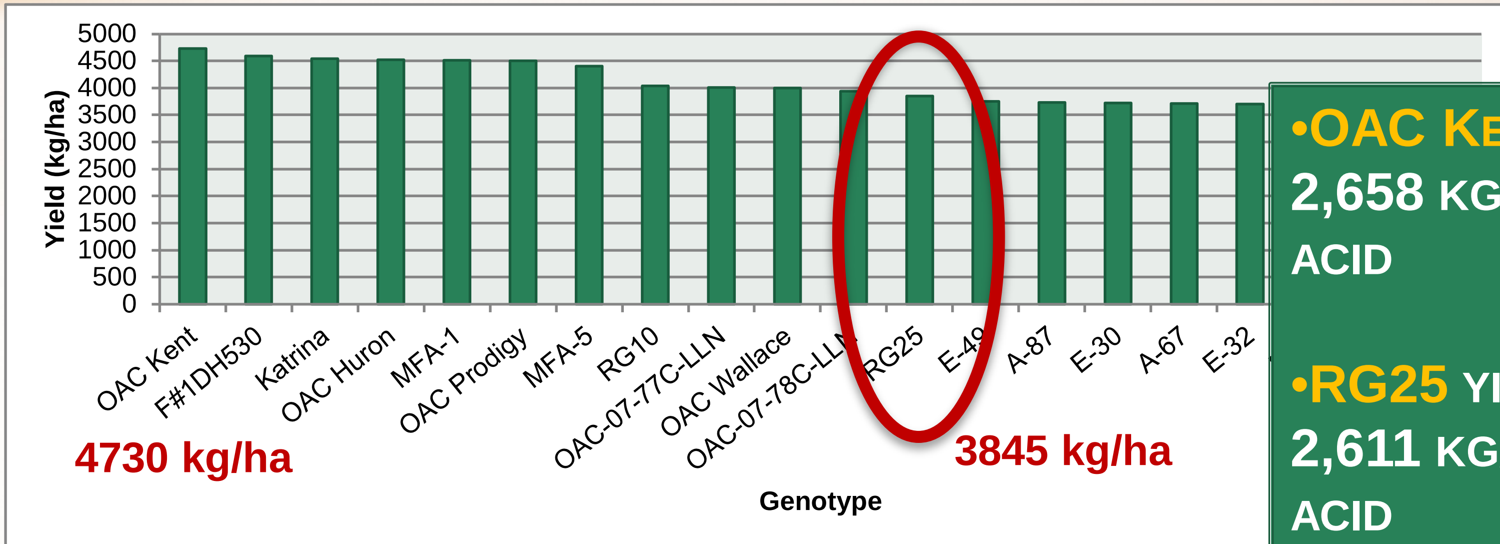

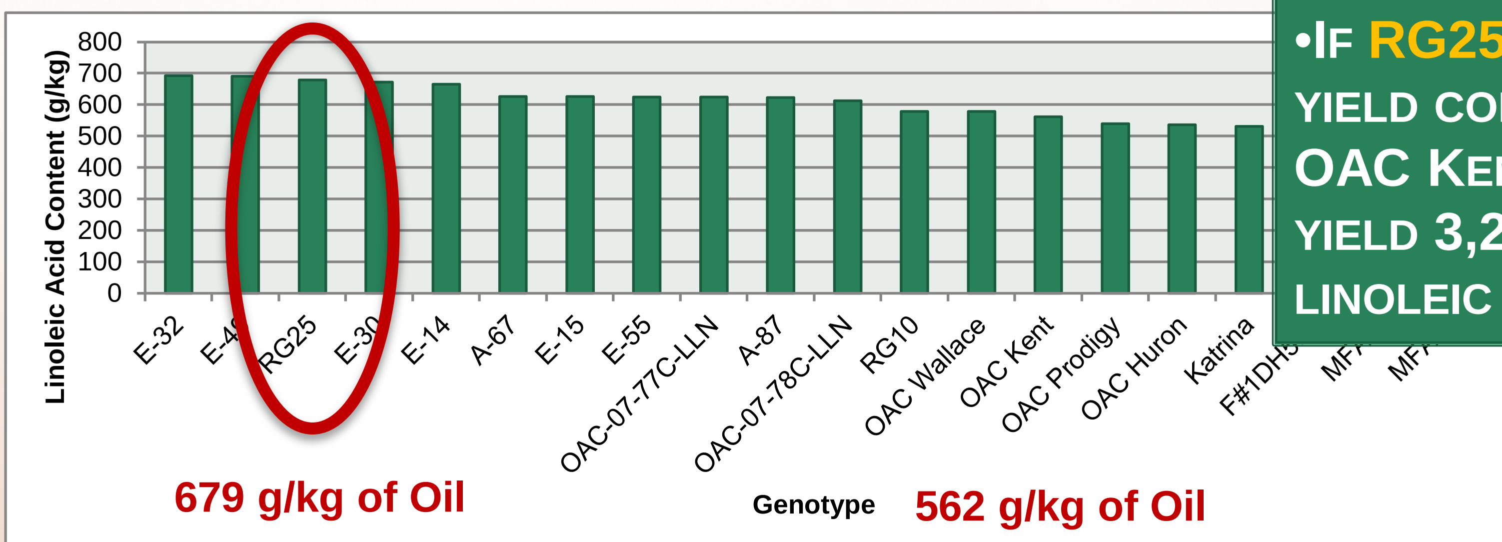

- **OAC KENT** YIELDS 2,658 KG/HA LINOLEIC ACID
- **RG25** YIELDS 2,611 KG/ HA LINOLEIC ACID
- IF **RG25** HAD THE SEED YIELD COMPARABLE TO OAC KENT IT WOULD YIELD 3,212 KG/HA LINOLEIC ACID

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HIGH LINOLEIC (18:2) SOYBEAN

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- **RG25** was the most promising soybean line tested for polyurethane production in this study
- Environment significantly influenced total seed yield and seed linoleic acid content
- A strong negative correlation exists between linoleic acid content and seed yield in this set of material
  - Independent mutations generated from EMS
  - Linkage of mutant fatty acid alleles with unfavorable yield alleles
  - Pleiotropic effects of mutant fatty acid alleles
- **Goal:** To incorporate mutant fatty acid alleles into a high yielding soybean background

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Current Research & Implications for Soybean Industry

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- Produced about 200 kg of seed of a new soybean line ***OAC 13-55C-HL*** with 69% linoleic acid for further testing in coatings, polyol and polyurethane industries
- Preliminary results on functionality:

Composition	Name	Formula	MW	Soybean Oil: Commodity Soy Oil	Soybean Oil: DuPont Plenish	Soybean Oil: Monsanto Vistive Gold	Soybean Oil: HOSO Average	Soybean Oil: High linoleic soybean	Beef Tallow	Canola
C16:0	Palmitic Acid	$C_{16}H_{32}O_2$	256.4	11.0%	7.0%	3.0%	5.0%	3.8%	27.0%	4.0%
C18:0	Stearic Acid	$C_{18}H_{36}O_2$	284.5	4.0%	4.0%	3.0%	3.5%	2.4%	19.0%	3.0%
C18:1	Oleic Acid	$C_{18}H_{34}O_2$	282.5	23.0%	76.0%	75.0%	75.5%	19.0%	50.0%	65.0%
C18:2	Linoleic Acid	$C_{18}H_{32}O_2$	280.5	54.0%	10.0%	15.0%	12.5%	69.3%	3.0%	19.0%
C18:3	Linolenic Acid	$C_{18}H_{30}O_2$	278.4	8.0%	3.0%	4.0%	3.5%	5.4%	1.0%	9.0%
Total				***100.0%***	*100.0%*	*100.0%*	***100.0%***	***100.0%***	***100.0%***	***100.0%***
Double Bond Content				1.6	1.1	1.2	1.1	1.7	0.6	1.3
Double Bond per cent increase over Commodity Soy Oil				**0%**	**-32%**	**-25%**	**-28%**	**12%**	**-62%**	**-16%**

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Organic Soybean Breeding (M.Sc. student, Torin Boyle)

- Organic agriculture: a management intensive approach to farming which seeks to take advantage of agroecosystems to enhance crop production (Heckman et al., 2009)

- Characterized by complex rotations, biological pest control and cyclical fertility systems involving integrated livestock systems

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Why Breed for Organic Production Systems?

- Organic farmers are currently utilizing conventionally bred varieties (Murphy et al., 2007)
- Crops in organic systems encounter more environmental stresses (Heckman et al., 2009)
  - Increased weed pressure
  - Reduced nutrient availability
- Is the genetics different for optimal organic performance?

Two experiments:

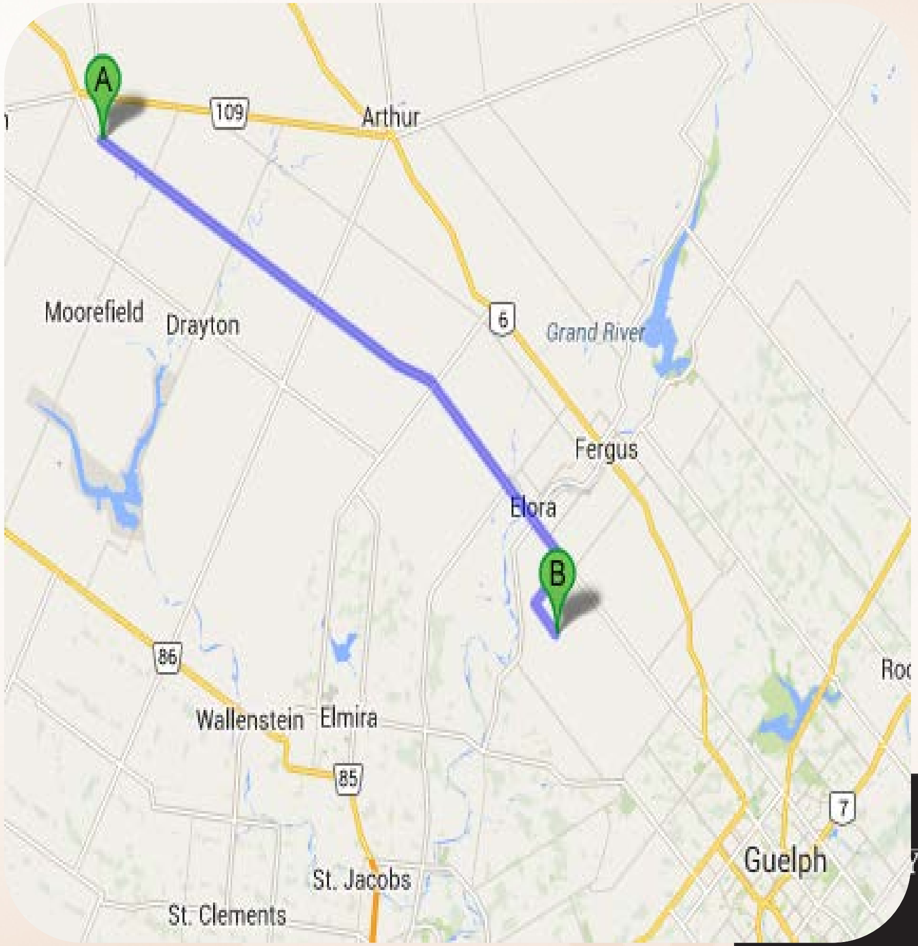
- 1. Yield Trials with conventionally developed cultivars
  - 30 Ontario soybean cultivars
  - Planted in a RCBD
  - Standard and organic traits were measured throughout development
- 2. Breeding trials
  - Two breeding populations
  - IR12-055 (OAC Sunny x S05-T6) and IR12-062 (OAC Calypso x DH618)
  - Both were selected under each environment and selections were compared

Yield Trials: Traits related to Organic Farming

- Weed Suppressive Ability(WSA) measured in early vegetative growth
- Nutrient Use Efficiency (NUE) measured at R5
- Root Morphology in the top 20 cm of soil at R5

Locations

- Organic Location (A)
  - Mapleton's Organic Farm Moorefield, ON
- Conventional Location (B)
  - Elora Research Station
  - Elora, ON
- About 25 mins apart



Root Morphology: Data Analysis

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- In Elora (conv. site), for all Root Morphology traits there was no difference among the cultivars
- In Moorefield (organic site), there were <u>significant differences between the varieties for all the Root Morphology traits</u>

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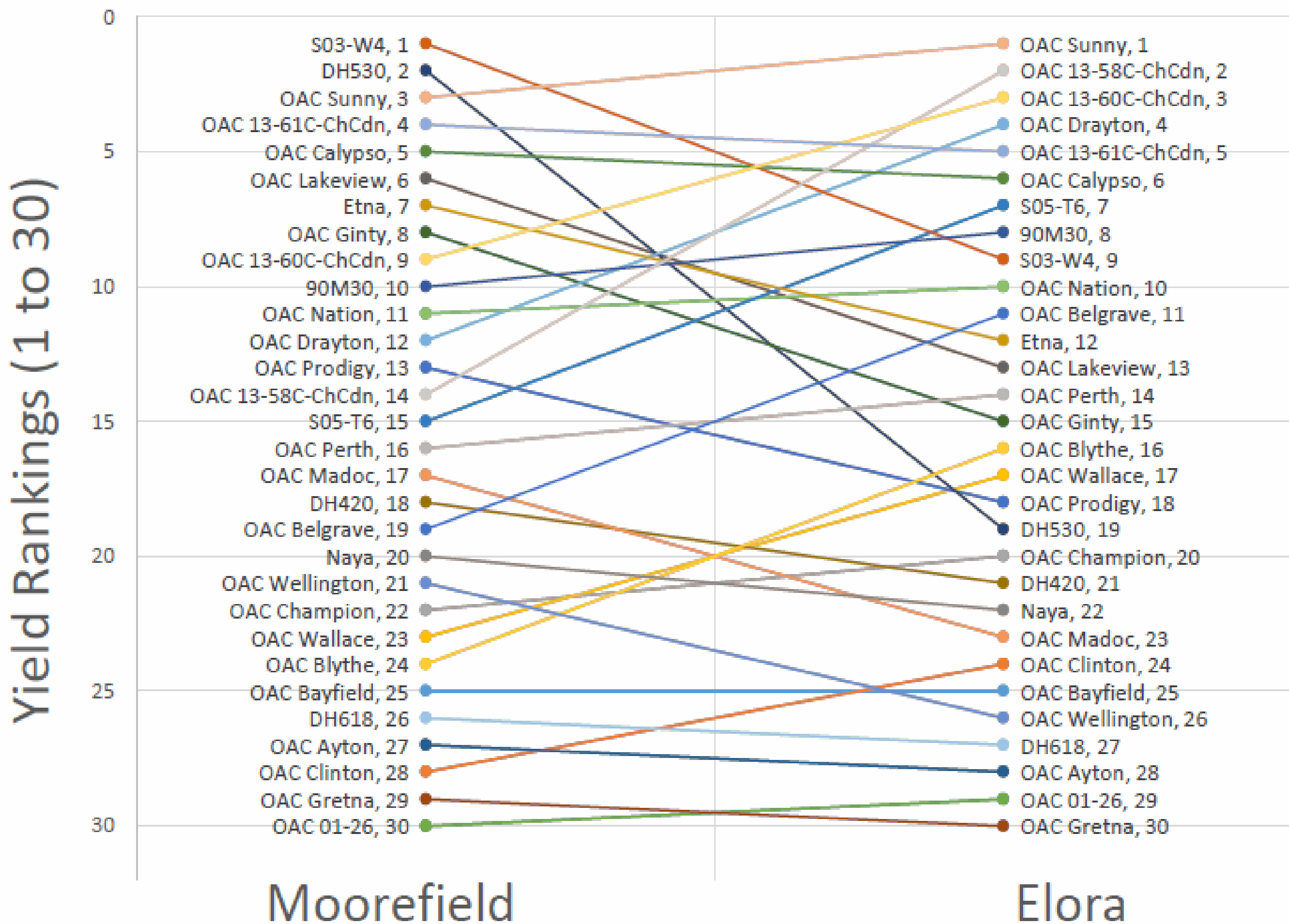
Kendall Tau Yield Rank Correlation and Diagram



Kendall Tau Rank Correlation Yield (Kg/ha)
0.55402
<.0001

Breeding Populations

- **2013 -F4 generation in Woodstock, ON**
  - Two crosses were selected
  - About 150 single plants were harvested from each cross:
  - OAC Calypso x DH618 and OAC Sunny x S05-T6

- **2014 -F5 generation in both Moorefield and Elora, ON**
  - F5 seed planted in single rows, selection based on visual performance
  - 25% selection pressure (35-40 lines advanced to F6)

Selections in Each Population

- IR12-062 (OAC Calypso x DH618) - 152 lines
  - Selection pressure of 25%
  - 8/38 **(21%) lines selected in each environment were the same**

- IR12-055 (OAC Sunny x S05-T6) a total of 162 lines
  - Selection pressure of 26%
  - 14/42 **(33%) lines selected in each environment were the same**

Understanding the Effects of Breeder Selections on Elite Soybean Germplasm Diversity

Robert Bruce
Ph.D. Candidate

Current Breeding Practice

- Elite germplasm is recycled for crossing
- Introduction of exotic germplasm may slow immediate progress in cultivar development
- Parental selections made purely based on agronomic performance; little genetic consideration

Breeding Diversity

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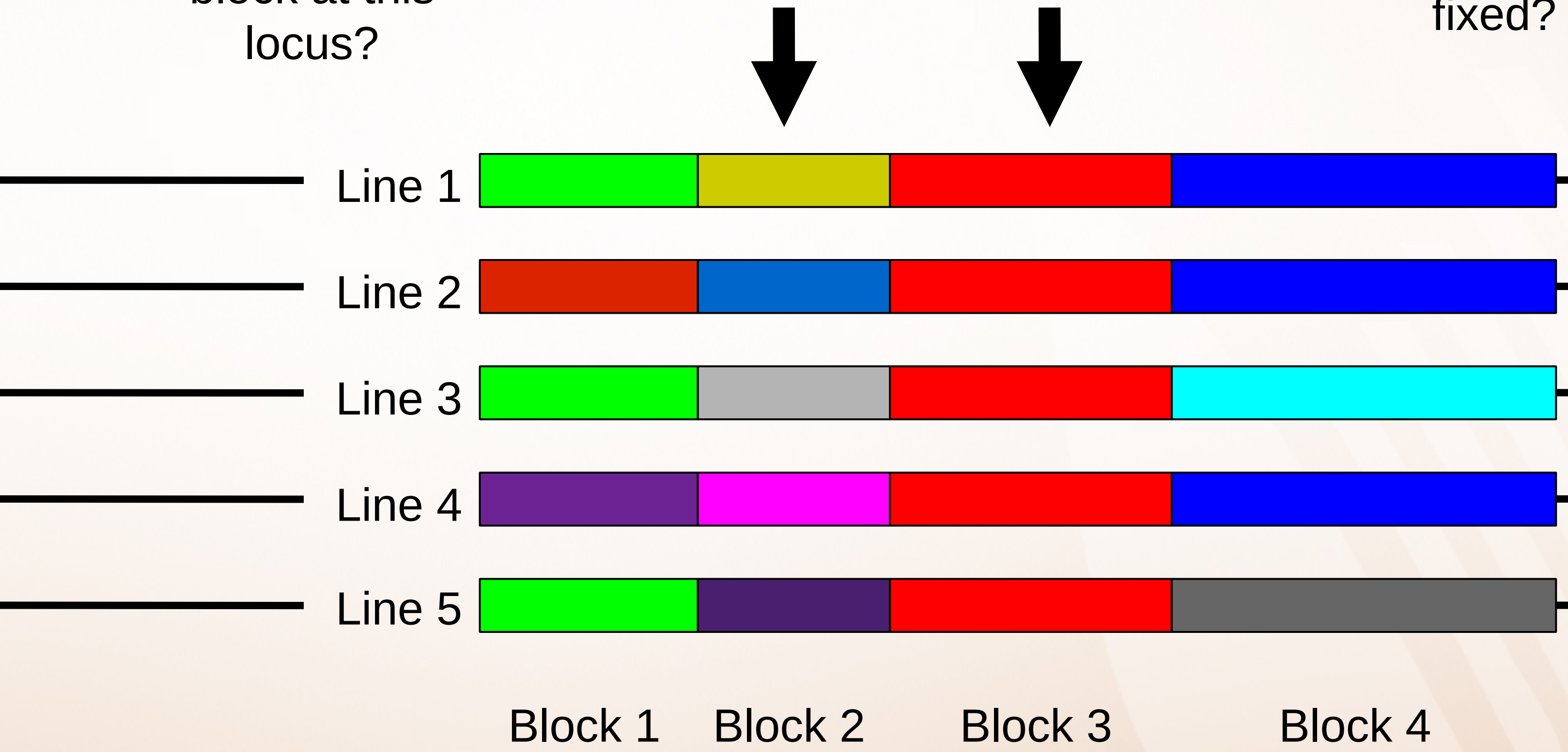
Is there no favourable block at this locus?

High Diversity

Low Diversity

Is the best block already fixed?



Frequency across breeding program? Over time? Between similar lines?

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Questions

- Have breeders' selections reduced diversity at loci for important agronomic traits in Ontario Soybean Germplasm?
- Is allelic structure conserved between breeding lines and cultivars for the same traits?
- Can targeted introgression of diverse alleles from PI lines further increase yield without compromising selection signatures?

Program Comparison

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Guelph (192 lines)

- Short Season (MG 00, 0 and I)
- Cultivars, breeding lines, Chinese germplasm, ancestors, tofu, natto
- G. soja x G. max RILs

Has selection for a trait in both programs altered diversity in the same pattern?

Ridgetown (96 lines)

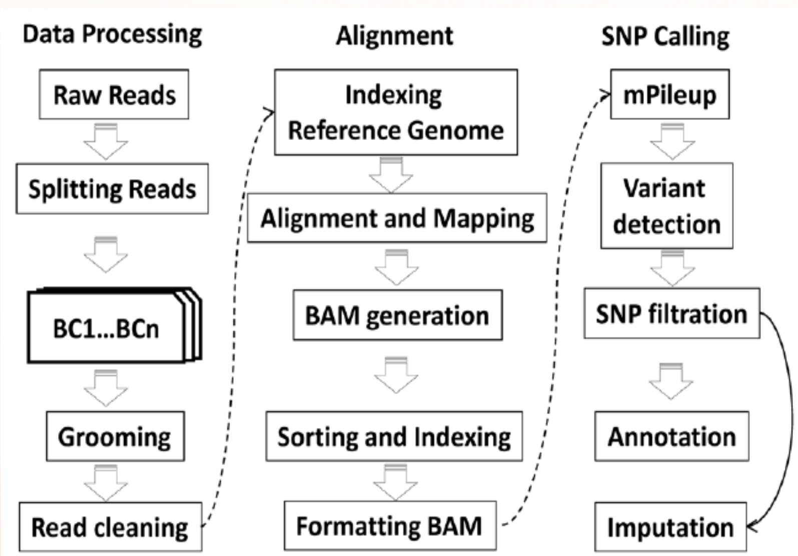
- Full Season Southern Ontario, MG 2 and 3
- Conventional, food grade soybeans

GBS

Line Selection and Growth

DNA Extraction

Illumina GAIIx Sequencing



Bioinformatics Pipeline

(Sonah et al. 2012)

Genotyping Using GBS (SNP)

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- Identify linkage blocks and their pedigree origin across the breeding programs
  - Signatures of selection
  - What genomic regions have altered diversity?
  - Can we associate diversity and genomic regions to traits of interest?

(USDA)

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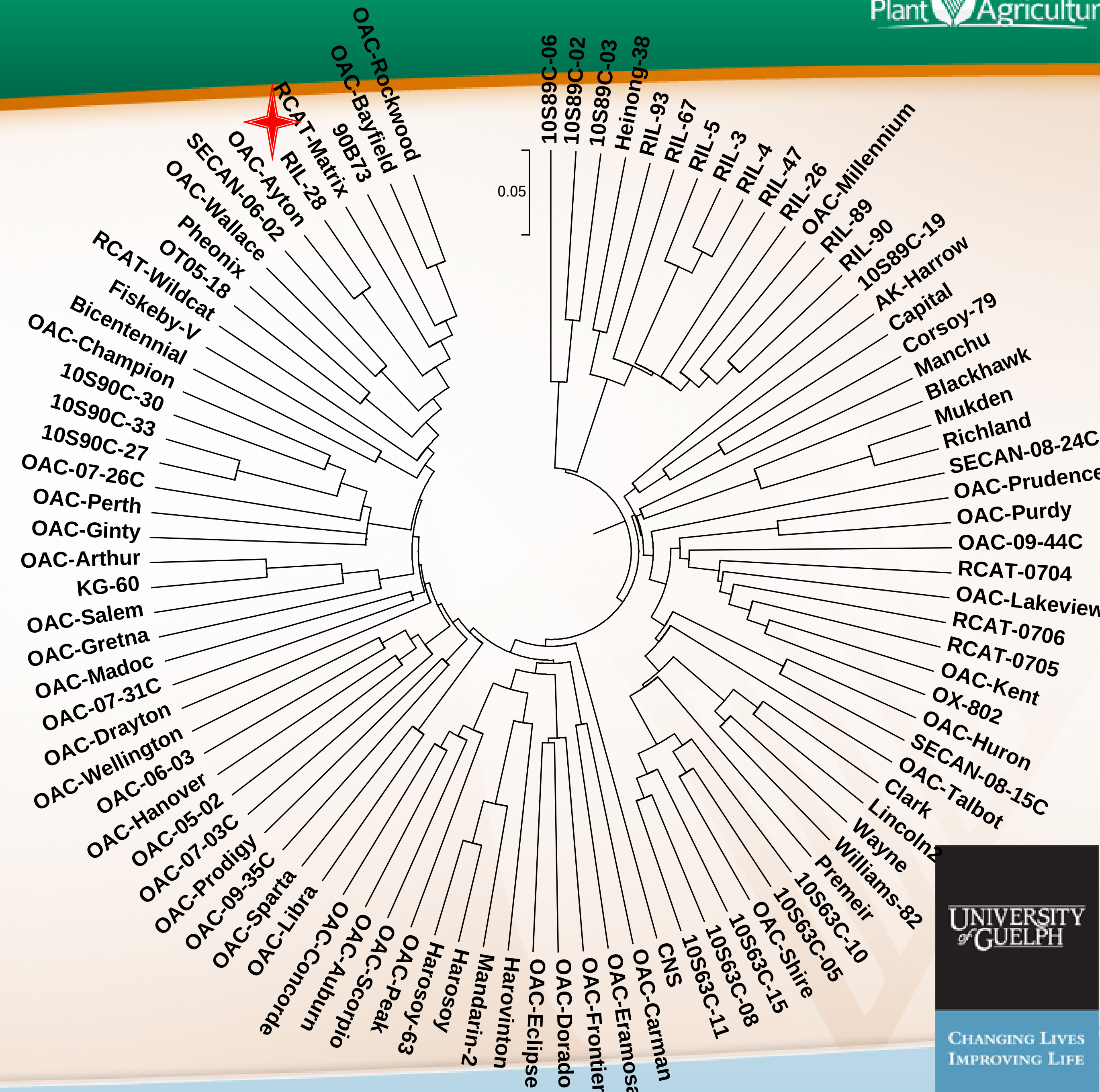

Figure. Circular tree diagram showing genetic relationships within the first 96-line panel. Clusters are similar to the pedigree relationships. SNP markers show that RIL 28 is not clustered with other RILs (red star) possibly due to a labeling or sampling error.

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Acknowledgements

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Many former and current graduate students, technicians, postdocs and collaborators

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Canada's Seed Partner

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Thank You

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