Progress on Herbicide Tolerance in Mustard

January 2016 SMDC Annual Meeting

Project Partners: M21, AAFC, Agrisoma, University of Florida, etc.

Mustard 21
Canada inc.

Mustard HT Project Objectives

1. **Overall Primary Objective: Develop a Herbicide Tolerant Mustard (HTM) Variety which is; i) Non GMO ii) with a NON HT canola Technology**
2. To create mutation for herbicide tolerance in Mustard Species.
3. To select for mutant genes for products which control the major broadleaf weed species found in mustard in western Canada: Kochia, wild mustard, stinkweed, thistle, etc.
4. To select mutant plant types with tolerance to at least 2X the rate of product required to control these weed species in the mustard crop.
5. To have acceptable consistent product tolerance and weed control over years and environments.
6. To identify the correct herbicide application rates for weed control for our selected herbicide products.

Mustard 21 Canada inc.

Identifying target herbicide technologies

- Suitable Group 2 & Group 4 products for the control of wild mustard and kochia.
- Candidate products;
- Group 2 = fluroxypyr, Florasulam, dicamba,
- Group #4 = imazethapyr, Solo.

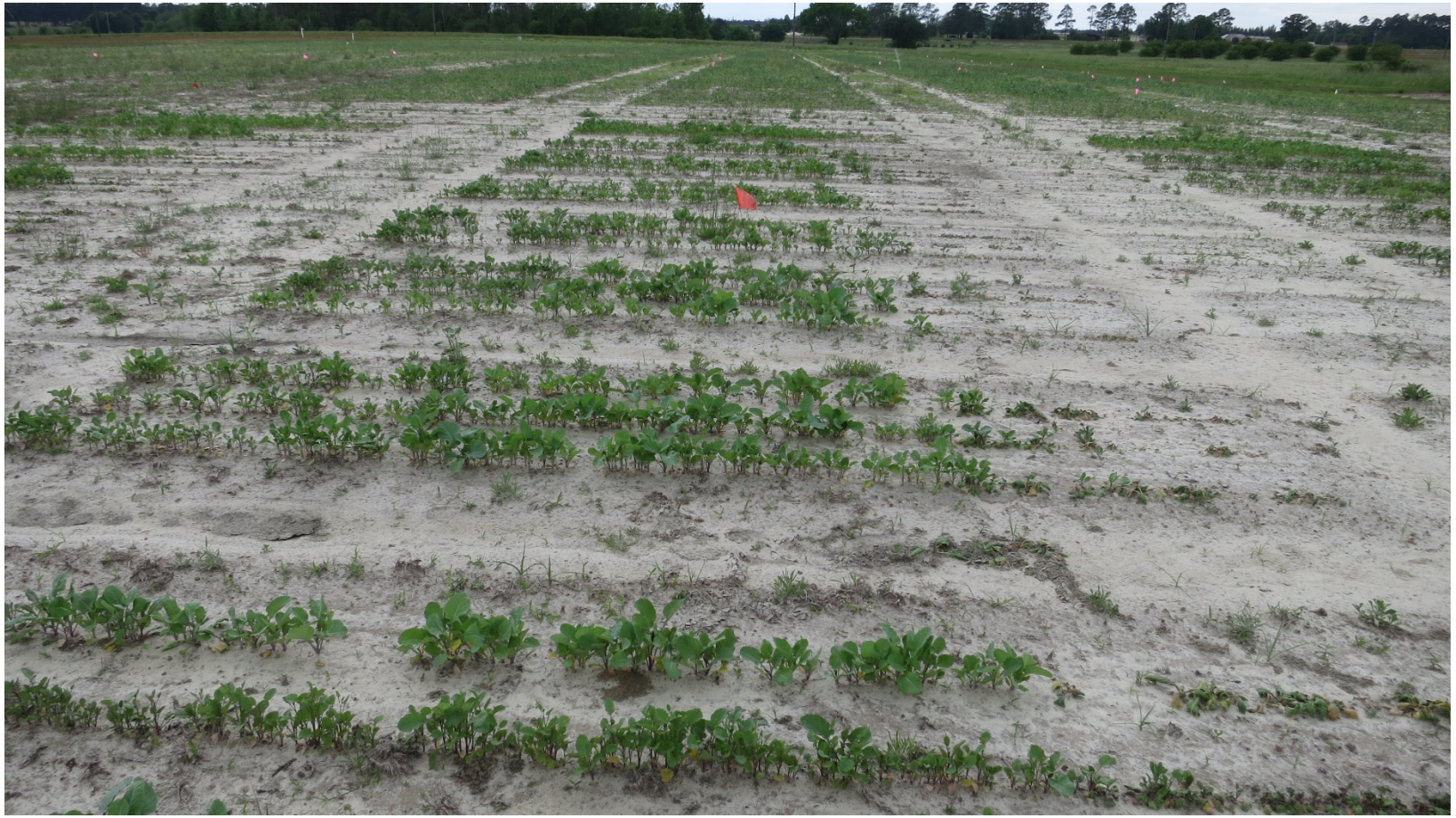
Mustard 21
Canada inc.

Creating mutations

- Mutation agent/means
  - Ultraviolet light to induce mutations
  - EMS (ethyl methanesulfonate)
  - Gamma radiation
- Mutation system (all non GM systems)
  - Seed mutation
  - Embryo mutation
- Know your starting species diversity for tolerance.
  - Initial species screening over rates and environments is required.

Mustard 21
Canada inc.

Searching through inherent species diversity



Native Germplasm Screening; specifically herbicide tolerance

Mustard 21 Canada inc.

Selecting Desirable Mutants

- Large scale selection of desired mutants. Many thousands of plants must be screened and carefully observed for tolerance. Escaped plants must be weeded out.
- Protocols for effective selection must be developed. These protocols depend on the mutation system being utilized and on the type of mutated population.
- Protocols will vary for greenhouse vs field screening and for mutant selection.
- Protocols must be tested over multiple environments and multiple years to prove their stability.

Mustard 21
Canada inc.

Selecting Mutants

A winner after 2X and 2X

More selections

Demonstrate safe tolerance level, desirable weed control and optimal product use rate

Rates and Environments

- Multiple sites within N. America: 3-4 sites each with herbicide and weed control expertise.
  - Determine weed control efficacy.
  - Determine acceptable crop tolerance levels. (vary by year).

Stability over years

- 3 years required at least to demonstrate crop tolerance and effective weed control over years (heat stress, rainfall levels, etc.)

Mustard 21
Canada inc.

Lewis 2015 HT Update_LEWIS2.pdf - Adobe Reader
File Edit View Window Help
Open 2 / 19 128% Tools Fill & Sign Comment

Herbicide Tolerance Work Flow

Y1
- Mutagen Treatment (M0)
- Seed Increase (M1)

Florida bulk screening

Y2
- Bulk Screen
- Field & Greenhouse Screen
- Florida Field Trials

Y3
- Field Trials
- GH Screening
- Dose Response
- Crossing
- Molecular Characterization
- Cross Tolerance

Y4
- Crossing
- Field - Test crosses
- Cross Tolerance
- Seed Increase

Y5
- Full Plot Multi-location Field Trials
- Phenotype, Yield, Herbicide Tolerance

Progress to Date – Microspore mutation

- UV mutation dose curve for LD50 for both B. juncea and B. carinata lines has been completed
- At microspore level, herbicide dose curves for LD 100 for both B. juncea and B. carinata lines has been completed
- Florasulam dose curve at microspore level for DH-AGR948.001 (in progress)
- At embryo level, herbicide dose curves for LD 100 for both B. juncea and B. carinata lines has been completed
- Routine herbicide screening for both species is in progress
- Three florasulam tolerant plants from EMS seed mutation derived donor plants have been identified
- In vitro seed herbicide screening technique has been developed

Mustard 21
Canada inc.

Microspore - Next Steps

- Herbicide screening at microspore and embryo level in B. juncea and B. carinata to achieve resistant plant to both group 2 and 4 herbicides will continue
- Development of suitable regeneration medium to get plantlet formation in Acc. 0060 DH-17 from fluroxypyr screened embryos
- Whole plant level screening of three tolerant plants to florasulam in Acc. 0060 DH-17
- Whole plant level screening of fluroxypyr tolerant plants achieved from 5 hour seed UV mutations and in vitro screening

Mustard 21
Canada inc.

Seed Mutation Projects – Progress summary

- All three mustard species have been included.
- Mutant populations have been made in all 3 species
- Greenhouse program;
  - Screening S. alba Group 2 at 1X rate, B. carinata Group 2 at .25X, 0.5X and 1X rates, B. juncea Group 4 at 2X rate
  - Dose response B. carinata Group 4 at 0 to 16X rates
  - Crossing projects; B. carinata elite breeding lines of both AAFC and Agrisoma X UF-S2,SA-S2 & UF-S3. B. juncea elite breeding line X S2 & S3
  - Seed increase of new selections from Florida

Mustard 21
Canada inc.

Summary of Seed Mutation Program

- UF - S2 and UF – S3 both exhibit improved group 4 tolerance when compared to native genotypes.
  - Quality data shows that these mutant lines are low oil content, etc. and that they are still segregating for herbicide tolerance levels.
  - UF – S3 exhibits tolerance to Group 2 herbicides, but the tolerance is at a lower level than required for commercial use in Canada.

- FUTURE work for 2016
  - Investigate mechanisms of tolerance in mutant lines
  - Create new mutant germplasm in all 3 species, especially in S. alba.
  - Initiate crossing program to transfer Group 4 tolerance now located in B. carinata to B. juncea if last field validation of tolerance in B. carinata is positive.

Mustard 21
Canada inc.

Commercialization Timeline

- 1st potential product should be Group 4 tolerant B. carinata
- Estimated year of commercial introduction for B. carinata is very difficult to predict; earliest possibility is 2019 – 2020.
- In all other species we have not yet identified suitable mutants.

Mustard 21
Canada inc.

Project Participants

- Eric Johnson, U. of S. (previous project lead)
- Murray Lewis, A.A.F.C. (current A.A.F.C. Project lead)
- Dr. Kevin Falk, A.A.F.C.
- Dr. Bifang Cheng, A.A.F.C.
- Ryan Vetter, A.A.F.C.
- Kayla Hawkins, A.A.F.C.
- Dr. Ravinder Gerwal, M21
- Daryl Males, M21
- Dr. Chris Bliss, Agrisoma
- Dr. Rick Bennett, Agrisoma
- Dr. Eddie Risseeuw, Agrisoma
- Erin Hill, Agrisoma
- Dr. David Wright, U. of Florida
- Dr. Ramone Leon, U. of Florida
- Marnard Douglas, U of Florida

Mustard 21
Canada inc.