NATIONAL SUNFLOWER ASSOCIATION OF CANADA

2019-2020 Development of Long-Type Confection Sunflower Hybrids

YEAR 2: 2019-20

ACTIVITY 1: SUMMER AND WINTER NURSERY

The objective of the summer and winter breeding nurseries is to develop elite parent lines, possessing genes for tolerance to sulfonylurea herbicides, rust and downy mildew that will, when crossed, produce herbicide tolerant experimental hybrids highly adapted to Canada, with a high level of resistance to downy mildew and rust, and possess improved seed types for Canadian processors and producers. For simplicity, the breeding of male parent lines and female parent lines in our program generally follows the same process.

The 2019 summer nursery was planted near Fargo, North Dakota with a total of 2,080 rows.

The goals for the 2019 nursery include:

- Growing out and fine tuning the males and female AxB lines used to make the 2019 preliminary nursery hybrids;
- Advance and identify several new A x B lines for potential use in making 2020 preliminary hybrids;
- Advance and identify several new males with genes for downy mildew and rust resistance for potential use in making 2020 preliminary hybrids;
- Carry out a small multiplication of the male and female parent lines used in the 2019 strip hybrid EX43400 for potential use in a future pilot production.;
- Tissue sample 450-500 plants for SNP marker screening
- Continue to develop new breeding populations for development of female parent lines containing genes for downy mildew and rust resistance.

Activity 1 met its Year 2 (2019-20) objectives to develop elite parent lines, possessing genes for tolerance to sulfonylurea herbicide (SU-7: non-transgenic), rust (R12) and downy mildew (PLARG) to provide a competitive production advantage to existing hybrids. The following quantities of finished, and unfinished parent lines were grown. The quantities of lines contained in the program are as follows:

- Finished male restorer lines - no dominant disease resistance genes. Elite (12)
- Finished male restorer lines - no dominant disease resistance genes. Semi- Elite (30)
- Finished male restorer lines fixed for gene PLARG or PLARG and R12 Elite (0)
- Finished male restorer lines fixed for gene PLARG or fixed for both PLARG and R12. Semi-Elite (48)
- Finished female A x B lines with cytoplasmic male sterile conversion completed. Elite (15)
- Finished female A x B lines with cytoplasmic male sterile conversion completed. Semi-Elite (31)

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<u>Deliverable</u>: Generation of new breeding populations (male restorer and female AxB lines) with desired characteristics from crossed plants

<u>Development of Male Restorer Lines:</u>

SUMMER 2019

- F_2 populations were bagged at R8. The bagged F_2 plants were rated for agronomics and seed type.
- Tissue samples were taken from the top rated F_2 plants and marker screened for dominant disease resistance genes.
- F_3 plants were selected and bagged in the winter nursery (Chile) based primarily on earliness and plant type.
- F_4 plants were bagged at R8. The bagged F_4 plants were rated for agronomics and seed type.

- Tissue samples were taken from the top rated F_4 plants and marker screened for dominant disease resistance genes.

WINTER 2019-2020

- F_5 plants were selected and bagged in the winter nursery (Chile) based primarily on earliness and plant type.
- F_5 plants were selected and bagged in the winter nursery based primarily on earliness and plant type.

Development of Female A x B Lines:

SUMMER 2019

- F_2 plants were selected before bloom based on earliness and plant type and a first cross was made to cytoplasmic male sterility.
- The top rated F_2 plants were advanced to winter nursery based on agronomics and seed type.
- F_4 plants were selected based on earliness and plant type with a third backcross made to cytoplasmic male sterility.
- The top rated F_4 plants were advanced to the winter nursery based on agronomics and seed type.

WINTER 2019-2020

- F_3 plants were selected based on earliness and plant type and a first backcross was made to cytoplasmic male sterility.
- F_5 plants were selected based on earliness and plant type with a fourth backcross made to cytoplasmic male sterility. If female conversion to sterility was complete, the new female A x B line was used to make experimental hybrids for testing in the 2020 summer nursery.

Deliverables met

- Selection in segregating populations for yield, seed type, agronomic integrity and presence of genes for herbicide tolerance and disease resistance
- Identified new homozygous parent lines for production of preliminary hybrids.
- Screened new parental line material for resistance to rust (R_{12} gene), downy mildew (PL_{ARG}).
- Screened material for the herbicide tolerance gene SU-7.
- Collected agronomic ratings on maturity, lodging, height, disease and a general visual screening of materials.

Figure 1: Herbicide damage

HERBICIDE TOLERANCE SCREENING

All parent lines in the NSAC program contain the SU-7 trait that is a single dominant gene from DuPont that conveys herbicide tolerance to tribenuron in sunflower. Both the summer and winter nurseries are sprayed with a 2x rate of tribenuron to confirm the presence of the trait within the

hybrids. Any hybrids that show injury are discarded. Figure 1 shows damage to plants that do not contain the trait.

DOWNY MILDEW, RUST AND SCLEROTINIA SCREENING

Breeding activities in the male parent program include the incorporation of genes for disease resistance (downy mildew: PL_{ARG}, rust: R_{12}). Male and female parent lines that contain the resistant genes were screened to confirm that the resistance genes were present. Plants that contain the resistance genes were selected for further advancement into the 2019-20 winter nursery

45 new herbicide tolerant male restorer lines (F_5) that are fixed for downy mildew resistance were identified. 21 of these lines were used in the winter breeding nursery to make experimental hybrids for testing in 2020 and were advanced to activity 2. SNP marker information confirmed that 12 of the new male restorer lines were also fixed for genes for rust resistance.

Based on agronomics, seed type and SNP marker information obtained from individual plants, 10 new F_4 female lines were identified that contain genes for downy mildew and/or rust resistance and were advanced to the 2019-20 winter nursery. Conversion to cytoplasmic male sterility (CMS) of the 10 new female lines was initiated in the 2018-19 winter nursery. CMS conversions will be completed in the 2020 summer nursery. The 10 new female lines containing genes for resistance will be advanced to the 2020-21 winter nursery and will be used to make experimental hybrids for testing in Manitoba in 2021.

These 10 female lines will be the first female parent lines to emerge from the program that contain PL_{ARG} or contain both PL_{ARG} and R_{12}. Having genes for disease resistance in both the male and female side of the program will provide more flexibility when selecting hybrid combinations since the female can now provide the resistance in combinations in which the male parent line may not carry PL_{ARG} and/or R_{12}.

Currently in the program, there are approximately 136 finished parent lines that have been utilized to make experimental hybrids used for testing in Canada (89 males, 47 females) since the program started in 2014. All 136 lines are herbicide tolerant and have been advanced through the program based on solid agronomics, seed type and adaptability to Canadian growing conditions. 47 of the male lines contain genes for resistance to downy mildew, or downy mildew and rust.

DISCUSSION

Based on comparative yields and seed types obtained from experimental hybrids tested in Manitoba in 2019, it appears that the program currently has the potential to produce a high number of experimental hybrids that can yield competitively and can produce improved seed types to commercial confection hybrids currently being grown in Canada. High yield performance and improved seed types in combination with herbicide tolerance and genetic disease resistance will provide attractive hybrid options for Canadian producers.

ACTIVITY 2: SUMMER AND WINTER NURSERY

The overall objective of the Canadian Testing Program is to isolate commercially viable experimental hybrids for advanced testing and eventual commercialization. While seed type and marketability are of extreme importance, the hybrids must also be early maturing, high yielding and have a strong agronomic package. Testing activities will include four levels of testing.

Preliminary Hybrid Screening

Planting Date: May 14 (Miami) and May 14 (Holland)

The NSAC transports our planting equipment from Fargo, ND in order to plant our nursery. This process has eliminated planting errors and ensures that the trials are planted at the desired time.

96 preliminary hybrids were selected from the 2018-2019 Summer and Winter nurseries and were tested at two locations in Manitoba. Each location had two rows and was replicated twice. The trials included two commercial performance checks (6946 DMR & Panther DMR) and one SU-7 herbicide check (P63ME70). The 2 commercial performance checks are not tolerant to the SU-7 herbicide, and therefore are strategically placed along the edges of the trials.

The trial was sprayed and tilled by a contract services company in both locations and plot maintenance including thinning was performed by NSAC's Research Agronomist. Benchmarks for a commercial confectionary hybrid were:

- Herbicide tolerance: resistance to sulfonylurea herbicide: SU-7
- Seed Type: Dark color, long (1.9-3.2 cm) seed with shoulder width
- Disease resistance: resistance to Downey Mildew (PL_{arg}) and Rust (R_{12})
- Early maturity: less than or equal to the commercially available hybrid check or about 117 days to R9 maturity
- Improved yield over commercially available hybrids
- General plant integrity/agronomics – acceptable height, good lodging tolerance, and good overall agronomic package

The NSAC crew hand clipped the sunflower heads from the selected lines on October 7th (Holland) and October 8th (Miami). The sunflower heads had to be dried, in order to thresh the seed and perform sizing calculations. The heads were threshed on October 21 with quality testing performed the following day, on October 22nd.

Of the 96 new lines tested in 2019, 36 were harvested and evaluated for yield, agronomic and seed quality traits. Based on the overall performance 12 hybrids were selected for advancement to the next level of testing in 2020. Seed of the selected 12 hybrids were produced in the 2019-2020 winter nursery in Chile for testing in Manitoba in 2020.

Variety Performance Trials (VPT)

Two new hybrids were tested at 4 locations within Manitoba: Carberry, Melita, Rossendale and Stonewall. Each trial included 3 replicates using a RCBD (Randomized Complete Block Design) for analysis. Data for only 2 locations were reported. The other 2 locations were lost due to dry planting conditions that resulted in variable and thin seedling emergence within the plots. The trials were harvested, and the results were communicated through the NSAC website and Seed Manitoba in December 2019.

The two NSAC hybrids (EX 43400; EX 88647) tested were lower in yield compared to the check; shorter maturity from 1-3 days and have overall bigger seed sizing.

TABLE 1 : 2019 VARIETY PERFORMANCE TRIAL RESULTS

SUNFLOWERS - NON-OIL TYPE

Comments:

These varieties were tested and data donated by the National Sunflower Association of Canada Inc. (NSAC)
All sunflowers varieties listed are susceptible to sclerotinia and sunflower rust strains present in Manitoba.
Genetic resistance to verticillium wilt is rated as moderately susceptible to moderately resistant for all sunflower varieties presented.

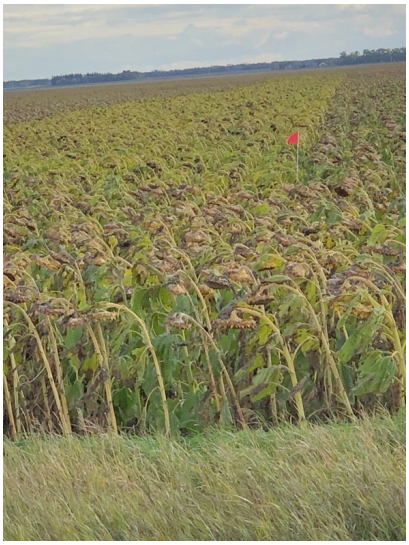
Summary Table

Company	Hybrid	Genetic Traits [1]	Site Years	Yield % Check	Maturity (days to R9)	Height (inches)	2019 Seed Sizing (%) [2] >22/64	>20/64	<20/64
NuSeed America	6946 DMR	DM	25	100	0	0	41	30	26
NuSeed America	Panther DMR	DM	33	100	1	-3	55	26	14
Experimental lines being tested/proposed for registration in Canada									
NSAC	EX 43400	ExSun	2	82	-1	3	47	32	22
NSAC	EX 88647	ExSun	2	91	-3	3	70	23	7
	CHECK CHARACTERISTICS								
	6946 DMR		25 site years	3195 lb/ac	121 days	68 inches			

1 Genetic traits include CL = Clearfield tolerance; ExSun = Express tolerance; DM = Downy Mildew Resistance.
2 Totals may not add to 100% due to rounding

Pre-Commercial Strip Trial Testing

One hybrid from the 2018 variety performance trial was advanced and tested at 2 locations within Manitoba in a head to head, field scale comparison to the commercial hybrid 6946 DMR. The experimental hybrid was treated the same as the commercial hybrid. Agronomic data was collected on both hybrids in the strip trial throughout the season. The trial was managed by the producer throughout the whole growing season. The hybrids were harvested and a weigh wagon captured the yield data. Seed samples were collected for seed quality (test weight, seed sizing, visual acceptance).



The experimental hybrid EX 43400 performed lower in yield compared to the commercial hybrid 6946 in side by side pre-commercial strip trials. The experimental hybrid was taller in height, producing a small head size but larger seed size. Based upon the final assessment of all the data collected from all the trials, the NSAC research committee will forgo the experimental hybrid in favor of more promising hybrids selected in the breeding pipeline.

TABLE 2: 2019 PRE-COMMERCIAL STRIP TRIAL RESULTS

Dundonald - 2019				Planted May 13		Harvested Nov. 4		Lodged	
Hybrid	Harv. Area (acre)	Yield (lbs/ac)	Harv. Mst (%)	Dockage (%)	Bus. Wgt (lbs/bus.)	Seed Sizing			
						<20/64	>20/64	>22/64	>24/64
6946	0.57	3529	16.8	1.4	27.5	33	52	13	2
43400	0.63	2286	16.9	4	22.4	31	40	26	4
Wawanesa - 2019				Planted May 14		Harvested Oct. 28		Cutworms	
Hybrid	Harv. Area (acre)	Yield (lbs/ac)	Harv. Mst (%)	Dockage (%)	Bus. Wgt (lbs/bus.)	Seed Sizing			
						<20/64	>20/64	>22/64	>24/64
6946	1.14	2690	12.4	2.2	28.8	46.2	35.3	14.8	3.7
43400	1.14	2315	13.9	1.8	26.3	31.6	34	24.9	9.5

KNOWLEDGE TRANSFER EVENTS:

The National Sunflower Association of Canada along with our breeder, held three knowledge transfer events communicating results with our membership and the sunflower industry:

September 18, 2019 - Nursery Tour with Manitoba sunflower industry representatives
September 25, 2019 - Nursery Tour with Manitoba sunflower industry representatives
February 12, 2020—NSAC Annual General Meeting with sunflower members and industry
March 5, 2020—Presentation at the DFCC Research Day in Saskatoon, SK

KNOWLEDGE TRANSFER EVENTS:

As the National producer organization for Canadian sunflower growers, knowledge transfer to our members is extremely important, as the NSAC must demonstrate producer's check-off dollars at work. For this project, there are two specific target audiences: producers and sunflower processors/buyers.

During the 2019-19 program year, the National Sunflower Association of Canada along with our breeder, held four knowledge transfer events communicating results with our membership and the sunflower industry:

- September 18, 2019 - Nursery Tour with Manitoba sunflower industry representatives
- September 25, 2019 - Nursery Tour with interested sunflower breeding company
- February 12, 2020—NSAC Annual General Meeting with sunflower members
- March 5, 2020—Presentation at the DFCC Research Day in Saskatoon, SK

FUNDING ACKNOWLEDGMENTS:

The National Sunflower Association of Canada grateful acknowledges the funding support from the Diverse Field Crops Cluster with funding from Agriculture and Agri-Food Canada's Canadian Agricultural Partnership program, Government of Manitoba & Government of Canada through the CAP-Ag Action Manitoba program and Western Grains Research Foundation.