Progress on Transferring Sclerotinia Resistance Genes from Wild *Helianthus* Species into Cultivated Sunflower

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BACKGROUND

- Cultivated sunflower lacks sufficient genes for Sclerotinia resistance
- Field and greenhouse evaluations have indicated excellent stalk and head rot resistance in wild perennial *Helianthus* species
- In the previous ten years, our project has focused on the introgression of the Sclerotinia resistance genes from the wild perennial *Helianthus* species into cultivated sunflower
- Crosses and backcrosses have been made for hexaploid, tetraploid, diploid wild species and amphiploids with NMS HA 89, HA 410, HA 441, or HA 451

OBJECTIVES

- Incorporate resistance genes from diverse wild perennial species into cultivated backgrounds
- Field and greenhouse evaluations to identify new resistant materials to Sclerotinia
- Genetic study of resistance and development of QTL mapping populations

MATERIALS
Phase 1 (since 2004)

12 sources used for stalk and head rot resistance genes

- 2 hexaploids (backcrossed with HA 410)

  H. californicus and *H. schweinitzii*

- 3 diploids (backcrossed with HA 410)

  H. maximiliani, *H. giganteus*, and *H. grosseserratus*

- 2 diploids (backcrossed with HA 441)

  H. nuttallii and *H. maximiliani*

- 5 amphiploids (backcrossed with HA 410)

  H. nuttallii/P21, *H. maximiliani*/P21, *H. gracilentus*/P21,
  H. grosseserratus/P21, and *H. strumosus*/P21

MATERIALS
Phase 2 (since 2010)

No.	Accession	Species	2n	Year
1	PI 547171	*H. hirsutus*	68	2011
2	PI 547174	*H. hirsutus*	68	2011
3	Ames 30340	*H. salicifolius*	34	2011
4	Ames 30348	*H. salicifolius*	34	2011
5	PI 494594	*H. occidentalis* subsp. *plantagineus*	34	2011
6	Ames 30317	*H. occidentalis* subsp. *plantagineus*	34	2011
7	Ames 30356*	*H. silphioides*	34	2011
8	Ames 30354*	*H. silphioides*	34	2011
9		*H. resinosus*	102	2012
10	PI 503209	*H. divaricatus*	34	2012
11	PI 503216	*H. divaricatus*	34	2012
12	PI 503218	*H. divaricatus*	34	2012

* Difficult to make crosses

MATERIALS
Phase 3 (since 2013)

No.	Accession	Species	2n	Year
1	PI 666724	*H. simulans*	34	2013
2	PI 547217	*H. strumosus*	102	2013
3	PI 547226	*H. strumosus*	102	2013
4	PI 547242	*H. tuberosus*	102	2013
5	PI 650089	*H. tuberosus*	102	2013
6	PI 650105	*H. tuberosus*	102	2013
7	PI 435880	*H. decapetalus*	68	2013

Note: All PIs immune to rust race 336.

METHODS

- Traditional crossing and backcrossing
- Embryo rescue
- Mitotic chromosome counting and pollen fertility examination
- Seed increase and field test for stalk and head rot
- GISH, FISH, and molecular markers

RESULTS

Seed increases in 2008-2014

Sources	2008	2009	2010	2011	2012	2013	2014
Hexaploid		160	112	37	32	36	52
Diploids-HA 410		153	164	43	55	53	6
Diploids-HA 441	169	70	181	99	120	36	96
Amphiploids		124	75	40	34	42	17
New diploids*						370	19
New tetraploids*						60	4
Total	169	507	532	219	241	597	194

* Phase 2 crosses

Field test in 2009-2014

Sources	Stalk rot (SR)						Head rot (HR)					
	2009	2010	2011	2012	2013	2014	2009	2010	2011	2012	2013	2014
Hexaploid	72	92	85	65	43	39		51	52	27	1	1
Diploids-HA 410	32	71	58	56	27	25		56	56	45	11	11
Diploids-HA 441	132	169	122	121	51	51	163	179	110	90	41	41
Amphiploids	77	81	59	42	18	16		23	22	11	0	0
New diploids						368						110
New tetraploids						60						0
Total	313	413	324	284	139	559	163	309	240	173	53	163

Replicated Sclerotinia head rot field evaluation of 2013 retests (Tests 1 and 2) at Carrington, ND and Staples, MN in 2014

Pedigree*	**Carrington, ND 2014**		**Staples, MN 2014**	
	Disease Rating	**Infected Plants**	**Disease Rating**	**Infected Plants**
TEST 1 **(Second retest)**	0-5	%	0-5	%
(NMS HA 89 x 1018 (MAX) x HA 441, BC1F2) HA 441,BC2F2	0	0	0.29	12
(NMS HA 89 x 1018 (MAX) x HA 441, BC1F2) HA 441, BC2F3	0	0	0	0
((NMS HA 89 x 1018 (MAX)) x HA 441, BC1F4	0	0	3.0	67
NMS HA 89 x 1018 (MAX) x HA 441 (3), BC3F2	0	0	0	0
((NMS HA 89 x 1018 (MAX)) x HA 441*3), BC3F3	0.19	4	2.8	69
(NMS HA 89 x 1323 (MAX)) x HA 441, BC1F4	0	0	0.67	17
(NMS HA 89 x 1324 (NUT)) x HA 441 (2), BC2F2	0.29	7	0.81	17
((NMS HA 89 x 1008 (NUT)) x HA 441), BC1F5	0	0	1.23	28
((NMS HA 89 x1018 (MAX) x HA 441) HA441, BC2F4	0.38	9	2.8	67
(NMS HA 89 x 1008 (NUT) x HA 441(2), BC2F2	0	0	1.00	25
➔ **Recurrent parent HA 441**	**0.36**	**8**	**1.01**	**38**
TEST 2 **(First retest)**				
((NMSHA 89 x H. GRO=PI613793) HA 410*2), BC2F3	0.37	9	3.23	17
(NMS HA 89 x H. GIG=PI547182) HA 410), BC1F4	0.50	10	2.50	80
((NMS HA 89 x H. GRO=PI613793) HA 410), BC2F3	0	0	0.67	17
((NMS HA 89 x H. GRO=PI613793) HA 410), BC1F4 *new 69*	0.65	8	1.92	45
➔ **Recurrent parent HA 410**	**2.61**	**57**	**3.63**	**83**
((NMS HA 89 x 1323(MAX) x HA 441) BC1F5	0.29	7	1.80	40
((NMS HA 89 x1324 (NUT) x HA 441) BC1F5	0	0	0.67	17
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F4	0.18	5	2.77	69
((NMS HA 89 x 1018 (MAX)) x HA 441) BC1F6	0	0	0.36	9
((NMS HA 89 x 1324 (NUT)) x HA 441) BC1F5	0	0	0.42	8
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441) BC2F4	0.39	17	1.46	54
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441) BC2F4	0	0	0.65	18
➔ **Recurrent parent HA 441**	**0.36**	**8**	**1.01**	**38**

Replicated Sclerotinia head rot field evaluation of new inter-specific crosses at Carrington, ND and Staples, MN in 2014

Pedigree*	**Carrington, ND 2014**		**Staples, MN 2014**	
	Disease Rating	**Infected Plants**	**Disease Rating**	**Infected Plants**
TEST 3 (New Selections)				
NMS HA 89 x ((SAL) x HA 410), F2	0.92	23	-	-
NMS HA 89 x ((SAL) x HA 410), F2	0.85	23	-	-
NMS HA 89 x ((SAL) x HA 410), F2	1.08	23	-	-
NMS HA 89 x ((SAL) x HA 410), F2	1.08	23	-	-
NMS HA 89 x ((SAL) x HA 410), F2	0.31	6	-	-
NMS HA 89 x ((OCC) x HA 410), F2	0.83	17	-	-
NMS HA 89 x ((OCC) x HA 410), F2	0.67	17	-	-
NMS HA 89 x ((OCC) x HA 410), F2	0.83	17	-	-
NMS HA 89 x ((OCC) x HA 410), F2	0	0	-	-
NMS HA 89 x ((OCC) x HA 410), F2	1.00	22	-	-
NMS HA 89 x ((OCC) x HA 410), F2	0.67	17	-	-
NMS HA 89 x ((OCC) x HA 410), F2	-	-	0.69	15
NMS HA 89 x ((SAL) x HA 410), F2	-	-	0	0
NMS HA 89 x ((SAL) x HA 410), F2	-	-	0.69	31
NMS HA 89 x ((SAL) x HA 410), F2	-	-	0.91	27
Recurrent parent HA 410	**2.61**	**57**	**3.63**	**83**
Checks				
Susceptible check HA 89 (S)	**3.31**	**72**	**2.56**	**67**
Susceptible check Mycogen (Cargill) 272 (S)	**3.50**	**77**	**4.14**	**88**
Resistant check Croplan 305 (R)	**0.95**	**21**	**3.14**	**82**
Resistant check Croplan 343 (R)	**0.24**	**27**	**0.86**	**29**
Amphiploid Bulk (R)	**0**	**0**	**0**	**0**

*The first three letters of the *Helianthus* species are used to identify the species source.
S=Susceptible, R=Resistant.

Replicated Sclerotinia stalk rot field evaluation of 2013 retest (Test 1) at Carrington, ND and Grandin, ND in 2014

Pedigree*	**Carrington, ND 2014**	**Grandin, ND 2014**
	Percent Infected Plants	**Percent Infected Plants**
TEST 1 (Second retest)	%	%
(DIV (68) x GRO (68) HA410(3), BC2F2) x HA 410	10	0
(STR (68) x HA 410 (3), BC2F2) x HA 410	0	0
(CAL 2376 x HA 410*5), BC4F5	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(CAL 2376 x HA 410*5), BC4F5	0	0
(CAL 2376 x HA 410*5), BC4F5	0	0
CAL 2376 x HA 410, BC4F2	0	0
CAL 2376 x HA 410 (5), BC4F3	0	0
CAL 2376 x HA 410 (5), BC4F3	0	0
CAL 2376 x HA 410 (5), BC4F3	0	0
CAL 2376 x HA 410 (5), BC4F3	0	0
MAX (68) sib x HA 410 (3), BC2F2	0	0
STR (68) x HA 410 (3), BC2F3	0	0
Recurrent parent HA 410	**9**	**31**

Replicated Sclerotinia stalk rot field evaluation of 2013 retests (Tests 1 and 2) at Carrington, ND and Grandin, ND in 2014

Pedigree*	**Carrington, ND 2014**	**Grandin, ND 2014**
	Percent Infected Plants	**Percent Infected Plants**
TEST 1 (Second retest)	%	%
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F3	0	0
(NMS HA 89 x 1018 (MAX)) x HA 441*2, BC2F4	0	0
(NMS HA 89 x 1018 (MAX)) x HA 441*2, BC2F4	0	0
(NMS HA 89 x 1314 (MAX)) x HA 441, BC2F4	0	0
(NMS HA 89 x 1323 (MAX)) x HA 441, BC3F3	0	0
((NMS HA 89 x 1018 (MAX)) x HA 441*3}, BC3F3	0	0
((NMS HA 89 x 1314 (MAX)) x HA 441*3}, BC3F2	0	0
((NMS HA 89 x 1323 (MAX)) x HA 441}, BC1F5	0	0
(NMS HA 9 x 1324 (NUT)) x HA 441, BC2F4	0	0
(NMS HA 89 x 1324 (NUT)) x HA 441, BC2F4	0	0
Recurrent parent HA 441	**6**	**9**
TEST 2 (First retest)		
((NMS HA 89 x GRO=PI613793) HA 410*2), BC1F4	0	0
((NMS HA 89 x GRO=PI613793) HA 410), BC1F4	0	0
(CAL 2376 x HA 410*5), BC4F5	0	0
(CAL 2376 x HA 410*5), BC4F4	0	0
(MAX 68 SIB x HA 410*3), BC2F4	0	0
(STR 68 x HA 410*3), BC3F3	0	0
Recurrent parent HA 410	**9**	**31**
((NMS HA 89 x 1323 (MAX) HA 441), BC2F4	0	0
(NMS HA 89 x 1323 (MAX) HA 441), BC1F5	0	5
Recurrent parent HA 441	**6**	**9**
CHECKS		
Susceptible check HA 89 (S)	**37**	**22**
Susceptible check Mycogen (Cargill) 272 (S)	**55**	**44**
Resistant check Croplan 305 (R)	**9**	**3**
Resistant check Croplan 343 (R)	**23**	**9**
Amphiploid Bulk (R)	**0**	**0**

Sclerotinia stalk rot field evaluation of new interspecific crosses at Carrington, ND and Grandin, ND in 2014

Pedigree*	**Percent Infected Plants and Number of Entries**					
CARRINGTON—New selections						
	0%	1-5%	6-10%	11-15%	16-20%	>20%
((NMS HA 89 x (OCC) x HA 410), F2	37	13	17	31	20	66
((NMS HA 89 x (SAL) x HA 410), F2	0	1	0	0	0	1
Recurrent parent HA 410=9%	–	–	–	–	–	–
((NMS HA89 x (OCC) x HA 451), F2	5	3	4	6	2	4
Recurrent parent HA 451=46%	–	–	–	–	–	–
TOTAL NUMBER	**42**	**17**	**21**	**37**	**22**	**71**
GRANDIN—New selections						
	0%	1-5%	6-10%	11-15%	16-20%	>20%
((NMS HA 89 x (SAL) x HA 410), F2	2	6	6	14	13	86
((NMS HA 89 x (HIR) x HA 410), F2	5	5	2	8	3	26
((NMS HA 89 x (OCC) x HA 410), F2	0	1	1	4	5	13
Recurrent parent HA 410 =31%						
(NMS HA 89 X (HIR) x HA 451), F2	0	0	1	1	0	9
Recurrent parent HA 451=42%	–	–	–	–	–	–
TOTAL NUMBER	**7**	**12**	**10**	**27**	**21**	**134**
GRAND TOTAL FOR ALL TESTS	**49**	**29**	**31**	**64**	**43**	**205**

Backcrosses between HA 410, HA 451, and NMS HA 89 and wild *H. hirsutus*, *H. salicifolius*, *H. occidentalis*, and *H. divaricatus* and seed set from 2013 to 2014

Parentage	BC_2F_1 (2013-2014)			BC_1F_2 (2013-2014)			BC_3F_1 (2014) (F_1 as female)			BC_2F_2 (2014) (F_1 as female)		
	Plants	2n	Seed set %	Plants	2n	Seed set %	Plants	2n	Seed set %	Plants	2n	Seed set %
H. hirsutus × HA 451	28	37-45	10.1	-	-	-	13	34-37	19.7	8	34-37	42.2
NMS HA 89 × *H. hirsutus*	31	34-46	9.0	-	-	-	18	34-39	12.7	5	34, 35	15.0
H. salicifolius × HA 410	26	34-36	34.3	5	34	9.0	16	34-36	24.0	19	34, 35	2.2
H. occidentalis × HA 410	12	34-36	25.7	12	34-36	22.8	27	34-36	23.2	16	34, 35	17.1
NMS HA 89 × *H. occidentalis*	7	34-36	34.7	-	-	-	1	35	80.0	3	34	11.0
H. divaricatus × HA 410	2	35	1.1	3	34	4.3	-	-	-	-	-	-
NMS HA 89 × *H. divaricatus*	25	34-36, 51	36.7	3	34	22.9	-	-	-	-	-	-

Seed set and pollen fertility of new interspecific crosses of wild perennials crossed with HA 410 in 2013-2014

Parentage	F_1 (2013-2014)			BC_1F_1 (2014)			
	Plants	BC_1F_1 seeds	% Seed set	Plants	% Fertility	BC_2F_1 seeds	% Seed set
H. simulans (PI 666724) × HA 410	25	28	0.03	10	86.44	Many	85.50
H. strumosus (PI 547217) × HA 410	17	775	5.17	29	3.01	24	0.13
H. strumosus (PI 547226) × HA 410	15	507	4.88	25	4.48	9	0.12
H. tuberosus (PI 547242) × HA 410	6	279	5.96	22	7.48	11	0.14
H. tuberosus (PI 650089) × HA 410	17	2149	18.56	21	3.07	64	0.91
H. tuberosus (PI 650105) × HA 410	14	1349	17.91	20	5.00	23	0.28
H. decapetalus (PI 435880) × HA 410	5	4	0.88	-	-	-	-

SUMMARY

- Replicated field evaluation in 2014 of 2013 retest for 131 families for stalk rot and 53 families for head rot confirmed successful introgression of resistance genes
- More than 400 new early generation families from Phase 2 crosses tested in 2014 suggested excellent stalk rot resistance
- Seed was increased in the field for nearly 200 progeny families in 2014
- Some progenies from five crosses of Phase 3 are established in greenhouse for further backcrossing

FUTURE WORK

- Families with better resistance than the recurrent parents were identified from the first retest, second retest and new selections will be retested again or released as a germplasm
- Initiate QTL mapping populations with selected resistant materials
- Continue to backcross the progenies derived from different sources to reduce 2n chromosomes to 34
- Characterize a newly discovered CMS from *H. salicifolius*

FUTURE WORK

- Study the chromosome non-reduction in interspecific crosses, which has been observed in the progenies derived from six wild perennials (See Poster)
- Identify chromosome addition lines, and characterize alien chromosomes or fragments in cultivated background

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Questions?

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