2016 Progress on Transferring Sclerotinia Resistance Genes from Wild *Helianthus* Species into Cultivated Sunflower

Zhao Liu (NDSU)

PI: Dr. Chao-Chien Jan (USDA-ARS, NCSL)

Co-PI: Gerald J. Seiler (USDA-ARS, NCSL)

Cooperators: Khalid Y. Rashid (Agric. & Agri-Food Canada)

Xiwen Cai (NDSU)

NDSU NORTH DAKOTA STATE UNIVERSITY

USDA

INTRODUCTION

- Sclerotinia, also called white mold, is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary
- It is a severe and widespread fungal disease attacking 64 plant families, and more than 360 species, causing both yield and quality reduction
- Moreover, species of the genus Sclerotinia can function either as soilborne or airborne pathogens
- It is difficult to control because it is a long-lived pathogen that is not host-specific, and the resistance is controlled by multiple genes
- Wild *Helianthus* species are important genetic resources for the improvement of cultivated sunflower

53 *Helianthus* Species

14 Diploid annuals (2n=2x=34)

26 Diploid perennials (2n=2x=34)

3 Tetraploid perennials (2n=4x=68)

7 Hexaploid perennials (2n=6x=102)

1 Mixploid perennials (2n=2x=34, 4x=68)

2 Mixploid perennials (2n=4x=68, 6x=102)

Wild *Helianthus* species resistant to sunflower diseases

Diseases	Wild species sources
Rust	*H. annuus*; *H. petiolaris*; *H. argophyllus*
Downy mildew	*H. annuus*; *H. petiolaris*; *H. praecox*; *H. hirsutus*; *H. laevigatus*; *H. californicus*; *H. ciliaris*; *H. pauciflorus*; *H. resinosus*; *H. strumosus*; *H. tuberosus*; *H.* x *laetiflorus*; *H. smithii*
Sclerotinia	*H. agrestis*; *H. argophyllus*; *H. debilis*; *H. neglectus*; *H. petiolaris*; *H. praecox*; *H. californicus*; *H. ciliaris*; *H. eggertii*; *H. pauciflorus*; *H. resinosus*; *H. tuberosus*; *H. hirsutus*; *H. maximiliani*; *H. nuttallii*; *H. giganteus*; *H. grosseserratus*; *H. salicifolius*; *H. smithii*
Phomopsis brown stem canker	*H. debilis*; *H. argophyllus*; *H. pauciflorus*; *H. tuberosus*; *H. hirsutus*; *H. pauciflorus*; *H. maximiliani*; *H. nuttallii*; *H. mollis*; *H. occidentalis*; *H. divaricatus*; *H. resinosus*; *H. strumosus*
Alternaria leaf spot	*H. praecox*; *H. debilis* subsp. *cucumerifolius*; *H. debilis* subsp. *silvestris* *H. tuberosus*; *H.* x *laetiflorus*
Powdery mildew	*H. debilis* subsp. *silvestris*; *H. praecox* subsp. *praecox*; *H. bolanderi*; 14 perennials
Rhizopus head rot	*H. divaricatus*; *H. hirsutus*; *H.* x *laetiflorus*; *H. resinosus*
Phoma black spot	*H. argophyllus*; *H. maximiliani*; *H. tuberosus*; *H. pauciflorus*
Charcoal rot	*H. tuberosus*; *H. mollis*; *H. maximiliani*; *H. resinosus*; *H. pauciflorus*
Broomrape	*H. anomalus*; *H. exilis*; all perennial species

Note: Wild annuals are in black, and wild perennials are in red.

Sources: Block CC et al. American Phytopathological Society Abstracts 102:S4.12 (2012)
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OBJECTIVES

- Identify resistant wild perennial *Helianthus* species populations and interspecific amphiploids
- Transfer resistance genes into a cultivated background
- Study the inheritance of resistance
- Release Sclerotinia head and stalk rot resistant germplasm

MATERIALS

- **Phase 1 (since 2004)**
  - 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
- **Phase 2 (since 2011)**
  - 1 hexaploid (backcrossed with HA 410)
    H. resinosus
  - 1 tetraploid (backcrossed with HA 410, HA451, NMS HA 89)
    H. hirsutus
  - 4 diploids (backcrossed with HA 410, HA451, NMS HA 89)
    H. salicifolius, *H. occidentalis* subsp. *plantagineus*, *H. silphioides*, and *H. divaricatus*

- **Phase 3 (since 2013)**
  - 2 hexaploids (backcrossed with HA 410, NMS HA 89)
    H. strumosus and *H. tuberosus*
  - 1 tetraploid (backcrossed with HA 410, NMS HA 89)
    H. decapetalus
  - 1 diploid (backcrossed with HA 410, NMS HA 89)
    H. simulans
- **Phase 4 (added in 2016)**
  - 1 amphiploid (backcrossed with HA 410, NMS HA 89)
    H. atrorubens/HA 89
  - 3 hexaploids (backcrossed with HA 410, NMS HA 89)
    H. smithii, *H. laevigatus*, and *H. pauciflorus* (*rigidus*)

Note: (1) For most species, 2-3 accessions were used.
(2) The materials in red were continued in 2016.

METHODS

- Traditional crossing and backcrossing
- Embryo rescue
- Mitotic chromosome counting
- Pollen fertility examination
- Seed increase in field and greenhouse
- Head and stalk rot field test
- Stalk rot greenhouse test
- GISH and FISH
- Molecular markers: SSR, SNPs

RESULTS AND DISCUSSION

Table 1. Seed increases for progeny families in 2012-2016

Sources	2012	2013	2014	2015	2016
Hexaploid	32	36	52	39	20
Diploids-HA 410	55	53	6	110	38
Diploids-HA 441	120	36	96	72	24
Amphiploids	34	42	17	157	16
New diploids*		370	19	108	21
New tetraploids*		60	4	66	1
New hexaploids*					32
Total	241	597	194	552	152

* Phase 2 and 3 crosses.

• Seed increase started in 2008.

Table 2. Field test of progeny families in 2012-2016

Sources	Stalk rot (SR)					Head rot (HR)				
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Hexaploid	65	43	39	9	9	27	1	1	0	0
Diploids-HA 410	56	27	25	18	18	45	11	11	6	3
Diploids-HA 441	121	51	51	5	5	90	41	41	14	7
Amphiploids	42	18	16	7	7	11	0	0	0	0
New diploids			368	318	30			110	316	35
New tetraploids			60	54	6			0	0	0
Total	284	139	559	411	75	173	53	163	336	45

- Disease evaluations started in 2009.

Table 3. Third year test of materials from Phase I crosses for head rot in 2016

Pedigree*	Carrington, ND 2016		Staples, MN 2016	
	Disease Severity	Disease Incidence	Disease Severity	Disease Incidence
TEST 2 (Second Retest)	0-5	%	0-5	%
((NMS HA 89 x GRO PI613793) HA 410*2), BC2F3 (47)	3.50	83	1.76	42
((NMS HA 89 x GRO PI613793) HA 410), BC2F3 (66)	1.83	38	1.94	44
((NMS HA 89 x GRO PI613793) HA 410), BC1F4 *new 69*	2.89	65	2.96	69
Recurrent parent HA 410	**4.88**	**92**	**3.71**	**89**
((NMS HA 89 x 1323(MAX) x HA 441), BC1F5 (7)	0.94	22	0.92	25
((NMS HA 89 x 1324(NUT) x HA 441), BC1F5 (8)	0.84	24	1.36	34
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F4 (9)	2.54	59	1.32	33
((NMS HA 89 x 1018 (MAX)) x HA 441), BC1F6 (10)	1.34	37	0.93	20
((NMS HA 89 x1324 (NUT) x HA 441), BC1F5 (11)	1.13	40	0.37	11
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441), BC2F4 (1	1.60	47	2.13	52
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441), BC2F4 (16)	2.58	55	1.82	54
Recurrent parent HA 441	**1.39**	**42**	**2.15**	**57**
Checks				
Susceptible check HA 89 (S)	**3.86**	**83**	**3.26**	**82**
Susceptible check Cargill/Mycogen (270/272) (S)	**2.13**	**54**	**4.04**	**82**
Resistant check Croplan 305 (R)	**1.86**	**41**	**1.95**	**47**
Resistant check Croplan 343 (R)	**0.87**	**3**	**0.95**	**27**

Entries close to or better than resistant check

Entries close to or better than recurrent parent

Table 4. Second year test of materials from Phase 2 crosses for head rot in 2016

Pedigree*	Carrington, ND 2016		Staples, MN 2016	
	Disease Severity	Disease Incidence	Disease Severity	Disease Incidence
TEST 3 (First Retest)	0-5	%	0-5	%
NMS HA 89 x (SAL x HA 410), F2 (1011)	1.94	52	1.18	30
NMS HA 89 x (SAL x HA 410), F2 (1014)	2.20	51	1.61	43
NMS HA 89 x (SAL x HA 410), F2 (1063)	4.17	85	3.08	77
NMS HA 89 x (SAL x HA 410), F2 (1069)	3.89	84	2.26	58
NMS HA 89 x (SAL x HA 410), F2 (1072)	1.69	44	1.40	36
NMS HA 89 x (SAL x HA 410), F2 (1074)	2.23	51	2.50	53
NMS HA 89 x (SAL x HA 410), F2 (1077)	3.94	82	3.67	89
NMS HA 89 x (OCC x HA 410), F2 (1047)	1.74	40	1.91	43
NMS HA 89 X (OCC X HA 410), F2 (1085)	3.68	84	1.61	41
NMS HA 89 X (OCC X HA 410), F2 (1097)	3.50	73	2.60	56
NMS HA 89 X (OCC X HA 410), F2 (1098)	4.59	95	3.24	79
Recurrent parent HA 410	**4.41**	**90**	**2.68**	**68**
Checks				
Susceptible check HA 89 (S)	**3.86**	**83**	**3.26**	**82**
Susceptible check Cargill/Mycogen (270/272) (S)	**2.13**	**54**	**4.04**	**82**
Resistant check Croplan 305 (R)	**1.86**	**41**	**1.95**	**47**
Resistant check Croplan 343 (R)	**0.87**	**3**	**0.95**	**27**

Table 5. First year test of materials from Phase 2 crosses for head rot in 2016

Pedigree*	Carrington, ND 2016		Staples, MN 2016	
	Disease Severity	Disease Incidence	Disease Severity	Disease Incidence
TEST 4 (New Selections)				
NMS HA 89 x (SAL x HA 410), F2 (1120)	2.59	47	1.90	44
NMS HA 89 x (SAL x HA 410), F2 (1121)	2.00	52	0.69	21
NMS HA 89 x (SAL x HA 410), F2 (1137)	4.05	86	2.97	71
NMS HA 89 x (SAL x HA 410), F2 (1138)	4.12	98	2.13	68
NMS HA 89 x (SAL x HA 410), F2 (1139)	4.25	85	2.19	54
NMS HA 89 x (OCC x HA 410), F2 (1163)	2.39	54	2.18	48
NMS HA 89 x (OCC x HA 410), F2 (1195)	3.53	75	2.04	51
NMS HA 89 x (OCC x HA 410), F2 (1208)	1.92	58	0.68	51
Recurrent parent HA 410	**4.24**	**85**	**2.73**	**60**
Checks				
Susceptible check HA 89 (S)	**3.86**	**83**	**3.26**	**82**
Susceptible check Cargill/Mycogen (270/272) (S)	**2.13**	**54**	**4.04**	**82**
Resistant check Croplan 305 (R)	**1.86**	**41**	**1.95**	**47**
Resistant check Croplan 343 (R)	**0.87**	**3**	**0.95**	**27**

*The first three letters of the *Helianthus* species are used to identify the species source: GRO=*H. grosseserratus*; MAX=*H. maximiliani*; NUT=*H. nuttallii*; SAL=*H. salicifolius*; and OCC= *H. occidentalis*.
(S)= Susceptible; (R)=Resistant. The number in parentheses at the end of each pedigree is the family ID of the selected family.

Table 6. Backcrosses between wild perennials and cultivated sunflower-Phase 3

Parentage	BC_1F_1 (2014 and 2016)				BC_2F_1 (2015)			
	Plants	Fertility %	BC_2F_1 seeds	Seed set %	Plants	Fertility %	BC_3F_1/BC_2F_2 seeds	Seed set %
H. strumosus (PI 547217) × HA 410	29	3.01	24[a] 3[b]	0.13 0.05	7	56.01	290	9.67
H. strumosus (PI 547226) × HA 410	25	4.48	9[a] 0[b]	0.12 0.00	3	77.49	43	2.90
H. tuberosus (PI 547242) × HA 410	22	7.48	11[a] 8[b]	0.14 0.15	4	7.80	34	1.77
H. tuberosus (PI 650089) × HA 410	21	3.07	64[a] 14[b]	0.91 0.24	32[a] 9[b]	- 33.56	Many 527	76.78 12.61
H. tuberosus (PI 650105) × HA 410	20	5.00	23[a] 901[b]	0.28 14.18	4[a] 6[b]	15.28 -	56 50	3.86 2.59

[a] Obtained by crossing HA 410 pollen to BC_1F_1 plants.
[b] Obtained by crossing BC_1F_1 pollen to NMS HA 89.

Backcrosses between wild perennials and cultivated sunflower-Phase 4

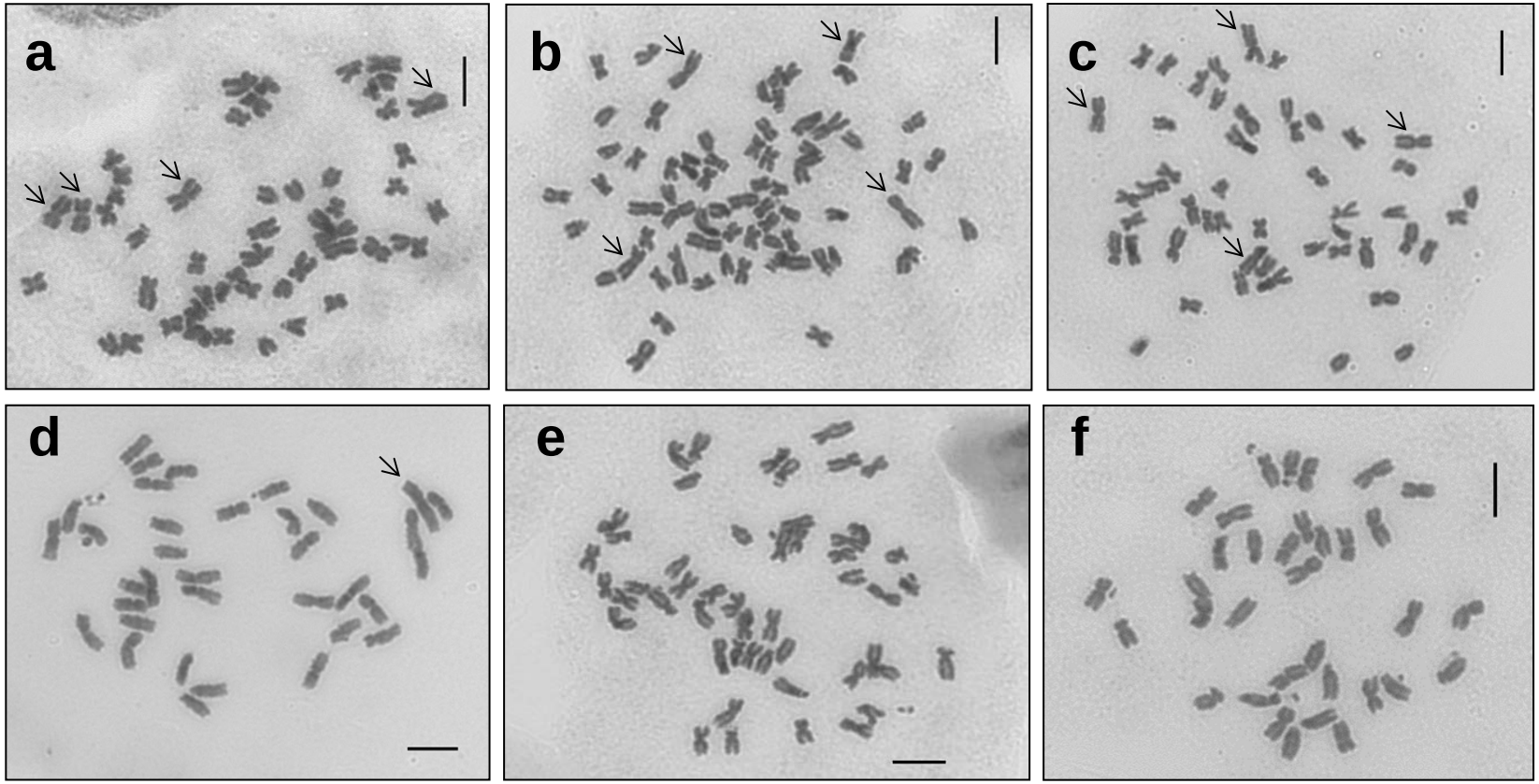

Figure 1. Chromosome spreads of several backcross progenies derived from *H. atrorubens* (a-d) and *H. laevigatus* (e and f). (a) G95/72 x HA 89, BC_1F_1, 2n=51; (b) G16/1039 x HA 410, BC_1F_2, 2n=68; (c) G16/1039 x HA 410, BC_2F_1, 2n=49; (d) NMS HA 89 x G16/1036-46, BC_2F_1, 2n=35; (e) G16/1053 x HA410, BC_2F_1, 2n=50; (f) NMS HA 89 x G16/1053-64, BC_2F_1, 2n=34. Notice that there are large chromosomes (arrows) from *H. atrorubens* in the cultivated background in Figures a-d. Bars=5 µm.

Table 7. Sclerotinia resistance germplasms crossed with HA 234 for RIL population development

Germplasms	Source	Pedigree for female parent	Selfed/Sib seed set (%)	Crossed seed set (%)
HR-MAX	09/4008	(NMS HA 89 x 1018 (MAX)) x HA 441^3, BC3F3	43.3	55
HR-NUT	09/4041	(NMS HA 89 x 1008 (NUT)) x HA 441, BC1F5	65	56.7
BSR-MAX	09/4011	(NMS HA 89 x 1018 (MAX)) x HA 441^3, BC3F3	15	26.9
BSR-NUT	10/4144	(NMS HA 89 x 1008 (NUT)) x HA 441, BC1F4	55	67
BSR-DIV	11/4484	[(DIV(68) x GRO (68)) x HA 410^3, BC2F2] x HA 410, BC3F2	43	65
BSR-STR	11/4489	[STR (68) x HA 410^3, BC2F2] x HA 410, BC3F2	51	55
BSR-CAL	09/4271	CAL 2376 x HA 410^5, BC4F4	-	20

(1) The germplasm release for these resistant sources are in progress.
(2) Root tips, leaf samples and pollen were collected from these sources.
(3) 1-3 plants from each resistant source were emasculated and crossed with cultivated HA 234 in 2016.

Table 8. Resistant and susceptible entries to stalk rot derived from *H. californicus* (CAL) for GISH and/or GBS analyses

Entry #	Source	Pedigree	SR Score (%)	Selfed seed set (%)
28	09/4104	CAL 2376 x HA 410^5, BC4F4	0	25.00
216	10/4386	CAL 2376 x HA 410^5, BC4F4	0	67.5
235	11/4470	CAL 2376 x HA 410^5, BC4F4	0	61.25
258	11/4478	CAL 2376 x HA 410^5, BC4F4	0	27.54
304	09/4306	CAL 2376 x HA 410^5, BC4F4	0	18.33
29	09/4106	CAL 2376 x HA 410^5, BC4F4	8-63	40.00
30	09/4110	CAL 2376 x HA 410^5, BC4F4	0-67	41.25
32	09/4114	CAL 2376 x HA 410^5, BC4F4	0-56	45.00
41	09/4163	CAL 2376 x HA 410^5, BC4F5	0-50	47.50
228	11/4466	CAL 2376 x HA 410^5, BC4F4	33-45	34.21
317	11/4497	CAL 2376 x HA 410^5, BC4F5	7-46	72.50

(1) These entries were selected based on field screening in 2009-2015.
(2) Root tips were collected for GISH analysis.
(3) Leaf samples were collected for GBS analysis.

SUMMARY

- In the last 12 years, the project has utilized 21 wild perennials, produced ~3100 progeny families, tested ~2500 families for stalk rot, ~1400 families for head rot in the field (including retests)
- The germplasm release for seven Sclerotinia resistant bulks are in progress
- In 2016, field evaluation identified several families derived from six diploid perennials with good resistance to head rot
- Seed was increased in the field for more than 150 progeny families
- The BC_1F_1 progenies (2n=51) derived from four perennials were advanced to the BC_2F_1 generation
- RIL population development was initiated for Sclerotinia resistance QTL mapping

FUTURE WORK

- ✓ Thirteen amphiploids derived from eight wild perennial species will be released as germplasms
- ✓ Additional families with better resistance than the recurrent parents identified in the different trials will be retested in 2017
- ✓ Continue to backcross the progenies derived from different sources to reduce 2n to 34
- ✓ Continue to develop QTL mapping populations by RIL and doubled-haploid approaches
- ✓ GISH and GBS comparisons between resistant and susceptible progenies derived from *H. californicus*
- ✓ Identify chromosome addition lines, and characterize alien chromosomes or fragments in cultivated background utilizing GISH and FISH techniques and molecular markers

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