Progress on the transferring Sclerotinia resistance genes from wild perennial *Helianthus* species into cultivated sunflower

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Background

- Sclerotinia is one of the most damaging and difficult-to-control sunflower diseases, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary
- Cultivated sunflower lacks sufficient genes for Sclerotinia resistance, but wild perennial *Helianthus* species are highly resistant
- Crosses and backcrosses for introgression of the resistance genes from hexaploid, tetraploid, diploid wild species and interspecific amphiploids into cultivated sunflower (HA 441 or HA 410 as the recurrent parents)
- Eleven accessions from five wild perennial and one annual *Helianthus* species have been established in the greenhouse in 2010

Objectives

- Incorporate resistance genes from diverse wild species into cultivated backgrounds
- Field evaluation to identify new materials with resistance genes to Sclerotinia
- Genetic study of resistance and QTL mapping

Materials and Methods

- Seed increase and field test for stalk and head rots for the materials obtained from earlier crosses
- New interspecific crosses using embryo rescue for 11 accessions of six wild *Helianthus* species
  - Mitotic chromosome counting and pollen fertility examination of the F1 plants
  - Backcross and embryo rescue of BC1 seedlings
- Genetic analysis and SSR marker detection of the addition lines obtained from former crosses

Results and Discussion

Seed increase and field test

- Hexaploids backcrossed with HA 410

  H. californicus and *H. schweinitzii*

- Diploids backcrossed with HA 410

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

- Diploids backcrossed with HA 441

  H. nuttallii and *H. maximiliani*

- Amphiploids backcrossed with HA 410

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

Hexaploid species

2004 ***H. californicus*** 2n=6x=102 × **HA 410** 2n=34

Embryo rescue

2005 **F_1** (2n=68) × HA 410

2006 **BC_1F_1** (2n=51) × HA 410

2006 **BC_2F_1** (2n=40-49) × HA 410

2007 **BC_3F_1** (2n=35-44) × HA 410

2007 **BC_4F_1** (2n=34-40) × HA 410

⊗

2008 **BC_4F_2** (2n=34-37)

(86 Lines 2n=34, 77 Lines 2n=35)

⊗

2008 **BC_4F_3** (2n=34-37)

(46 Lines 2n=34, 35 Lines 2n=35)

⊗

2009

154 Lines for seed increase in field

6 Lines for seed increase in greenhouse

72 Lines for field test

2009

BC_4F_3 (2n=34-37)

⊗

2010

112 Lines for seed increase in field

76 Lines for stalk rot field test 1

16 Lines for stalk rot field test 2

51 Lines for head rot field test 2

⊗

2011

37 Lines for seed increase in field

69 Lines for stalk rot field test 1

16 Lines for stalk rot field test 2

52 Lines for head rot field test 2

H. schweinitzii 2n=6x=102 × **HA 410** 2n=34

Embryo rescue

F_1 (2n=68) × HA 410

BC_1F_1 (2n=49-135) × HA 410

BC_1F_2 × HA 410

145 seeds from 2n=49 -53

550 seeds from 2n=68

0 seed from 2n=134 -135

BC_2F_1

26 seedlings with 2n=49 -53

⊗ ×HA 410

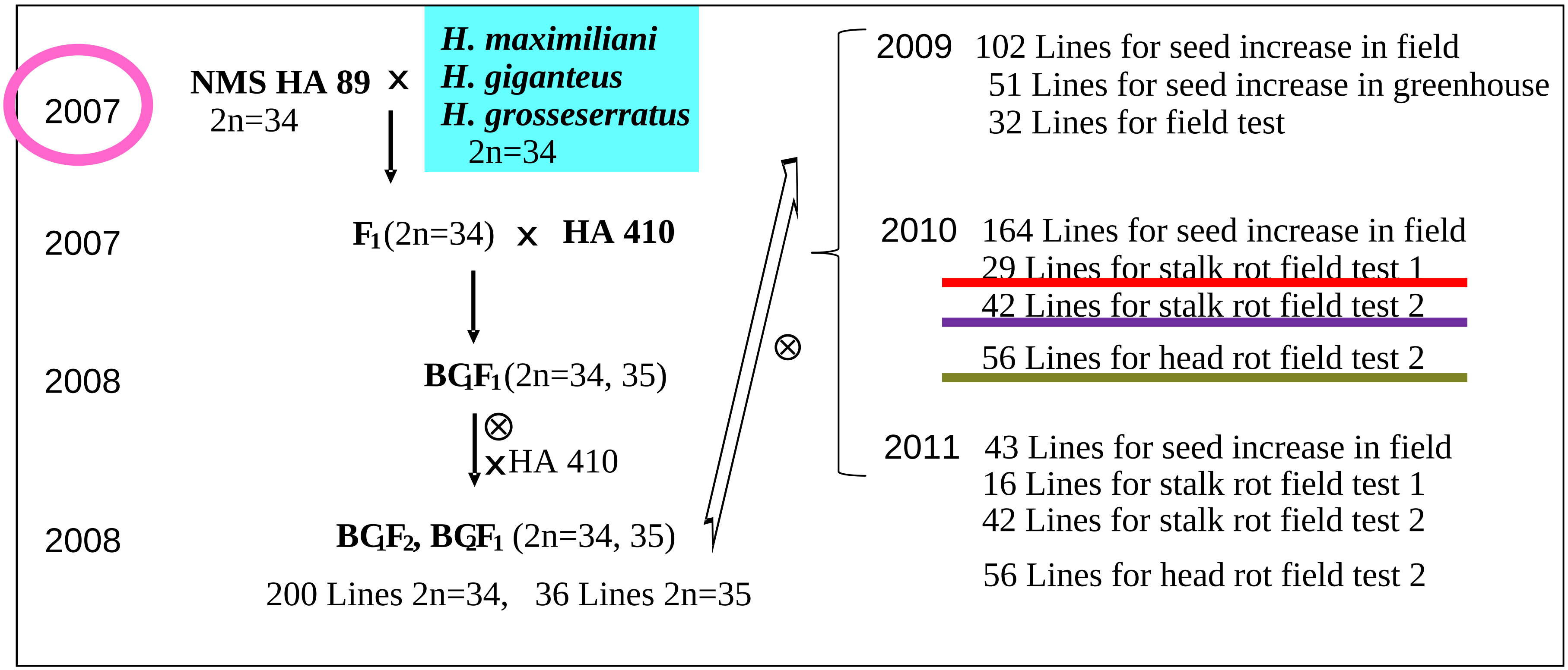
43 plants with 2n=34 -72

⊗ ×HA 410

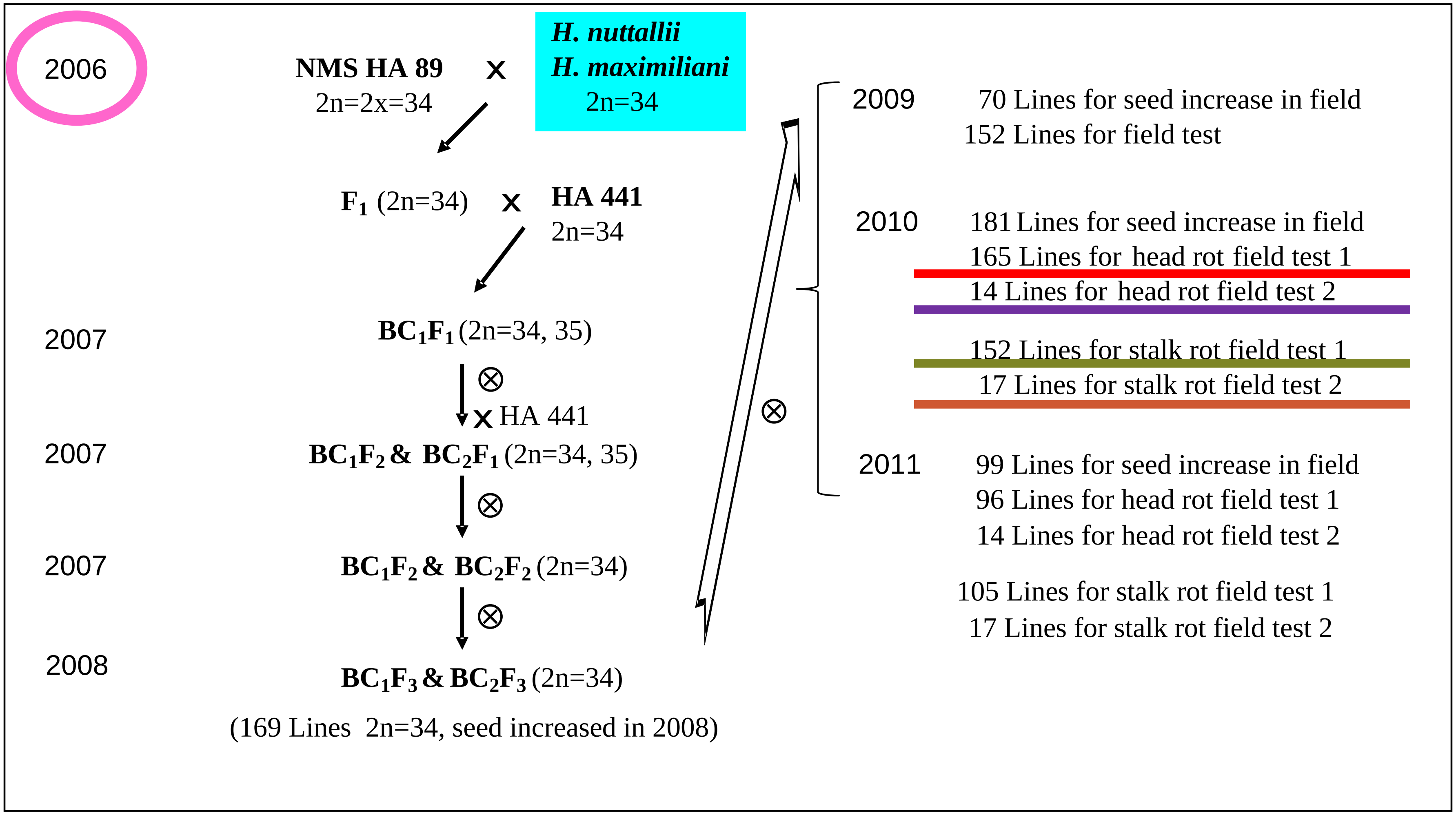
BC_3F_2 & BC_4F_1

47 seedlings

Diploid species (HA 410)



Diploid species (HA 441)



Interspecific amphiploids

2005
***H. nuttallii* /P21,** 2n=68
***H. maximiliani* /P21,** 2n=68
***H. gracilenatus* /P21,** 2n=68
***H. grosseserratus* /P21,** 2n=68
***H. strumosus* /P21,** 2n=102

x **HA 410** 2n=2x=34

2006 **F_1** (2n=51, 68) x HA 410

2007 **BC_1F_1** (2n=35-44) x HA 410

2007 **BC_2F_1** (2n=34-37)
(43 Lines 2n=34, 29 Lines 2n=35)

2008 ⊗, x HA 410

2008 **BC_2F_2, BC_3F_1** (2n=34-36)
(106 Lines 2n=34, 58 Lines 2n=35)

⊗

2009
118 Lines for seed increase in field
6 Lines for seed increase in greenhouse
77 Lines for field test

2010
75 Lines for seed increase in field
61 Lines for stalk rot field test 1
20 Lines for stalk rot field test 2
23 Lines for head rot field test 2

2011
40 Lines for seed increase in field
39 Lines for stalk rot field test 1
20 Lines for stalk rot field test 2
22 Lines for head rot field test 2

Field Sclerotinia head rot evaluations at Carrington, ND in 2009 with 163 entries and Staples, MN in 2011 with 87 entries replicated twice, plus two susceptible checks, HA 89 and Cargill 270, two resistant checks Croplan 343 and Croplan 305, and the recurrent parent HA 441 each year

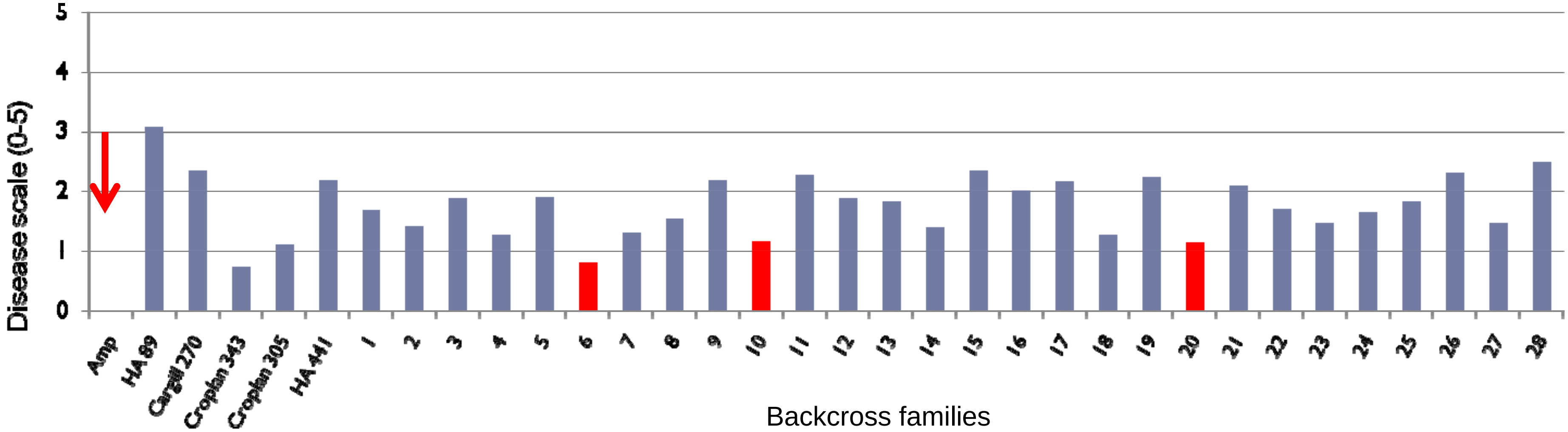
Pedigree	Plant Disease Rating (No. of Entries)						
(NMS HA 89 x Nut 1008) x HA 441,BC1F4-BC3F3 **(2009)**	1.68 (6)	1.41 (8)	1.87 (3)	1.25 (1)	1.90 (1)	0.80 (2)	1.29 (13)
(NMS HA 89 x Nut 1008) x HA 441, BC1F4-BC3F3 **(2011)**	1.07 (3)	2.21 (11)	1.03 (3)	0.63 (1)	1.64 (3)		
(NMS HA 89 x Max 1018) x HA 441, BC2F2-BC3F2 **(2009)**	1.52 (6)	2.17 (8)	1.15 (4)	2.26 (2)	1.87 (1)	1.82 (1)	1.38 (2)
“ **(2009)**	2.33 (1)	2.00 (15)	2.15 (3)				
(NMS HA 89 x Max 1018) x HA 441, BC2F2-BC3F2 **(2011)**	1.77 (5)	2.72 (2)	2.47 (8)	1.55 (5)	0.20 (3)	1.83 (2)	
(NMS HA 89 x Max 1314) HA 441, BC1F4-BC3F2 **(2009)**	1.26 (5)	2.23 (7)					
(NMS HA 89 x Max 1314) HA 441, BC1F4-BC3F2 **(2011)**	0.75 (3)	1.04 (3)					
(NMS HA 89 x Max 1323) HA 441, BC1F4-BC3F3 **(2009)**	1.13 (6)	2.07 (10)	1.70 (9)				
(NMS HA 89 x Max 1323) HA 441, BC1F4-BC3F3 **(2011)**	3.13 (1)	2.56 (5)	2.40 (4)	3.12 (3)	2.75 (2)		
(NMS HA 89 x Nut 1324) HA 441, BC1F4-BC2F4 **(2009)**	1.46 (3)	1.64 (17)	1.83 10)	2.29 (3)	1.45 (1)	2.47 (15)	
(NMS HA 89 x Nut 1324) HA 441, BC1F4-BC2F4 **(2011)**	2.22 (4)	2.83 (1)	3.13 (2)	2.08 (3)	2.50 (1)	2.28 (9)	

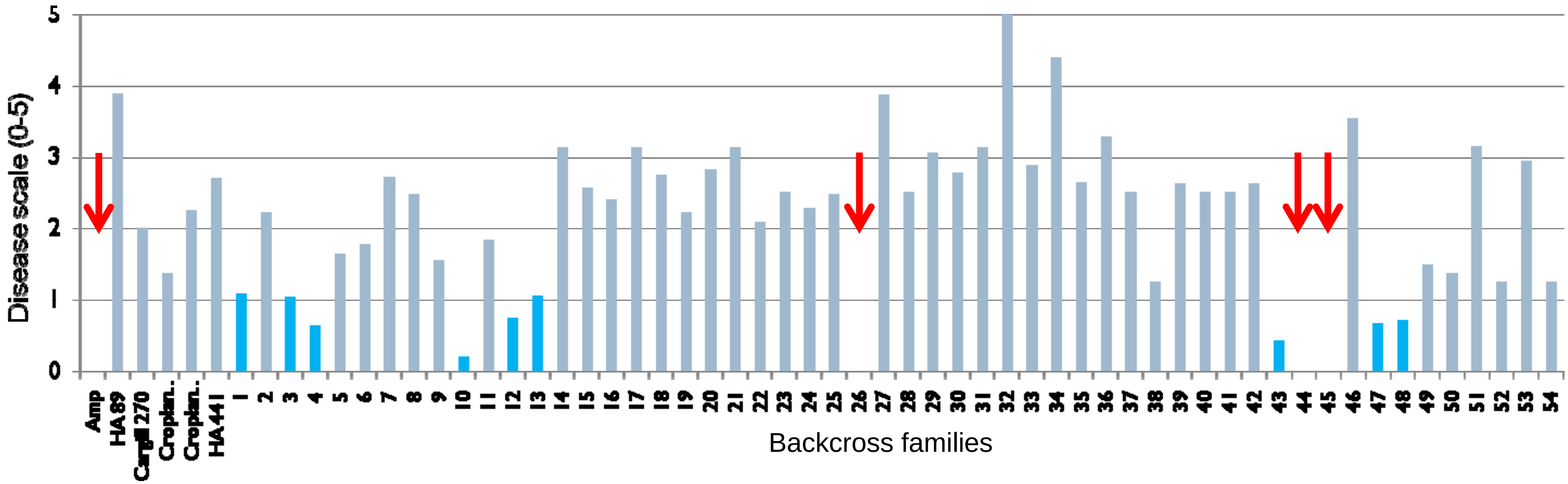
Infection of susceptible checks HA 89 (3.07; 3.88) and Cargill 270 (2.33; 2.00), resistant checks Croplan 343 (0.73; 1.37) and Croplan 305 (1.09; 2.24), recurrent parent HA 441 (2.16; 2.70), and the bulk amphiploid (Amp) (0; 0). The first 3 letters of the *Helianthus* species are used followed by an accession number. Checks for 2009 are listed first in parentheses followed by 2011 highlighted in yellow.

Field Sclerotinia head rot evaluation of new interspecific combinations at Staples, MN in 2011, with 120 entries replicated twice, plus two susceptible checks, HA 89 and Cargill 270, two resistant checks, Croplan 343 and Croplan 305, and the recurrent parent HA 441

Pedigree	Plant Disease Rating (No. of Entries)				
((Div 68 x Gro 68, Amp) HA410, BC2F2	2.48 (3)				
(Gro 68 x P21, Amp) HA 410, BC3F2	0 (1)				
(Max 68 x P21, Amp) HA 410, BC2F2	3.87 (2)	2.50 (1)			
(Nut 68 x P21, Amp) HA 410, BC2F2	3.05 (3)	2.77 (3)			
(Str 68 x P21, Amp) HA 410, BC2F2	3.12 (6)	5.00 (2)			
Cal 2376 x HA 410, BC4F2-BC5F3	2.88 (28)	4.38 (2)	2.64 (6)	3.28 (3)	2.50 (1)
(NMS HA 89 x Nut 1008) x HA 441, BC1F4-BC2F3	1.25 (4)				
(NMS HA 89 x Nut 1324) x HA 441, BC1F4-BC2F4	2.62 (1)	2.50 (1)			
(NMS HA 89 x Max 1018) x HA 441, BC1F5-BC2F4	2.50 (1)	2.62 (1)	0.42 (1)	0 (1)	
(NMS HA 89 x Max 1323) x HA 441, BC1F4-BC3F3	0 (1)				
(NMS HA 89 x Gig PI 547182) HA 410, BC2F2	3.53 (6)	0.67 (3)			
(NMS HA 89 x Gro PI 613793) HA 410, BC1F3	0.72 (8)	1.48 (17)	1.36 (2)	3.14 (3)	
(NMS HA 89 x Max PI 586892) HA 410, BC1F3-BC2F2	1.25 (3)	2.95 (4)	1.25 (2)		

Field Sclerotinia head rot evaluations at Carrington, ND in 2009 and Staples, MN in 2011





New interspecific crosses involving 11 accessions of five perennial and one annual *Helianthus* species

No.	Accession	Species	2n	Physiological type
1	PI 547171	*H. hirsutus*	68	Perennial
2	PI 547174	*H. hirsutus*	68	Perennial
3	Ames 30340	*H. salicifolius*	34	Perennial
4	Ames 30348	*H. salicifolius*	34	Perennial
5	PI 494594	*H. occidentalis* subsp. *plantagineus*	34	Perennial
6	Ames 30317	*H. occidentalis* subsp. *plantagineus*	34	Perennial
7	Ames 30356	*H. silphioides*	34	Perennial
8	Ames 30354	*H. silphioides*	34	Perennial
9		*H. resinosus*	102	Perennial
10	PI 468415	*H. agrestis*	34	Annual
11	PI 468416	*H. agrestis*	34	Annual

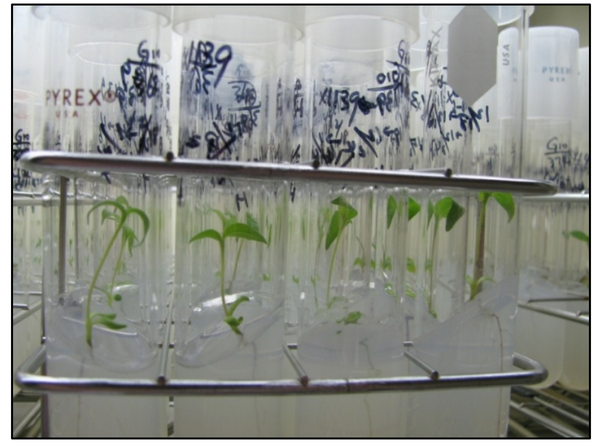
Embryo rescue process for interspecific crosses

Emasculation /pollen collection

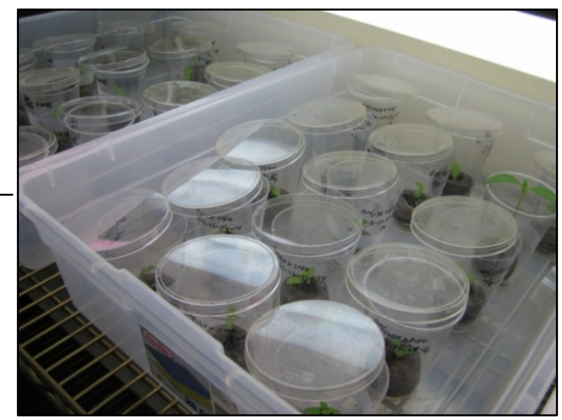
↓

Crossing →

Culture medium #I



Culture medium #II



Jiffy-7

Sunshine mix

Greenhouse

Embryo rescue to obtain the F1 plants

No.	Female	Male	Seeds	Florets	Embryos	F1
1	*H. hirsutus*	HA 410	173	1715	140	27
2	*H. hirsutus*	HA 451	69	446	63	34
3	NMS HA 89	*H. hirsutus*	1492	6044	470	81
4	*H. salicifolius*	HA 410	280	4700	241	26
5	*H. salicifolius*	HA 451	23	795	15	0
6	NMS HA 89	*H. salicifolius*	41	11300	31	5
7	*H. occidentalis*	HA 410	285	1210	217	15
8	*H. occidentalis*	HA 451	269	1060	182	27
9	*H. occidentalis*	*H. nuttallii* 102	10	180	10	4
10	NMS HA 89	*H. occidentalis*	10	3650	4	3
11	*H. resinosus*	HA 451	63	1875	8	8
12	*H. silphioides*	HA 410	277	2540	234	2
13	*H. silphioides*	HA 451	123	2415	110	1
14	*H. silphioides*	*H. nuttallii* 102	161	540	155	96
15	NMS HA 89	*H. silphioides*	88	10960	54	0
16	*H. agrestis*	HA 410, HA 451, and *H. nuttallii* 102	112	1170	111	0
17	NMS HA 89	*H. agrestis*	4	4700	3	0
	Total		3480	55300	2048	329

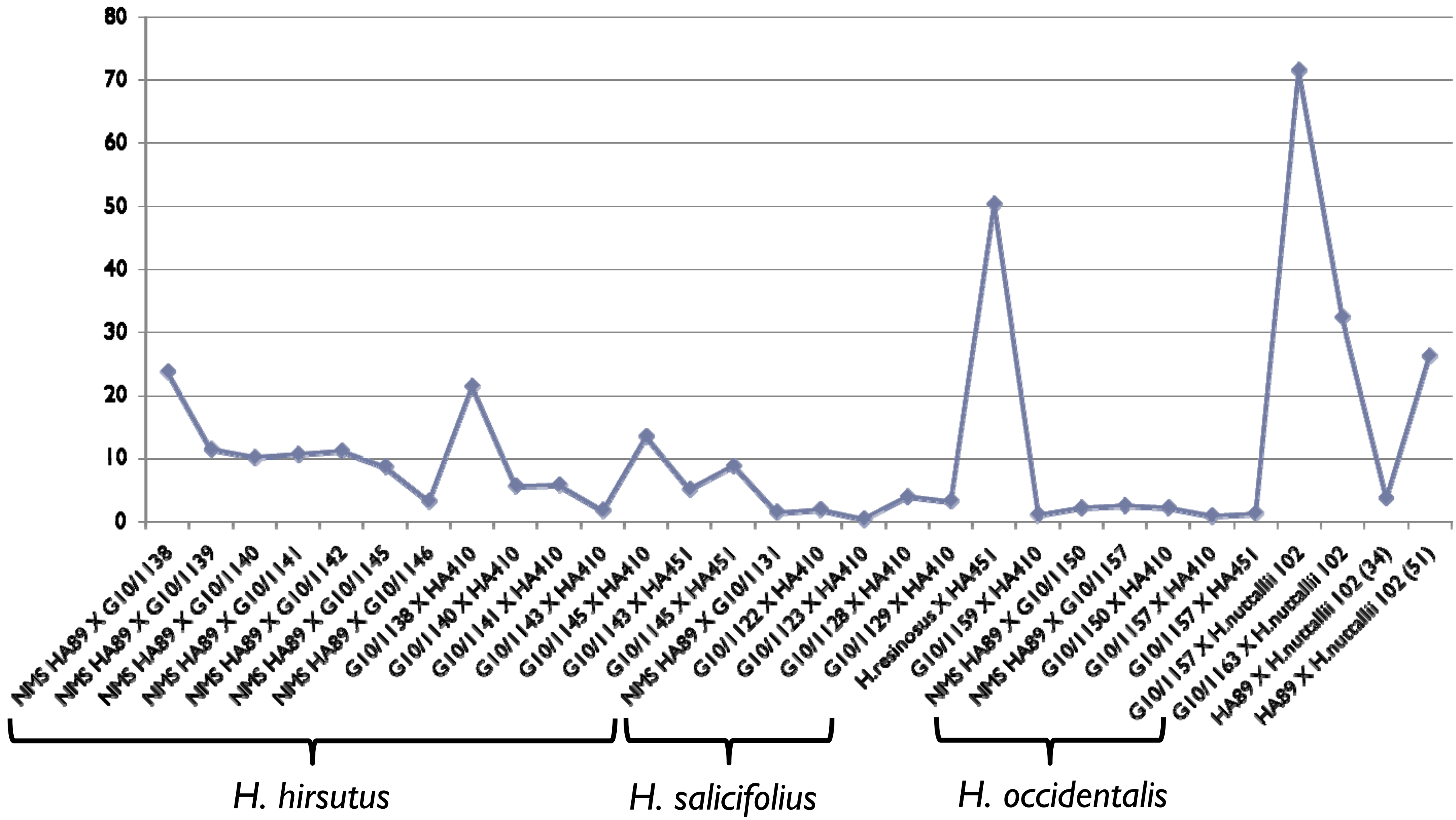
Wild parents and the F1 plants

H. hirsutus

H. salicifolius

H. occidentalis

Comparison of the pollen stainability of the F1s derived from different crosses



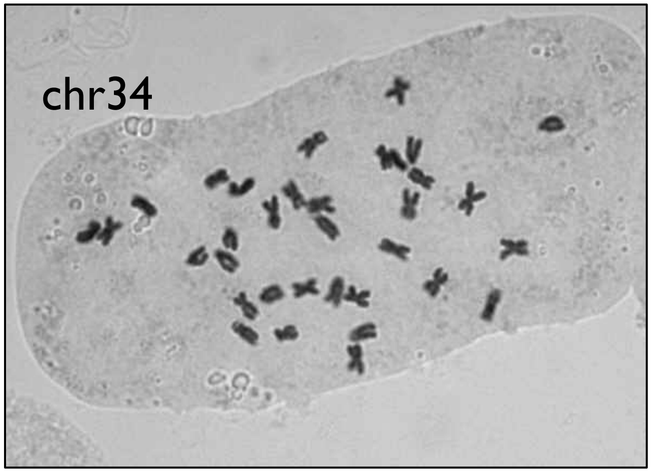
BC seed set of different crosses

Female	Male	Recurrent	Seeds	Florets	Seed set (%)
NMS HA89	*H. hirsutus*	HA 410	67	17954	0.3732
H. hirsutus	HA 410	HA 410	44	6418	0.6856
H. hirsutus	HA 451	HA 410	1	1187	0.0842
H. hirsutus	HA 451	HA 451	98	23750	0.4126
NMS HA 89	*H. hirsutus*	F1-C sib or self	7	19277	0.0363
NMS HA 89 /*H. hirsutus*	*H. hirsutus*/ HA 410	F1 sib	0	14718	0.0000
H. salicifolius	HA 410	HA 410	48	14244	0.3370
NMS HA 89	*H. salicifolius*	HA 410	2	1590	0.1258
H. occidentalis	HA 410	HA 410	10	9225	0.1084
H. occidentalis	HA 451	HA 451	21	5923	0.3546
H. silphoides	HA 410	HA 410	4	670	0.5970
H. silphoides	HA 451	HA 410	1	155	0.6452
Total			303	115111	0.2632

Embryo rescue of BC1 crosses

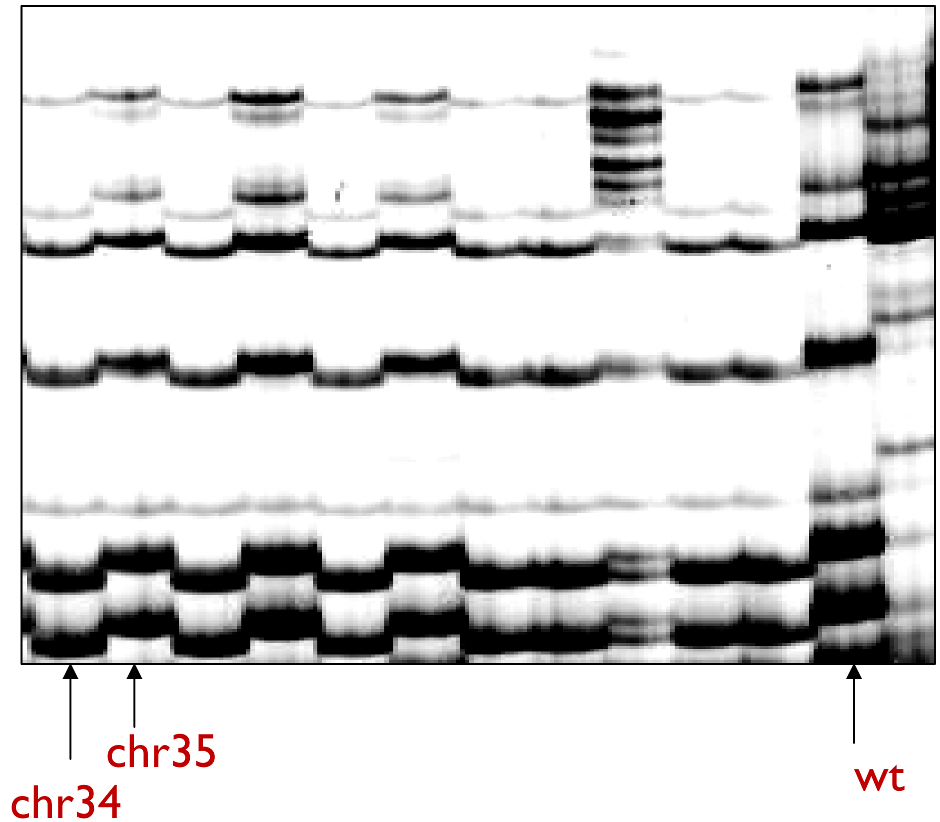
	Female	Male	Recurrent	Seeds	Florets	Embryos	Test tubes
1	*H. hirsutus*	HA 410	HA 410	67	3790	55	26
2	*H. hirsutus*	HA 451	HA 451	19	2450	16	9
3	NMS HA 89	*H. hirsutus*	HA 410	32	3525	30	4
4	NMS HA 89	*H. hirsutus*	HA 451	13	740	8	1
5	*H. salicifolius*	HA 410	HA 410/HA 451	137	6698	88	36
6	NMS HA 89	*H. salicifolius*	HA 410	5	350	5	3
7	*H. occidentalis*	HA 410	HA 410	30	3732	23	6
8	*H. occidentalis*	HA 451	HA 410/HA 451	22	2580	20	9
9	*H. occidentalis*	*H. nuttallii* 102	HA 451	49	700	46	9
10	NMS HA 89	*H. occidentalis*	HA 410	3	350	4	3
11	NMS HA 89	*H. occidentalis*	HA 451	3	250	3	0
12	*H. silphoides*	HA 410	HA 410/HA 451	8	640	5	0
13	HA 89	*H. nuttallii* 102	HA 410	75	470	63	45
14	HA 89	*H. nuttallii* 102	HA 451	212	905	205	102
	Total			675	27180	571	253

Identification of alien chromosome addition lines by linkage group specific SSR markers



93 BC plants from 5 amphiploids were investigated, with 4 polymorphic markers from each LG.

The alien chromosome is related to LG 5



Conclusion and Future work

- Seed increase in 2008-2011 provided sufficient seeds for field evaluation
- Replicated field tests in 2009 and 2011 for head rot resistance identified families with moderate to good resistance indicating successful gene introgression
- The progenies with 2n=34 chromosomes obtained from the crosses will be field evaluated further for both head and stalk rot resistance in 2012

- More than 300 F1 plants were obtained from the crosses between the cultivated sunflower and wild perennial *Helianthus* species, except annual *H. agrestis*
- *H. silphoides* was difficult to cross with cultivated sunflower, but was easier to cross with *H. nuttallii*. *H. nuttallii* could be considered as a bridge parent for the cross
- Continue to identify addition lines, and analyze alien chromosomes or fragments in cultivated background utilizing GISH and FISH

Acknowledgements

Funding: National Sclerotinia Initiatives

Lisa Brown

Alicia Garcia

Dr. Xuelin Fu

Marjorie Olson

Dr. Nikolay Balbyshev

Leonard Cook

Megan Ramsett

Many others …….

Thank you for your attention!