ADVANCEMENT ON TRANSFERRING SCLEROTINIA RESISTANCE GENES FROM WILD *HELIANTHUS* SPECIES INTO CULTIVATED SUNFLOWER

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INTRODUCTION

- Sclerotinia is one of most damaging and difficult-to-control diseases for sunflower
- Cultivated sunflower lacks sufficient genes for Sclerotinia resistance
- Perennial *Helianthus* species are highly resistant to Sclerotinia
- Crosses and backcrosses have been made to introgress the resistance genes from haxaploid, diploid wild species and interspecific amphiploids, using HA 441 or HA 410 as the recurrent parents (Phase 1)
- New crosses and backcrosses have been initiated between six wild *Helianthus* species and cultivated sunflower (Phase 2)

OBJECTIVES

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

PROGRESS-PHASE 1

- 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

Seed increase in 2008-2013

Sources	2008	2009	2010	2011	2012	2013
Hexaploid		160	112	37	32	36
Diploids-HA 410		153	164	43	55	53
Diploids-HA 441	169	70	181	99	120	36
Amphiploids		124	75	40	34	42
Total	169	507	532	219	241	167

Field test in 2009-2013

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

Unexpected adverse field conditions in 2009-2013

	SR	HR
2009	OK	OK
2010	Failed due to midge damage and adverse environmental conditions	Failed due to midge damage and adverse environmental conditions
2011	Hail damage	OK
2012	Good	Good
2013	Discarded, low germination	Fair, low germination

Replicated field Sclerotinia head rot evaluations of 2012 retests (Test 1) and new interspecific crosses (Test 2) at Staples, MN in 2013

Pedigree*	**Disease Rating**	**Percent Infected Plants**
TEST 1 (Retests)	0-5	%
(NMS HA 89 x 1008 (NUT))* x HA 441*3, BC3F2-BC2F4	0	0
((NMS HA 89 x 1018 (MAX)) x HA 441, BC1F4-BC1F6	0	0
((NMS HA 89 x 1018 (MAX)) x HA 441, BC1F2) x HA 441, BC2F2-BC2F4	0.17	2
(NMS HA 89 x 1314 (MAX)) x HA 441*3, BC2F4-BC3F2	0.36	7
(NMS HA 89 x 1323 (MAX)) x HA 441, BC1F5	0.33	8
(NMS HA 89 x 1323 (MAX)) x HA 441*3, BC3F3-BC3F4	0	0
(NMS HA 89 x 1324 (NUT)) x HA 441*2, BC1F5-BC2F2	0.15	9
Amphiploid Bulk	0	0
Recurrent parent HA 441	**0.41**	**14**
Susceptible check HA 89	**0.34**	**9**
Susceptible check Cargill 270	**0.82**	**29**
Resistant check Croplan 305	**0.63**	**15**
Resistant check Croplan 343	**0.12**	**4**

Replicated field Sclerotinia head rot evaluations of 2012 retests (Test 1) and new interspecific crosses (Test 2) at Staples, MN in 2013 (continued)

Pedigree*	**Disease Rating**	**Percent Infected Plants**
TEST 2 (New Selections)		
(NMS HAR9 x GIG=PI 547182) x HA 410*2, BC2F3	0	0
(NMS HA 89 x GRO=PI 613793) x HA 410 , BC1F4	0	0
((NMS HA 89 x GRO=PI 613793) x HA 410*2, BC2F3	0.24	8
CAL 2376 x HA 410*5, BC4F5	0	0
Recurrent parent HA 410	1.2	25
(NMS HA 89 x 1008 (NUT)) x HA 441*2, BC2F4	0	0
(NMS HA 89 x 1018 (MAX)) x HA 441, BC1F6	0.20	5
(NMS HA 89 x 1323 (MAX)) x HA 441, BC1F5	0.36	7
(NMS HA 89 x 1324 (NUT)) x HA 441, BC1F5	0.30	7
Recurrent parent HA 441	**0.41**	**14**
Susceptible check HA 89	**0.34**	**9**
Susceptible check Cargill 270	**0.82**	**29**
Resistant check Croplan 305	**0.63**	**15**
Resistant check Croplan 343	**0.12**	**4**

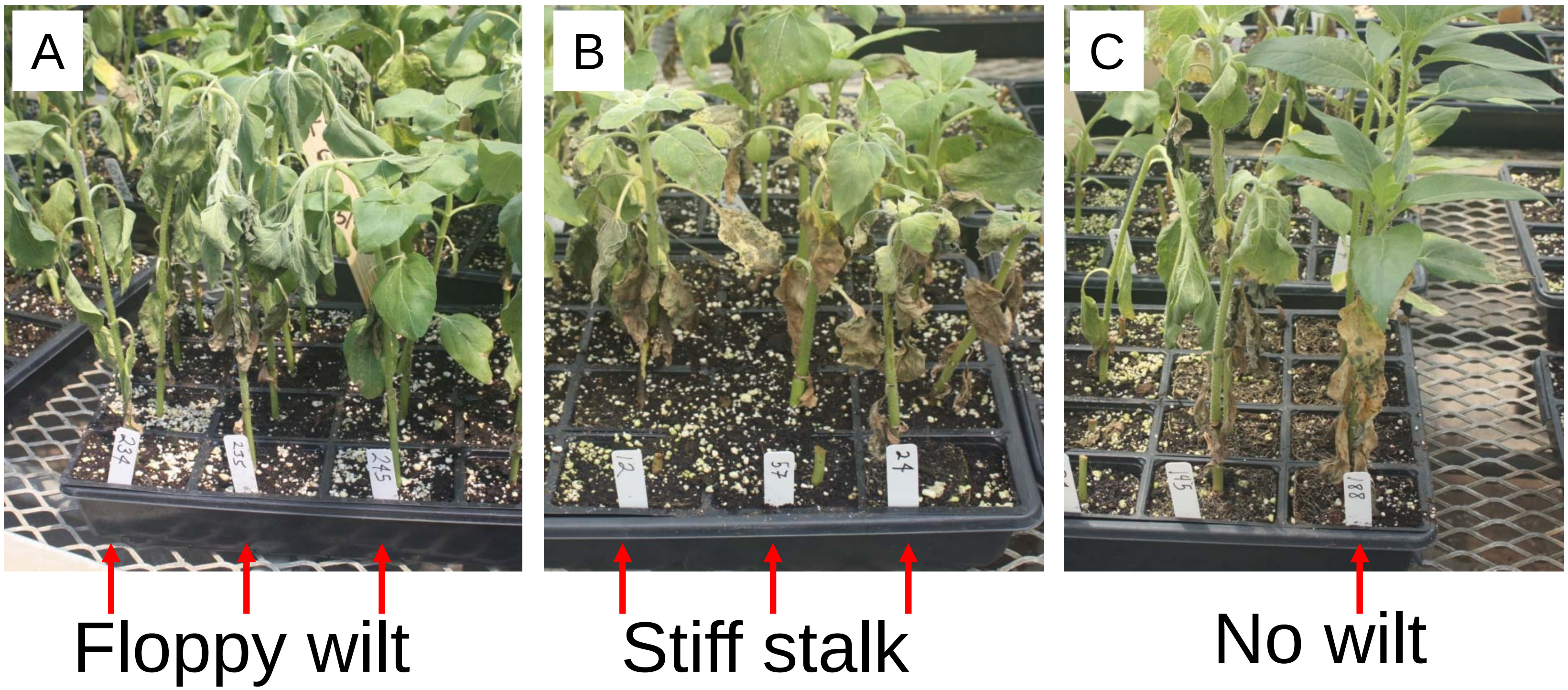
Stalk rot tested in greenhouse

- Materials: 34 families from Test 1 and 17 families from Test 2 of 2012
- Checks:

  Susceptible check: Cargill 270

  Resistant checks: F30294 and Croplan 305
- 1-9 reps (4 plants per rep)-randomized, plants were inoculated at the 6-8 leaf growth stage
- The observation was stopped after 20 days

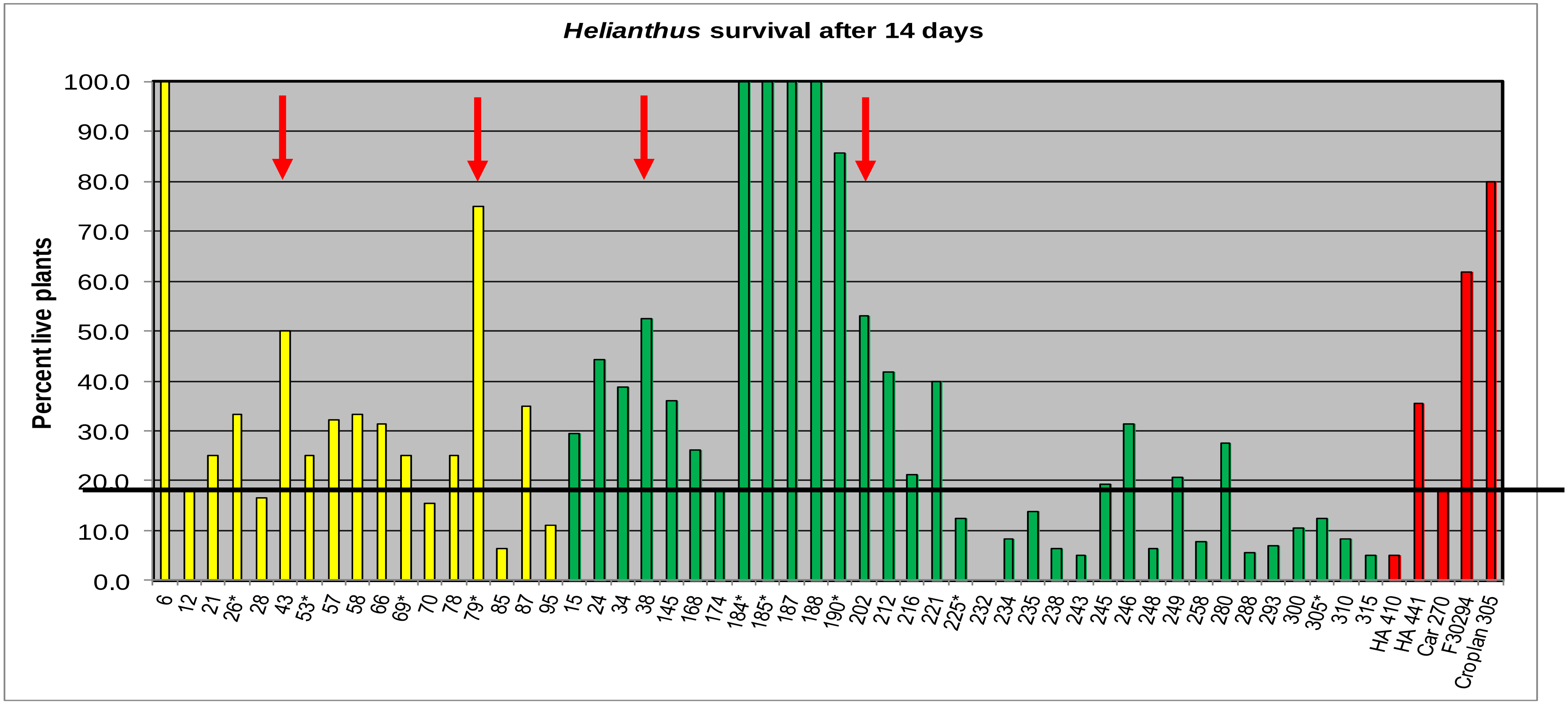
Different reaction types to stalk rot tested in greenhouse



2013 greenhouse screening for stalk rot resistance

Entry	PEDIGREE	Live plants at 14 days		Entry	PEDIGREE	Live plants at 14 days
		%				%
6	AMPHIPLOID BULK	100.0		202	STR (68) x HA 410 (3), BC2F2	53.1
12	{(NMS HA 89 x 1324(N)) x HA 441}, BC1F5	17.9		212	(GRO (68)Sib x HA 410), BC2F1) x HA 410	41.7
21	{(NMS HA 89 x 1323(M)) x HA 441}, BC2F4	25.0		216	{H. CA 2376 x HA 410*5}, BC4F4	21.1
26*	{H.CA 2376 x HA 410*5}, BC4F4	33.3		221	{H. CA2376 x HA 410*5}, BC4F4	40.0
28	{H.CA 2376 x HA 410*5}, BC4F4	16.7		225*	{H. CA 2376 x HA 410*5}, BC4F4	12.5
43	{H.CA 2376 x HA 410*6}, BC5F4	50.0		232	{H.CA 2376 x HA 410*5}, BC4F4	0.0
53*	{(NMS HA 89 x H.GRO=PI 613793)HA 410}, BC1F4	25.0		234	H. CA 2376 x HA 410 (5), BC4F3	8.3
57	{(NMS HA 89 x H.GRO=PI 613793)HA 410*2}, BC2F3	32.1		235	H. CA 2376 x HA 410 (5), BC4F3	13.9
58	{(NMS HA 89 x H.GRO=PI 613793)HA 410*2}, BC2F3	33.3		238	{H. CA 2376 x HA 410*5}, BC4F4	6.3
66	{(NMS HA 89 x H.GRO=PI 416793)HA 410}, BC1F4	31.3		243	H. CA 2376 x HA 410 (5), BC4F3	5.1
69*	{(NMS HA 89 x H.GIG=PI 547182)HA 410}, BC1F4	25.0		245	H. CA 2376 x HA 410 (5), BC4F3	19.4
70	{(NMS HA 89 x H.GIG=PI 547182)HA 410}, BC1F4	15.6		246	{H.CA 2376 x HA 410*5}, BC4F4	31.3
78	{(NMSHA 89 x H.GRO=PI 613793)HA 410}, BCF3	25.0		248	{H. CA 2376 x HA 410*5}, BC4F4	6.3
79*	{(NMSHA 89 x H.GRO=PI 613793)HA 410}, BC1F4	75.0		249	H. CA 2376 x HA 410 (5), BC4F3	20.8
85	{(NMSHA 89 x H.GRO=PI 613793)HA 410*2}, BC2F3	6.3		258	H. CA 2376 x HA 410 (5), BC4F3	7.7
87	{(NMSHA 89 x H.GRO=PI 613793)HA 410*2}, BC1F4	35.0		280	MAX (68) sib x HA 410 (3), BC2F3	27.5
95	{MAX68 SIB x HA 410*3}, BC3F3	11.1		288	(STR (68) x HA 410 (3), BC2F2) x HA 410	5.6
15	{(NMS HA 89 x 1018(M)) x HA 441}, BC1F5	29.4		293	STR (68) x HA 410 (3), BC2F3	7.1
24	{(NMS HA 89 x 1018(M)) x HA 441*3}, BC3F3	44.4		300	{H. CA 2376 x HA 410*5}, BC4F5	10.5
34	(NMSHA 89 x 1323(M) x HA 441, BC1F4	38.9		305*	{H. CA 2376 x HA 410*5}, BC4F5	12.5
38	(NMSHA 89 x 1323(M)) x HA 441 2, BC2F2	52.5		310	{H.CA 2376 x HA 410*5}, BC4F5	8.3
145	(NMSHA 89 x 1314(M)) x HA 441 3, BC3F2`	36.1		315	{H. CA 2376 x HA 410*5}, BC4F5	5.0
168	{(NMSHA 89 x 1324(N)) x HA 441, BC2F4	26.1				
174	{(NMSHA 89 x 1324(N)) x HA 441, BC2F4	18.4		Check	HA 410 (Recurrent parent)	5.0
184*	GRO (68) Sib	100.0		Check	HA 441 (Recurrent parent	35.4
185*	HIR 1126 (102) Sib	100.0		Check	Cargill 270 (Susceptible)	18.3
187	MAX (68) sib x HA 410 (3), BC2F2	100.0		Check	F30294 (Resistant)	61.7
188	NUT (68) Sib	100.0		Check	Croplan 305 (Resistant)	80.0
190*	STR (68) Sib	85.7				

Greenhouse stalk rot resistance confirmation at 14 days after inoculation



Green bars are retests from 2012 field tests (test 1), yellow bars are new lines selected from 2012 field tests (test 2), and red bars are checks

Summary

- Over 160 advanced backcross progeny families from Phase I were grown in Fargo in 2013 to provide a continuous supply of materials for field evaluation
- Field test results showed many families with better disease rating than recurrent parent or resistant checks, suggesting successful resistance gene introgression

- Greenhouse test indicated that over 50% tested families showed better resistance to stalk rot compared to the susceptible check.
- The families with good resistance will be prepared for germplasm release, further characterization, and the initiation of QTL mapping populations

PROGRESS-PHASE 2

- Interspecific crosses were established between six perennial *Helianthus* species and cultivated sunflower in 2011-2012
- Backcrosses and embryo rescue for F_1 or BC_1 progenies for 12 accessions of six perennial *Helianthus* species

Wild *Helianthus* species involved in crosses in Phase 2

No.	Accession	Species	2n	Cross
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

Backcrosses between HA 410, HA 451, and NMS HA 89 and wild *H. hirsutus*, *H. salicifolius*, *H. occidentalis*, and *H. divaricatus* and seed set of further backcrossing with HA 410 or HA 451 from 2011 to 2013

Parentage	BC_1F_1 (2011-2013) (F_1 as female)			BC_2F_1 (2013)			BC_1F_2 (2013)			BC_1F_1 (2012-2013) (F_1 as male)			
	Plants	2n	Seed set %	Plants	2n	Seed set %	Plants	2n	Seed set %	BC_1 Embryo /florets	Plants	2n	Seed set %
H. hirsutus × HA 410	0	-	-	-	-	-	-	-	-	139/4000	45	34	66.0
H. hirsutus × HA 451	11	44-68	3.6	28	37-45	10.1	-	-	-	19/3200	11	34	73.2
NMS HA 89 × *H. hirsutus*	24	38-68	8.4	31	34-46	9.0	-	-	-	-	-	-	-
H. salicifolius × HA 410	19	34-52	9.1	26	34-36	34.3	5	34	9.0	254/7200	172	34-51	56.6
NMS HA 89 × *H. salicifolius*	1	34	1.3	-	-	-	-	-	-	-	-	-	-
H. occidentalis × HA 410	4	34-35	7.1	12	34-36	25.7	12	34-36	22.8	355/3200	216	34	68.3
H. occidentalis × HA 451	3	35-46	0.6	-	-	-	-	-	-	64/4000	25	34	74.8
NMS HA 89 × *H. occidentalis*	1	34	25.3	7	34-36	34.7	-	-	-	-	-	-	-
H. divaricatus × HA 410	2	34	4.5	-	-	-	-	-	-	-	-	-	-
NMS HA 89 × *H. divaricatus*	14	34, 35, 51	16.6	-	-	-	-	-	-	-	-	-	-

16

104

17

469

Summary

- 137 BC_1F_1, BC_2F_1 or BC_1F_2 plants were planted in greenhouse for further backcrossing in 2013, with seed set of 9-34%
- More than 200 BC_1F_2, BC_2F_1, BC_2F_2 or BC_1F_3 plants were established in greenhouse in 2013
- 469 BC_1F_1 plants were planted in greenhouse in 2012, with seed set of 56-75%
- Seeds for 430 BC_1F_2 families were increased in the field in 2013

CONCLUSION AND FUTURE WORK

- Field and greenhouse evaluations showed progeny families with good head and stalk rot resistance, indicating successful introgression of resistance genes
- The outstanding families will be confirmed and prepared for germplasm release, further characterization, and used to initiate QTL mapping populations
- More than 400 BC_1F_2 families were planted in the field in 2013 to increase seed
- Continue to backcross the progenies with 2n>34. More than 200 backcross progenies were established in greenhouse in 2013

- Continue to identify addition lines, and characterize alien chromosomes or fragments in cultivated background utilizing GISH and FISH techniques
- Previously produced BC_1F_1 seed of a few wild *Helianthus* species crossed with HA 89 will be added to the Sclerotinia project in 2014

  (*H. decapetalus, H. laevigatus, H. smithii, H. giganteus, H. pauciflorus* (*rigidus*), and *H. resinosus*)

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