Novel Sources of Sclerotinia Basal Stalk Rot and Head Rot Resistance from Crop Wild Relatives of Sunflower (*Helianthus annuus* L.)

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Abstract

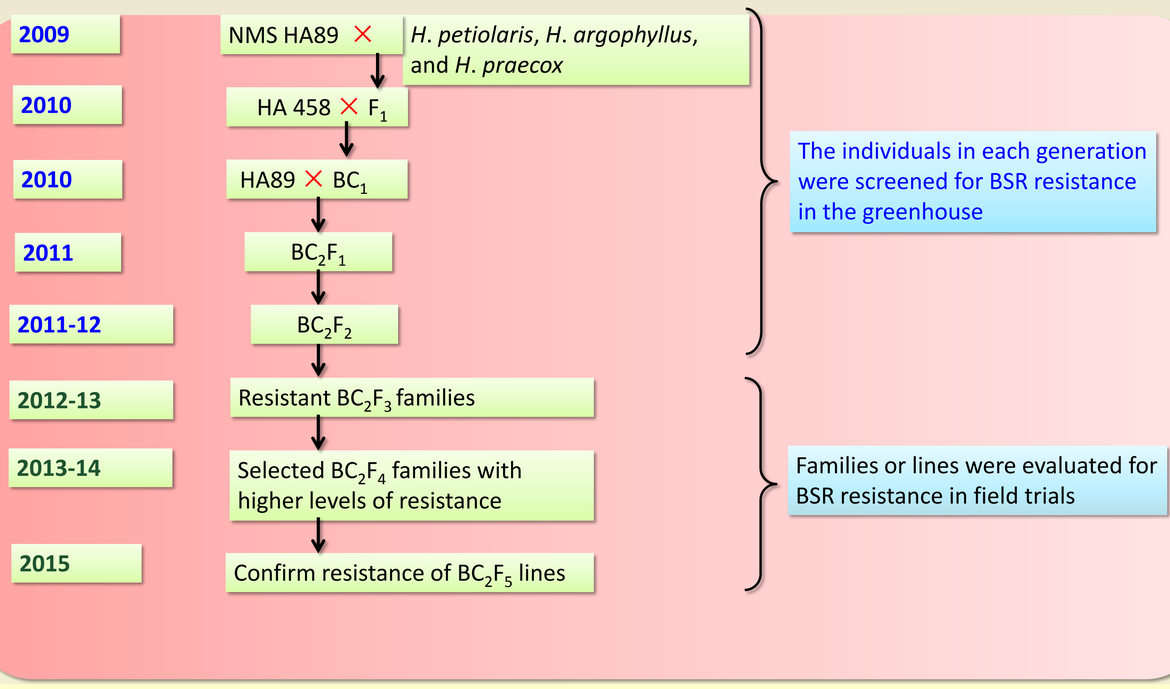
Sclerotinia sclerotiorum is the causal agent of a serious sunflower disease epidemic worldwide. Among Sclerotinia basal stalk rot, head rot, and mid-stalk rot, the former two are the most damaging, accounting for over 80% of the disease incidence. The genetics of resistance to basal stalk rot (BSR) and head rot (HR) is quantitative, requiring many genes, which complicates breeding efforts. There are 53 *Helianthus* species (39 perennial and 14 annual) of sunflower crop wild relatives that represent a considerable amount of genetic diversity available for improvement of cultivated sunflower, which has a very narrow genetic base. The objective of the study was to evaluate interspecific germplasm in various stages of breeding in artificially inoculated field trials for BSR incidence at three locations, Carrington, ND, Grandin, ND, and Crookston, MN. Sclerotinia BSR resistance was successfully transferred from three wild annual *Helianthus* species into cultivated sunflower, with two *H. petiolaris*, six *H. argophyllus*, and five *H. praecox* introgression lines. Whole genome scans using genotyping-by-sequencing were used to detect the presence of the wild introgression segments in the selected lines. Single nucleotide polymorphism markers revealed the presence of wild segments in the cultivated sunflower background located on linkage groups 1, 3, 8, 9, 10, and 11. Additionally, 411 progeny families from crosses of amphiploids, hexaploid, and diploid perennials with cultivated lines were screened for BSR and HR resistance. More than 150 early generation interspecific families of perennial *H. hirsutus*, *H. salicifolius*, and *H. occidentalis* tested in replicated BSR field trials suggested excellent BSR resistance, further confirming successful gene introgression. Five BSR and two HR germplasms are scheduled for release based on their higher levels of resistance. Accessions of sunflower crop wild relatives from the USDA-ARS-NPGS genebank continue to contribute specific traits to combat emerging pests and environmental challenges, and at the same time preserve them for future generations.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is the most destructive pathogen of sunflower (*Helianthus annuus* L.) causing two important diseases, stalk rot and head rot. The mode of infection and the genetics of resistance are completely different for the two diseases, effectively doubling the effort required to control the diseases. Resistance to *S. sclerotiorum* is under polygenic control (Talukder et al., 2014) and no major resistance gene is known against this pathogen in cultivated sunflower. Therefore, breeding for Sclerotinia resistance relies on incorporating genetic factors from partially resistant lines. The genus *Helianthus* is native to North America and comprises 14 annual diploids (n=17) and 39 perennial species. Wild annual and perennial species have been a good source of resistance genes for economically important sunflower diseases (Seiler and Rieseberg, 1997). Basal stalk rot (BSR) resistance was identified in wild annual species of *H. argophyllus*, *H. praecox*, and *H. petiolaris.* Wild annual and perennial species were selected to transfer Sclerotinia BSR and head rot (HR) resistance into cultivated sunflower. In this poster we report the progress of transferring Sclerotinia BSR and HR resistance into cultivated sunflower and monitoring the introgressed alien segments using a high throughput SNP marker resource and the current status of pre-breeding novel Sclerotinia resistance sources from wild annual and perennial species into cultivated sunflower.

Materials and Methods

Introgression of Sclerotinia resistance from annual crop wild relatives



Results and Discussion

Transfer of Sclerotinia BSR resistance from wild annual species into cultivated sunflower is moving forward. Wild introgressed families consistently showed superior Sclerotinia BSR resistance in the field evaluations tested over the years **(Table 1).** In 2014, 23 BC_2F_4 families derived from *H. argophyllus*, *H. petiolaris*, and *H. praecox* were evaluated for BSR resistance in multi-location field trials **(Fig. 1).** Across environments, all the introgressed BC_2F_4 families showed significantly higher Sclerotinia BSR resistance than both the susceptible checks, Cargill 270 and HA89 (36% DI). The level of resistance in the introgressed families was similar to the resistant check, Croplan 305 (9.6% DI). However, ten BC_2F_4 families, five each derived from *H. argophyllus* and *H. praecox* had significantly higher BSR resistance (0 to 3.6% DI) than the other resistant check, HA 441 (18.3% DI).

Resistance donor	Disease incidence (%) 2012 Pedigree	2012 BC_2F_3	2013 Pedigree	2013 BC_2F_4	2014 BC_2F_4
H. petiolaris ssp. *fallax*	11-256-049	0	12F-3405-2	4.0	8.4
PI 435843	11-256-053	0	12F-3406-5	5.6	-
H. argophyllus	11-275-037	0	12F-3416-4	9.3	2.9
PI 494573	11-283-037	0	12F-3424-4	0	10.0
	11-291-01	6.6	12F-3438-2	3.1	9.5
	11-291-09	4.5	12F-3442-1	6.7	4.5
	11-291-17	1.7	12F-3443-1	4.2	6.3
H. praecox ssp.	11-291-45	5.3	12F-3451-4	3.9	7.9
runyonii	11-291-57	1.5	12F-3456-1	8.3	-
PI 468853	11-291-65	4.2	12F-3459-1	0	0.7
	11-291-67	2.3	12F-3460-4	0	7.9
	11-292-33	0	12F-3467-1	3.3	0
	11-294-21	3.1	12F-3482-1	3.3	3.3
Susceptible checks	Cargill 270	34.8	Cargill 270	72.6	36.0
	HA 89	23.7	HA 89	51.6	35.7
Resistant checks	Croplan 305	12.4	Croplan 305	34.9	9.6
	HA 441	27.4	HA 441	28.6	18.3

Table1. Evaluation of the 13 most resistant BC_2F_3 families and derived BC_2F_4 lines for Sclerotinia basal stalk rot resistance in the field from 2012 to 2014.

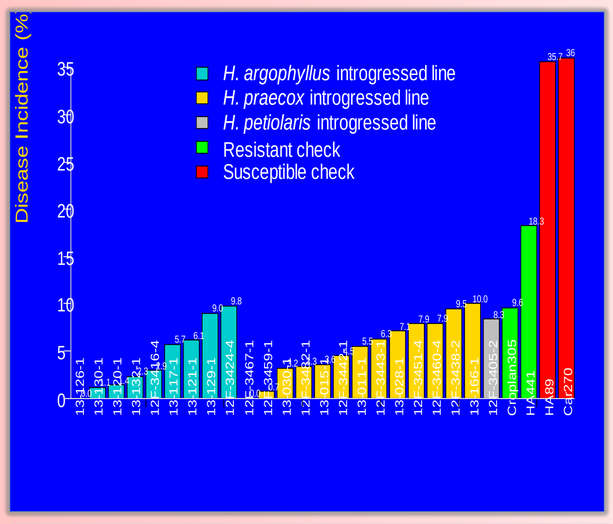


Figure 1. Mean performance of 23 BC_2F_4 families for Sclerotinia basal stalk rot resistance at two locations in North Dakota during 2014.

In 2015, six *H. argophyllus*, two *H. petiolaris*, and eight *H. praecox* derived BC_2F_5 wild introgression lines were evaluated for BSR resistance at two North Dakota locations. Wild introgression lines consistently showed superior BSR resistance in the field evaluations tested over the years. A one-way analysis of variance of DI data from seven environments revealed that all the BC_2F_5 introgression lines showed significantly higher BSR resistance than the resistant inbred check, HA 441 **(Fig. 2).** Most of the introgression lines either showed significantly higher or similar levels of BSR resistance than the resistance hybrid check, Croplan 305.

To detect the presence of *H. argophyllus*, *H. petiolaris,* and *H. praecox* chromosome segments in the highly BSR resistant wild introgression sunflower lines, a whole genome scan was performed using genotyping-by-sequencing (GBS) approach. Polymorphic single nucleotide polymorphism (SNP) markers revealed the presence of introgressed segments in the cultivated sunflower background predominantly located on linkage groups 1, 3, 8, 9, 10, and 11. Some of these introgressed segments might be associated with BSR resistance **(Fig. 3).**

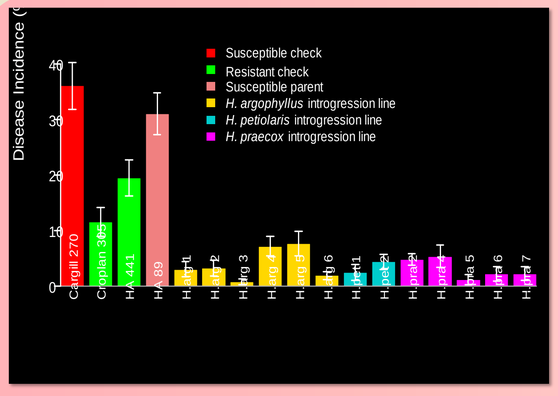


Figure 2. Mean BSR disease incidence (DI) of the most resistant wild sunflower introgression lines evaluated in seven environments during 2012-2015.

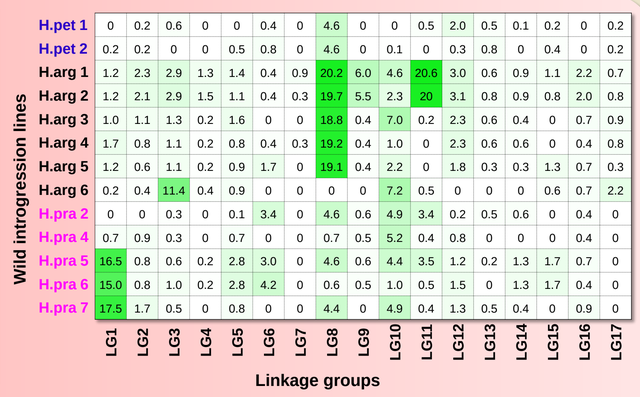


Figure 3. Tracking of *H. argophyllus, H. petiolaris,* and *H. praecox* chromosome segments in highly BSR resistant wild sunflower introgression lines using SNP markers.

Introgression of Sclerotinia resistance from perennial crop wild relatives

Several of the interspecific lines derived from wild perennial species showed very good resistance to head rot (HR) compared to the recurrent parent and checks **(Table 2).** Additionally, 441 families from amphiploid, hexaploid, and diploid perennial crosses were tested for BSR resistance at Carrington, ND and Grandin, ND. Families with better BSR resistance than the recurrent parents were identified **(Table 3).** More than 150 early generation interspecific families of perennial *H. salicifolius*, *H. hirsutus*, and *H. occidentalis* tested in replicated BSR field trials in 2015 suggested excellent BSR resistance, further confirming successful gene introgression **(Table 3)**. Five Sclerotinia BSR and two HR tolerant germplasms from wild perennial species field evaluated from 2009-2015 are scheduled for release in 2016-2017 **(Table 4)**.

Table 2. Replicated Sclerotinia head rot field evaluation of 2014 interspecific lines derived from perennial sunflower crop wild relatives at Carrington, ND and Staples, MN in 2015.

Pedigree*	Carrington, ND 2015 Disease Rating	Carrington, ND 2015 Infected Plants	Staples, MN 2015 Disease Rating	Staples, MN 2015 Infected Plants
TEST 2 (Second Retest)	0-5	%	0-5	%
((NMS HA89 x H. GRO=PI613793) HA 410*2), BC2F3	2.21	58	0	0
((NMS HA89 x H. GRO=PI613793) HA 410), BC2F3	2.76	74	0	0
((NMS HA89 x H. GRO=PI613793) HA 410), BC1F4	1.87	58	1.46	33
Recurrent parent HA 410	**3.67**	**92**	**0.25**	**6**
((NMS HA89 x 1323 (MAX) x HA 441) BC1F5	1.91	53	0	0
((NMS HA89 x 1324 (NUT) x HA 441) BC1F5	2.7	60	0	0
((NMS HA89 x 1008 (NUT)) x HA 441) HA 441, BC2F4	0.81	17	0.19	4
((NMS HA89 x 1018 (MAX)) x HA 441) BC1F6	1.82	38	0.37	12
((NMS HA89 x 1324 (NUT) x HA 441) BC1F5	0.68	21	0.50	11
Recurrent parent HA 441	**1.94**	**55**	**0.51**	**11**
TEST 3 (First Retest)				
NMS HA89 X (SAL X HA 410), F2	0.50	13	-	-
NMS HA89 X (SAL X HA 410), F2	0.92	22	-	-
NMS HA89 X (SAL X HA 410), F2	-	-	0	0
NMS HA89 X (SAL X HA 410), F2	-	-	0.32	12
NMS HA89 X (SAL X HA 410), F2	-	-	0	0
NMS HA89 X (SAL X HA 410), F2	-		0.33	8
NMS HA89 X (OCC X HA 410), F2	-	-	0.25	8
NMS HA89 X (OCC X HA 410), F2	-	-	0.21	7
NMS HA89 X (OCC X HA 410), F2	-	-	0.18	7
Recurrent parent HA 410	**4.71**	**97**	**0.20**	**5**
TEST 4 (New Selections)				
NMS HA89 x ((SAL) x HA 410), F2	-	-	0.29	**7**
NMS HA89 x ((SAL) x HA 410), F2	-	-	0.29	**7**
NMS HA89 x ((SAL) x HA 410), F2	-	-	0	**0**
NMS HA89 x ((OCC) x HA 410), F2	1.08	25	-	-
NMS HA89 x ((OCC) x HA 410), F2	-	-	0.27	**7**
NMS HA89 x ((OCC) x HA 410), F2	-	-	0.29	**7**
Recurrent parent HA 410	**4.89**	**100**	**0.29**	**7**
Checks				
Susceptible check HA89 (S)	**4.54**	**96**	**1.38**	**37**
Susceptible check Mycogen (Cargill) 272 (S)	**1.83**	**45**	**2.58**	**67**
Resistant check Croplan 343 (R)	**1.08**	**26**	**0.0**	**0.0**

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.

Table 3. Replicated Sclerotinia stalk rot field evaluation of 2014 interspecific lines derived from perennial sunflower crop wild relatives at Carrington, ND, and Grandin, ND in 2015.

Pedigree*	Carrington, ND Percent Infected Plants	Grandin, ND Percent Infected Plants
TEST 2 (Second Retest)	%	%
(NMS HA89 x GIG=PI 547182) HA 410*2, BC2F3	0	0
(NMS HA89 x GIG=PI 547182) HA 410, BC1F4	0	10
(NMS HA89 x GRO=PI 416793) HA 410, BC1F4	10	0
CAL 2376 x HA 410*5), BC4F3-F5	2	0
(NMS HA89 x GRO=PI 613793) HA 410, BC2F3	4	5
(NMS HA89 x GRO=PI 613793) HA 410, BC1F4	2	2
MAX68 SIB x HA 410*3, BC2F4	0	0
NUT68 x HA 410*3, BC2F4	0	15
(NMS HA89 x MAX=PI 586892) HA 410*2, BC2F2-F3	6	13
STR (68) x HA 410 (3), BC2F4	0	0
Recurrent parent HA 410	**11**	**12**
(NMS HA89 x 1323 (MAX)) HA 441,BC2F4	0	3
(NMS HA89 x 1323 (MAX)) HA 441,BC1F5	4	7
(NMS HA89 x 1324 (NUT)) HA 441,, BC1F5	0	8
Recurrent parent HA 441	**8**	**5**
TEST 3 (First Retest)		
NMS HA89 X (HIR. X HA 410), F2	3	3
NMS HA89 X (HIR. X HA 410), F2	3	2
(NMS HA89 X (SAL X HA 410), F2	2	0
(NMS HA89 X (SAL X HA 410), F2	0	3
(NMS HA89 X (OCC X HA 410), F2	4	0
(NMS HA89 X (OCC X HA 410), F2	3	0
(NMS HA89 X (OCC X HA 410), F2	5	0
(NMS HA89 X (OCC X HA 410), F2	0	0
Recurrent parent HA 410	**11**	**12**
NMS HA89 X (HIR X HA 451), F2	6	15
NMS HA89 X (HIR X HA 451), F2	6	10
(NMS HA89 X (OCC X HA 451), F2	4	10
(NMS HA89 X (OCC X HA 451), F2	4	19
(NMS HA89 X (OCC X HA 451), F2	3	10
Recurrent parent HA 451	**22**	**29**
CHECKS		
Susceptible check HA89 (S)	**15**	**25**
Susceptible check Mycogen (Cargill) 272 (S)	**18**	**14**
Resistant check Croplan 305 (R)	**8**	**8**

The first three letters of the *Helianthus* species are used to identify the species source: GIG=*H. giganteus*; GRO=*H. grosseserratus*; CAL=*H. californicus*; MAX=*H. maximiliani*, NUT=*H. nuttallii*; STR=H. strumosus; HIR=*H. hirsutus*; SAL=*H. salicifolius;* and OCC= *H. occidentalis*. (S)= Susceptible; (R)=Resistant.

Table 4. Scheduled releases of HR and BSR tolerant germplasms in 2017 based on field screening from 2009-2015.

Proposed Release Name	Disease Rating	Infected Plants
HEAD ROT RELEASES	0-5	%
HR MAX*-1 (4)**	0.9	23
HR NUT-1 (3)	1.1	24
Recurrent parent HA 441	**2.3**	**52**
Checks		
Susceptible check HA89 (S)	**3.4**	**71**
Susceptible check Mycogen (Cargill) 272 (S)	**3.3**	**70**
Resistant check Croplan 305 (R)	**2.7**	**59**
Resistant check Croplan 343 (R)	**1.7**	**46**
STALK ROT RELEASES		
SR MAX-1 (7)	–	3
SR NUT-1 (3)	–	3
Recurrent parent HA 441	–	11
SR CAL-1 (23)	–	2
SR DIV-1 (1)	–	7
SR STR-1 (2)	–	2
Recurrent parent HA 410	–	**23**
Checks		
Susceptible check HA89 (S)	–	**33**
Susceptible check Mycogen (Cargill) 272 (S)	–	**37**
Resistant check Croplan 305 (R)	–	**9**
Resistant check Croplan 343 (R)	–	**17**

*The first three letters of the Helianthus species are used to identify the species source: MAX=*H. maximiliani*; NUT=*H. nuttallii*; CAL=*H. californicus*; DIV=*H. divaricatus*, and STR=*H. strumosus*.
** Number of families in the release. ***(S)=Susceptible; (R)= Resistant.

Summary

1. Replicated field evaluations in 2014-2015 confirmed the successful introgression of Sclerotinia BSR and HR resistance genes from annual and perennial sunflower crop wild relatives.

2. GBS revealed the presence of *H. argophyllus* segments in LGs 3,8,9,10 and 11 of the sunflower genome with LG 8 with the highest frequency.

3. Progeny families with increased resistance for BSR and HR will be selected for the development of QTL mapping populations.

4. Germplasm lines with increased levels of resistance for Sclerotinia BSR and HR will be released in the near future.

References

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