

# Deployment of Novel Sources of Sclerotinia Stalk Rot Resistance in Sunflower

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Sclerotinia head rot



Sclerotinia stalk rot



Field score, Oct. 2009,



# Searching for *Sclerotinia* stalk rot resistance from wild annual species

- ◆ Wild sunflower and the sunflower crop are native to North America
- ◆ There are 51 species of wild *Helianthus* - 14 annual and 37 perennial
- ◆ Wild annual species are a valuable sources of resistance genes
- ◆ Easy to cross to the cultivated sunflower (all diploid)
- ◆ Genetic recombination in the progenies could be achieved through homologous pairing



## Identifying Sclerotinia stalk rot resistances from wild annual species

- ◆ A total of 460 accessions from 14 wild annual species were evaluated for their reaction to Sclerotinia stalk rot in 2007, 2008, and 2009 in the greenhouse and field
- ◆ Eighty-five plants selected from 21 resistant accessions were saved and shipped from Ames to Fargo for greenhouse crossing



Table 1. F<sub>1</sub> hybrids, BC<sub>1</sub> and BC<sub>2</sub> progenies from the crosses of HA 89 with selected stalk rot resistant plants from *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*

Crosses	No. accession	Total florets pollinated	F <sub>1</sub>		BC <sub>1</sub>	BC <sub>2</sub>
			No. seed	Seed set (%)	No. seed	No. seed
NMS HA89 × <i>H. argophyllus</i>	4	6,556	316	0.00-40.24	211	>200
NMS HA89 × <i>H. debilis</i>	6	6,986	81	0.17-2.40	161	>200
NMS HA89 × <i>H. praecox</i>	5	8,516	629	1.22-13.83	109	>200
NMS HA89 × <i>H. petiolaris</i>	6	13,519	670	0.13-13.53	102	>200





# Sclerotinia stalk rot screen in F<sub>1</sub> and BC<sub>1</sub>

Experiment 1 (F<sub>1</sub> hybrids, Feb. 2010):

- a. 80 g dry mycelium-bearing millet seed
- b. Soil temp. 22-24°C in greenhouse



Inoculation tray filled with mycelium-bearing millet seed



Susceptible check Car270 shows disease symptom seven days after inoculation



(NMSHA 89 X *H. praecox*) F<sub>1</sub>

Susceptible  
check Car270



(NMSHA 89 X *H. petiolaris ssp. fallax*) F<sub>1</sub>

Susceptible  
check Car270



Comparison of susceptible check Car270 with F<sub>1</sub> hybrids 18 days after inoculation.  
96% of Car270 plants died, whereas most F<sub>1</sub> plants survived well



Table 2. The measurements of the reaction of F<sub>1</sub> plants and their parent to Sclerotinia stalk rot infection at 7, 11, 14, and 18 days after inoculation

Materials	No. plant tested	Stalk rot incidence (%)			
		7 d	11 d	14 d	18 d
HA441 (R-check)	48	4	6	16	16
Car 270 (S-check)	48	73	98	98	100
Seeds 2000 4129 (S-check)	48	81	94	94	94
HA89 (Parent)	38	0	3	16	26
(NMS HA89 x <i>H. petiolaris</i> ssp. <i>fallax</i> PI435815) F <sub>1</sub>	44	0	0	5	7
(NMS HA89 x <i>H. petiolaris</i> ssp. <i>fallax</i> PI435843) F <sub>1</sub>	21	0	0	5	5
(NMS HA89 x <i>H. petiolaris</i> PI451978) F <sub>1</sub>	44	0	5	9	11
(NMS HA89 x <i>H. petiolaris</i> ssp. <i>fallax</i> PI 468811) F <sub>1</sub>	44	0	0	2	2
(NMS HA89 x <i>H. argophyllus</i> PI 494573) F <sub>1</sub>	22	0	0	0	0
(NMS HA89 x <i>H. argophyllus</i> PI435623+PI 649863) F <sub>1</sub>	38	0	0	5	11
(NMS HA89 x <i>H. debilis</i> ssp. <i>cucumerifolius</i> PI 435654) F <sub>1</sub>	10	0	0	0	0
(NMS HA89 x <i>H. debilis</i> ssp. <i>silvestris</i> PI 468685) F <sub>1</sub>	9	0	0	0	0
(NMS HA89 x <i>H. praecox</i> ssp. <i>runyonii</i> PI 435849) F <sub>1</sub>	36	0	0	0	0
(NMS HA89 x <i>H. praecox</i> ssp. <i>runyonii</i> PI 468853) F <sub>1</sub>	36	0	16	19	22
(NMS HA89 x <i>H. praecox</i> ssp. <i>hirtus</i> PI 468847) F <sub>1</sub>	28	4	4	18	25
(NMS HA89 x <i>H. praecox</i> ssp. <i>hirtus</i> PI 435855) F <sub>1</sub>	36	0	0	0	0

Stalk rot incidence: the percentage of dead plants



## Experiment 2 (BC<sub>1</sub> progenies, Aug. 2010):

a. 150 g mycelium-bearing millet seed

b. Soil temp. 20-22°C in growth chamber



150 g mycelium-bearing millet seed per tray



Mycelial growth after three days incubated at 22°C



All roots are in direct contact with inoculum



Table 3. The measurements of the reaction of BC<sub>1</sub> plants and their parent to *Sclerotinia* stalk rot infection at 7, 10, and 14 days after inoculation

Materials	No. plant	Stalk rot incidence (%)		
		7 d	10 d	14 d
HA441 (R-check)	23	100	100	100
Car270 (S-check)	24	95.8	100	100
HA89 (Parent)	23	91.3	100	100
HA458 (Parent)	23	100	100	100
HA458/(NMS HA89 x <i>H. petiolaris</i> ssp. <i>fallax</i> PI435815) BC <sub>1</sub>	23	95.7	100	100
HA458/(NMS HA89 x <i>H. petiolaris</i> ssp. <i>fallax</i> PI435843) BC <sub>1</sub>	23	78.3	78.3	78.3
HA458/(NMS HA89 x <i>H. petiolaris</i> PI451978) BC <sub>1</sub>	22	100	100	100
HA458/(NMS HA89 x <i>H. argophyllus</i> PI 494573) BC <sub>1</sub>	23	69.6	69.6	69.6
HA458/(NMS HA89 x <i>H. argophyllus</i> PI435623+PI 649863) BC <sub>1</sub>	20	80	80	80
HA458/(NMS HA89 x <i>H. debilis</i> ssp. <i>silvestris</i> PI 468685) BC <sub>1</sub>	4	100	100	100
HA458/(NMS HA89 x <i>H. debilis</i> ssp. <i>cucumerifolius</i> PI 468667) BC <sub>1</sub>	20	80	80	80
HA89/(NMS HA89 x <i>H. debilis</i> ssp. <i>silvestris</i> PI 468680) BC <sub>1</sub>	10	70	70	70
HA458/(NMS HA89 x <i>H. praecox</i> ssp. <i>runyonii</i> PI 435849) BC <sub>1</sub>	10	80	80	80
HA458/(NMS HA89 x <i>H. praecox</i> ssp. <i>runyonii</i> PI 468853) BC <sub>1</sub>	22	81.8	81.8	81.8
HA458/(NMS HA89 x <i>H. praecox</i> ssp. <i>hirtus</i> PI 468847) BC <sub>1</sub>	23	91.3	91.3	91.3
HA458/(NMS HA89 x <i>H. praecox</i> PI 413176) BC <sub>1</sub>	14	92.9	92.9	92.9

# Optimize greenhouse method

Experiment 3 (six selected inbred lines, Oct.-Nov. 2010):

- Three sets of 80 g, 120 g and 160 g mycelium-bearing millet seed
- Two replicates with randomized complete block design
- Soil temp. 22-24°C in greenhouse



80 g

120 g

160 g





## Seedling age of three and one-half weeks



160 g mycelium-bearing millet seed  
14 d after inoculation





Table 4. The measurements of the reaction of inbred lines to Sclerotinia stalk rot infection at 14 and 18 days after inoculation

Materials	No. plant	Stalk rot incidence (%)					
		80 g millet		120 g millet		160 g millet	
		14 d	18 d	14 d	18 d	14 d	18 d
HA 441 (R)	16	0	0	6.3	6.3	50.0	56.3
RHA 280	16	0	0	0	12.5	6.3	25.0
RHA 801	16	12.5	18.8	18.8	18.8	6.3	6.3
HA 458 (S)	16	12.5	25.0	62.5	87.5	62.5	75.0
RHA 439 (S)	16	25.5	62.5	62.5	75.0	87.5	87.5
HA 89 (S)	16	62.5	75.0	56.3	75.0	75.0	81.3



# Correlation between susceptibility assessed in the field and with the greenhouse method

Experiment 4 (selected RIL lines, Nov.-Dec. 2010):

- a. 120 g mycelium-bearing millet seed
- b. Two replicates with randomized complete block design
- c. Soil temp. 20-23°C in greenhouse

Four recombinant inbred lines (RILs), RIL243, RIL253, RIL262, and RIL293, were selected from the cross RHA280 x RHA801 and are resistant to stalk rot

Another four RILs, 8-11-1, 9-10-1, 12-17-1 and 15-16-1, were selected from the cross HA441 x RHA439 and are resistant to head rot



120 g mycelium-bearing millet seed  
18 d after inoculation



RIL253

HA 89



RIL 243

9-10-1



Table 5. Correlation between susceptibility assessed in the field and with the greenhouse method

Materials	2009			2010	
	Carrington	Crookston	Average	Carrington	GH
HA 89	21.0 (10)	-	21.0 (10)	9.3 (17)	75.0 (2)
RAH280	16.7 (2)	7.7 (1)	12.2 (3)	0 (2)	41.7 (2)
RHA801	7.7 (2)	2.8 (2)	5.3 (4)	0 (2)	54.2 (2)
RIL243	0.0 (4)	6.7 (4)	3.4 (8)	1.3 (4)	8.3 (2)
RIL253	0.0 (4)	3.7 (4)	1.9 (8)	0 (4)	8.7 (2)
RIL264	1.5 (4)	2.1 (4)	1.8 (8)	0 (4)	0.0 (2)
RIL293	9.8 (4)	17.5 (4)	13.7 (4)	8.9 (4)	50.0 (2)
RHA439	-	-	-	2.7 (2)	72.3 (2)
HA441	-	-	-	2.4 (2)	37.5 (2)
8-11-1	-	-	-	6.3 (4)	62.5 (2)
9-10-1	-	-	-	0 (4)	20.9 (2)
12-17-1	-	-	-	1.4 (4)	47.8 (2)
15-16-1	-	-	-	0 (4)	37.5 (2)

The number in parentheses represents the number of replicates

# Future plan

1. Screen BC<sub>2</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>2</sub> populations for stalk rot resistance in the greenhouse
2. Screen advanced generations in the field to validate the greenhouse results
3. Investigate inheritance of Sclerotinia resistance in introgressed lines
4. Develop an advanced backcross population for QTL mapping of stalk rot resistance genes from *H. argophyllus*





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