

Deployment of Novel Sources of Sclerotinia Resistance in Sunflower - 2013 Progress

Yunming Long¹, Thomas J. Gulya², Charles C Block³,
Brent S. Hulke², Lili Qi²

¹Department of Plant Sciences, NDSU, Fargo, ND

²USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

³USDA-ARS, Plant Introduction Station, Ames, IA



Project background

Two distinctly different diseases caused by Sclerotinia occur on sunflower:

- 1) Stalk rot-----unique to sunflower
root infection caused by mycelia of *Sclerotinia sclerotiorum*
 - 2) Head rot (middle stalk rot)-----similar to all other hosts
head infection caused by ascospores of *S. sclerotiorum*
- ♦ ♦ The genetics of resistance to the two diseases is different



Sclerotinia head rot



Sclerotinia stalk rot



Project background

- ◆ *Helianthus* native to North America comprises 14 annual and 37 perennial species
- ◆ Wild annual species are valuable resistance sources
 - Easy to cross to the cultivated sunflower (all diploid)
 - Homologous recombination easily occurs
- ◆ Stalk rot resistance was identified in wild annual species of *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*

Wild annual species were selected to transfer Sclerotinia stalk rot resistance into cultivated sunflower



Objectives of the project

1. Pre-breed novel Sclerotinia resistance from wild annual species of *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris* into cultivated sunflower, and develop an advanced backcross population for QTL mapping
2. Investigate inheritance of Sclerotinia resistance in introgression lines



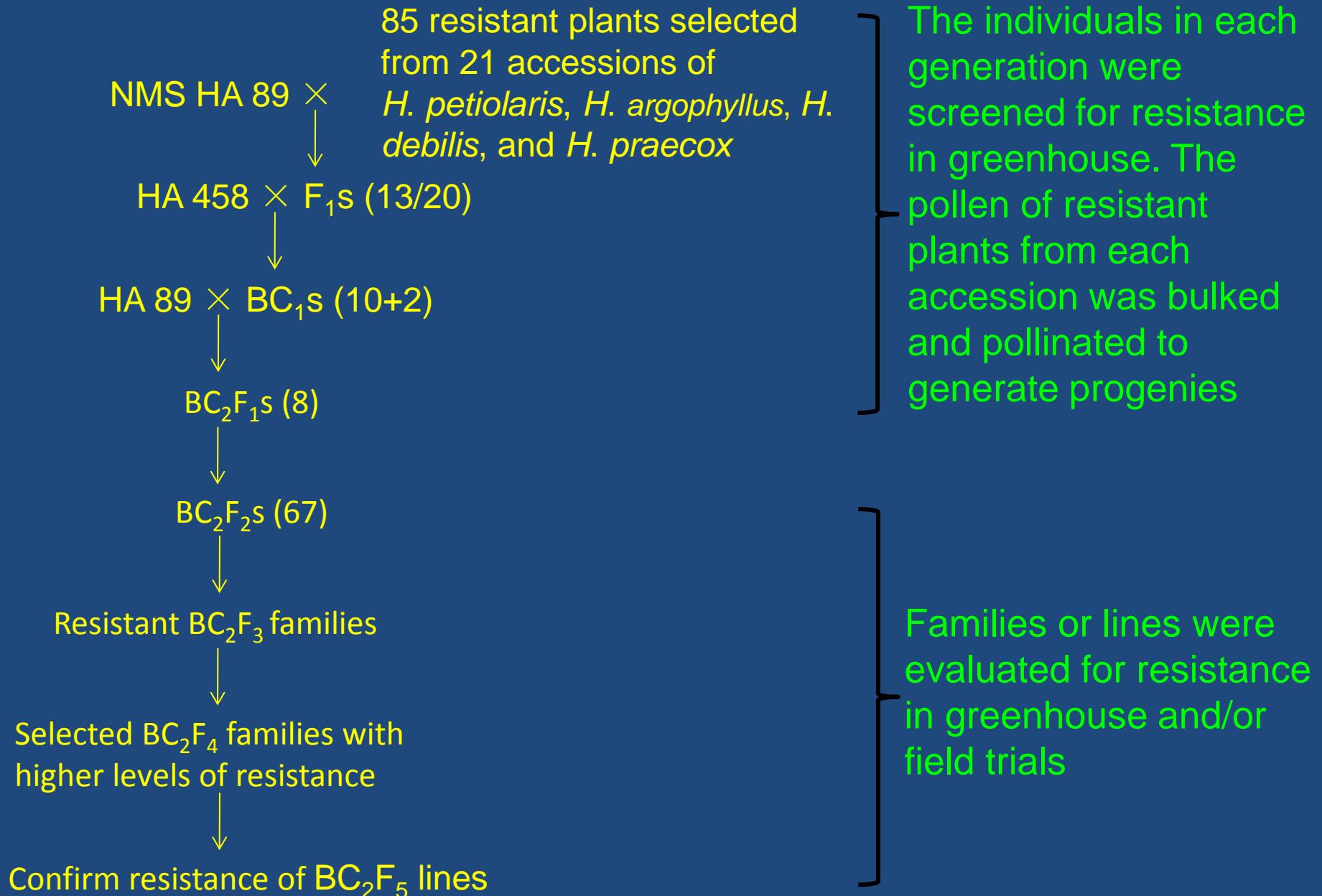


Fig. 1 Schematic of introgression of Sclerotinia resistance from wild species

Test of stalk rot resistance in greenhouse



Mycelium-bearing
millet seed per tray



Mycelia growth after
three days incubation
at 22°C in darkness



The roots of 3-week
seedlings
directly contact mycelia



Seedlings at 14 days after inoculation at 22-24°C in greenhouse

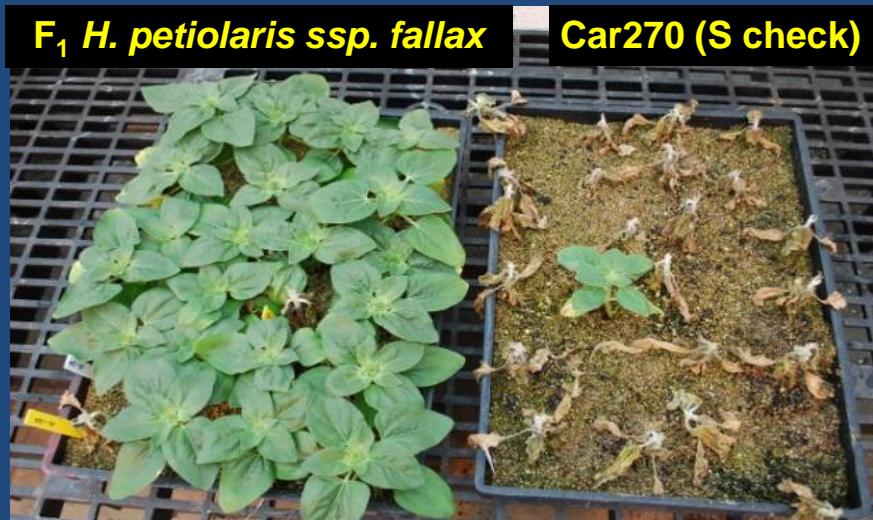


Table 1. Introgression of resistance to Sclerotinia stalk rot from wild annual species

Materials	F ₁		BC ₁ F ₁		BC ₂ F ₁		BC ₂ F ₂
	No. Plant Tested	Disease Incidence (%)	No. Plant Tested	Disease Incidence (%)	No. Plant Tested	Disease Incidence (%)	
HA 441 (R-check)	48	14.6	22	68.2	48	20.9	
Corplan305 (R-check)	44	18.2	-	-	24	8.3	
HA 89 (Parent)	38	15.8	22	95.5	48	13	
Car 270 (S-check)	48	97.9	23	100	24	29.2	
<i>H. petiolaris</i> ssp. <i>fallax</i> PI435815	44	4.5	23	100	-	-	
<i>H. petiolaris</i> ssp. <i>fallax</i> PI435843	21	4.8	23	78.3	72	16.7	5
<i>H. petiolaris</i> PI451978	44	9.1	22	100	-	-	
<i>H. petiolaris</i> ssp. <i>fallax</i> PI 468811	44	2.3	-	-	-	-	
<i>H. argophyllus</i> PI 494573	22	0	23	69.6	72	12.3	14
<i>H. argophyllus</i> PI435623+PI 649863	38	5.3	20	80	72	15.3	5
<i>H. debilis</i> ssp. <i>cucumerifolius</i> PI 435654	10	0	4	100	-	-	
<i>H. debilis</i> ssp. <i>cucumerifolius</i> PI 468667	-	-	20	80	72	11.2	7
<i>H. debilis</i> ssp. <i>silvestris</i> PI 468680	-	-	10	70	72	17.9	9
<i>H. debilis</i> ssp. <i>silvestris</i> PI 468685	9	0	-	-	-	-	
<i>H. praecox</i> ssp. <i>runyonii</i> PI 435849	36	0	10	80	72	20.9	7
<i>H. praecox</i> ssp. <i>runyonii</i> PI 468853	36	19.4	22	81.8	71	5.8	12
<i>H. praecox</i> ssp. <i>hirtus</i> PI 468847	28	17.9	23	91.3	72	18.2	8
<i>H. praecox</i> ssp. <i>hirtus</i> PI 435855	36	0	-	-	-	-	
<i>H. praecox</i> PI 413176	24	0	14	92.9	-	-	

Hyphen indicates lack of seeds

Table 2. Development of stalk rot resistance families

Wild species donor	BC ₂ F ₂		BC ₂ F ₃		BC ₂ F ₄	
	No. family	No. plant tested	No. family advanced	No. family selected	No. family selected	No. family selected
<i>H. petiolaris</i> ssp. <i>fallax</i> PI 435843	5	768	23	2	2	
<i>H. argophyllum</i> PI 494573	14	640	41	9		2+
<i>H. argophyllum</i> PI 435623 + PI 649863	5	480	13	0		-
<i>H. debilis</i> ssp. <i>cucumberifolius</i> PI 468667	7	720	16	1		underway
<i>H. debilis</i> ssp. <i>silvestris</i> PI 468680	9	480	56	0		-
<i>H. praecox</i> ssp. <i>runyonii</i> PI 435849	7	336	53	5		underway
<i>H. praecox</i> ssp. <i>runyonii</i> PI 468853	12	480	78	17		9+
<i>H. praecox</i> ssp. <i>hirsutus</i> PI 468847	8	384	22	0		-
Total	67	4288	302 (7%)	34		13+



Table 3. Evaluation of the 13 most resistant BC₂F₃ families and derived BC₂F₄ lines for their reaction to Sclerotinia stalk rot in field trials in the years 2012 and 2013

Resistance donor	Average disease incidence (%)			
	2012		2013	
	Pedigree	BC ₂ F ₃	Pedigree	BC ₂ F ₄
Cargill 270 (S check)	34.78	Cargill 270 (S check)	72.63	
HA 89 (S check)	23.66	HA 89 (S check)	51.58	
Croplan 305 (R check)	12.36	Croplan 305 (R check)	34.87	
HA 441 (R check)	27.43	HA 441 (R check)	28.6	
<i>H. petiolaris</i> ssp. <i>fallax</i>	11-256-049	0	12F-3405-2	4.02
PI435843	11-256-053	0	12F-3406-5	5.64
<i>H. argophyllus</i> PI	11-275-037	0	12F-3416-4	9.31
494573	11-283-037	0	12F-3424-4	0
<i>H. praecox</i> ssp. <i>runyonii</i>	11-291-01	6.63	12F-3438-2	3.13
PI 468853	11-291-09	4.5	12F-3442-1	6.67
	11-291-17	1.67	12F-3443-1	4.17
	11-291-45	5.26	12F-3451-4	3.85
	11-291-57	1.47	12F-3456-1	8.33
	11-291-65	4.17	12F-3459-1	0
	11-291-67	2.28	12F-3460-4	0
	11-292-33	0	12F-3467-1	3.33
	11-294-21	3.13	12F-3482-1	3.33

Table 4. Evaluation of the 27 additional BC₂F₃ families for their reaction to Sclerotinia stalk rot in field trials in 2013

Wild species donor	No. families screened in greenhouse	No. families selected in greenhouse	No. families tested in field	Disease incidence (%)*			
				0-10	10.1-20	20.1-30	>30
<i>H. petiolaris</i>	12	0	0				
<i>H. argophyllus</i>	42	11	9	6	2	1	0
<i>H. praecox</i>	65	21	6	0	2	0	4
<i>H. debilis</i>	112	15	12	0	1	0	11
Total	231	47	27	6	5	1	15

*The disease incidence of checks:

Cargrill 270 (72.63%), HA 89 (acceptor, 51.58%)

Croplan 305 (34.87%), HA 441 (28.6%)

Screening of polymorphism between HA 89 and wild species

Table 5. Detection of SSR marker polymorphism between HA 89 and sunflower wild species

Wild species	No. SSRs tested	No. polymorphic SSRs			Total
		Co-dominant	Dominant		
<i>H. petiolaris</i> ssp. <i>fallax</i> PI 435843	507	202	44		246
<i>H. argophyllus</i> PI 494573	869	427	97		524
<i>H. argophyllus</i> PI 435623	507	255	50		305
<i>H. debilis</i> ssp. <i>cucumerifolius</i> PI 468667	507	215	35		250
<i>H. praecox</i> ssp. <i>runyonii</i> PI 435849	507	227	35		262
<i>H. praecox</i> ssp. <i>hirsutus</i> PI 468847	507	198	27		225



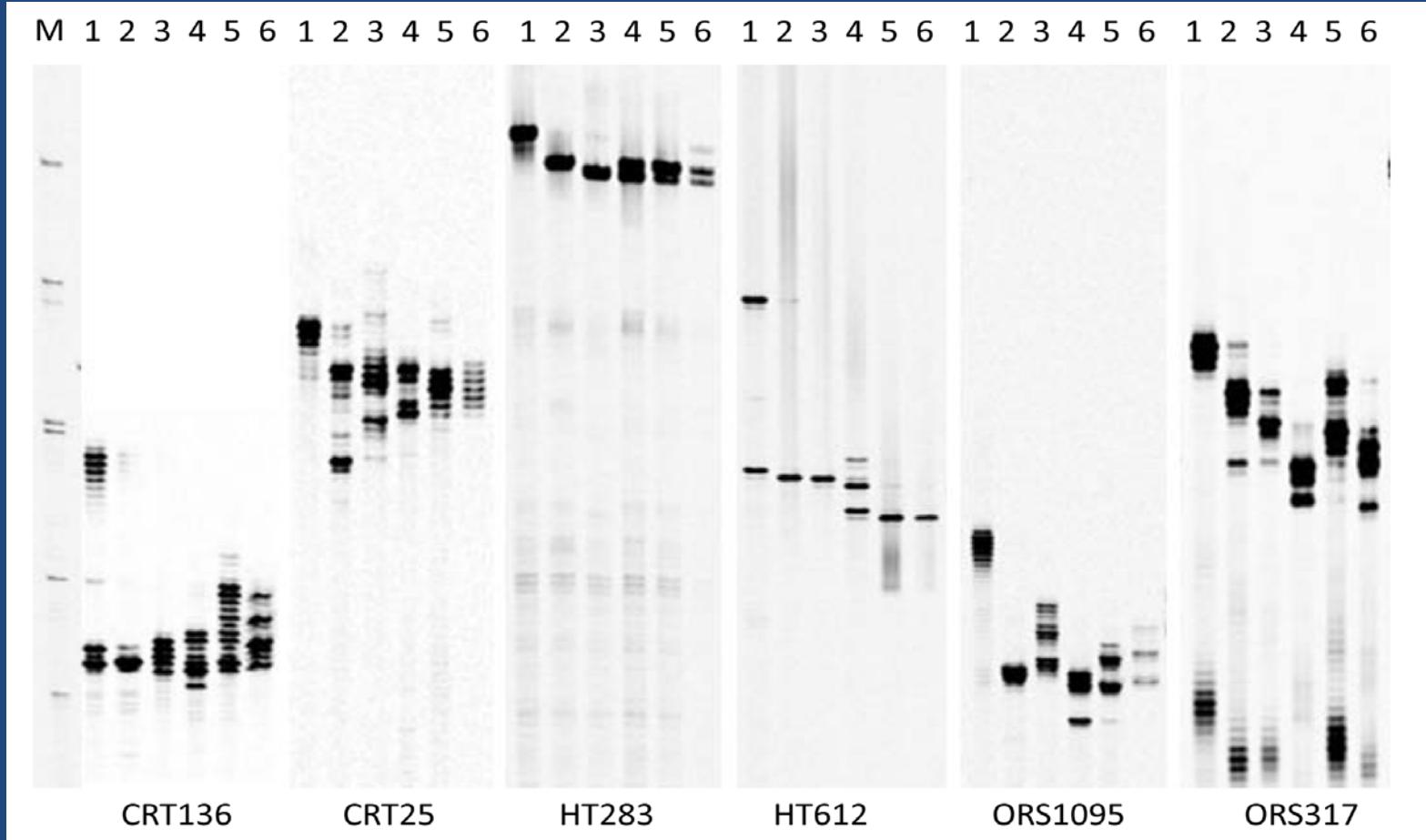


Fig. 2 Images for PCR patterns of some SSR markers among acceptor HA 89 and four wild species
1, HA 89; 2, *H. petiolaris* ssp. *fallax*; 3, *H. argophyllus*; 4, *H. debilis* ssp. *cucumberifolius*; 5, *H. praecox* ssp. *runyonii*; 6, *H. praecox* ssp. *hirsutus*



Table 6. Tracking of chromosome segments in introgression lines using SSR markers

Resistant donor	Pedigree	Generation	No. SSRs screened	No. wild alleles	Chromosome
<i>H. petiolaris</i> ssp. <i>fallax</i>	12F-3405-2	BC ₂ F ₄	508	1	15
PI435843	12F-3406-5	BC ₂ F ₄	508	1	15
<i>H. argophyllus</i> PI 494573	12F-3416-4	BC ₂ F ₄	256	23	8, 9, 10, 11

Conclusion

1. Translocated chromosome segments probably associate with stalk rot resistance.
2. These SSR markers would be used in breeding resistance

Acknowledgment

Angelia Hogness
Dr. Nikolay Balbyshev
Megan Ramsett
Michelle Gilley
Christopher Misar

National Sclerotinia Initiative



Thank you for your attention