

# Update on Transferring Sclerotinia Resistance Genes from Wild *Helianthus* Species into Cultivated Sunflower

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## Abstract

With the aim to introgress Sclerotinia resistance genes from wild perennials into cultivated sunflower, crosses and backcrosses have been made between interspecific amphiploids, and perennials with cultivated sunflower. The backcross progenies with 2n=34 chromosomes derived from different crosses were evaluated in replicated trials in 2009-2016. In 2016, 75 families were tested for stalk rot at Carrington, ND and Staples, MN, and 45 families for head rot at Carrington, ND and Staples, MN. However, the stalk rot trials were not successful, so only the results of the head rot trials will be presented. Several families of the first or second retests and new selections had good resistance to head rot. Families with better resistance than the recurrent parents identified in the different trials will be retested in 2017. The pollen of the BC<sub>1</sub>F<sub>1</sub>s derived from *H. strumosus* and *H. tuberosus* (2n=51) was crossed to NMS HA 89 in 2016 to obtain more BC<sub>2</sub>F<sub>1</sub> seeds. Previously produced BC<sub>1</sub>F<sub>1</sub> seeds (2n=51) of four perennials crossed with HA 89 were planted in 2016, which were crossed with HA 410 and NMS HA 89, respectively with the BC<sub>2</sub>F<sub>1</sub> seeds planted in the greenhouse. Seeds of more than 150 progeny families were field-increased. These materials will provide additional potential pools of resistance genes and increase the probability of identifying useful resistance QTLs. Crosses between seven Sclerotinia resistant sources and cultivated HA 234 were made in 2016 to develop the recombinant inbred line (RIL) populations for Sclerotinia resistance QTL mapping. Five resistant and six susceptible BC<sub>4</sub>F<sub>4</sub>/BC<sub>4</sub>F<sub>5</sub> progenies derived from *H. californicus* were planted for further analyzing the alien fragments using genomic *in situ* hybridization (GISH) and genotyping by sequencing (GBS) techniques. Thirteen amphiploids derived from eight wild perennial species will be released as germplasms.

## Introduction

Sclerotinia, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, attacks sunflower (*Helianthus annuus* L.) causing root, stalk, and head rot, and is one of the most damaging and difficult-to-control sunflower diseases (Gulya, 2004). Many wild perennial *Helianthus* species have been confirmed to be highly resistant to this fungus (Block et al., 2011; Feng et al., 2007a, b). In the last 12 years, our lab has focused on the introgression of resistance genes from the wild perennial *Helianthus* species into cultivated sunflower, via the traditional backcross method (Liu et al. 2011, 2013, 2015). The objectives of this study were to: (1) identify resistant wild perennial *Helianthus* species populations and interspecific amphiploids; (2) transfer resistance genes into a cultivated background; and (3) study the inheritance of resistance.

## Materials and Methods

- 1. Head rot resistance evaluation:** Replicated field evaluations for 45 families of 2015 retests and new selections derived from Phase 1 and 2 crosses were conducted at Carrington, ND and Staples, MN in 2016.
- 2. Mitotic chromosome counts:** Conducted using the standard Feulgen staining method.
- 3. Seed increase and backcrosses:** Seed of 152 backcross families derived from previous crosses were increased in the field in 2016. In the greenhouse, the pollen of the BC<sub>1</sub>F<sub>1</sub>s derived from *H. strumosus* and *H. tuberosus* (2n=51) was crossed to NMS HA 89 in 2016 to obtain more BC<sub>2</sub>F<sub>1</sub> seeds. The BC<sub>1</sub>F<sub>1</sub> plants (2n=51) of *H. smithii*, *H. atrorubens*, *H. laevigatus*, and *H. pauciflorus* (*rigidus*) with HA 89 were crossed with HA 410 and NMS HA 89, respectively.
- 4. QTL mapping population development:** Crosses between seven Sclerotinia resistant sources and cultivated HA 234 were made in 2016.

## Results

### 1. Sclerotinia head rot resistance evaluation of 2015 retests and new selections

**Table 1.** Replicated Sclerotinia head rot field evaluation of 2015 retest (Tests 2 and 3) and new interspecific crosses (Test 4) at Carrington, ND and Staples, MN in 2016.

Pedigree*	Carrington, ND 2016		Staples, MN 2016	
	Disease Rating	Infected Plants	Disease Rating	Infected Plants
<b>TEST 2 (Second Retest)</b>	0-5	%	0-5	%
((NMS HA 89 x GRO P1613793) HA 410*2), BC2F3 (47)	3.50	83	1.76	42
((NMS HA 89 x GRO P1613793) HA 410), BC2F3 (66)	1.83	38	1.94	44
((NMS HA 89 x GRO P1613793) HA 410), BC1F4 *new 69*	2.89	65	2.96	69
<b>Recurrent parent HA 410</b>	<b>4.88</b>	<b>92</b>	<b>3.71</b>	<b>89</b>
((NMS HA 89 x 1323(MAX) x HA 441), BC1F5 (7))	0.94	22	0.92	25
((NMS HA 89 x 1324(NUT) x HA 441), BC1F5 (8))	0.84	24	1.36	34
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F4 (9)	2.54	59	1.32	33
((NMS HA 89 x 1018 (MAX)) x HA 441), BC1F6 (10)	1.34	37	0.93	20
((NMS HA 89 x 1324 (NUT) x HA 441), BC1F5 (11))	1.13	40	0.37	11
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F4 (15)	1.60	47	2.13	52
((NMS HA 89 x 1008 (NUT)) x HA 441) HA 441, BC2F4 (16)	2.58	55	1.82	54
<b>Recurrent parent HA 441</b>	<b>1.39</b>	<b>42</b>	<b>2.15</b>	<b>57</b>
<b>TEST 3 (First Retest)</b>				
NMS HA 89 x (SAL x HA 410), F2 (1011)	1.94	52	1.18	30
NMS HA 89 x (SAL x HA 410), F2 (1014)	2.20	51	1.61	43
NMS HA 89 x (SAL x HA 410), F2 (1063)	4.17	85	3.08	77
NMS HA 89 x (SAL x HA 410), F2 (1069)	3.89	84	2.26	58
NMS HA 89 x (SAL x HA 410), F2 (1072)	1.69	44	1.40	36
NMS HA 89 x (SAL x HA 410), F2 (1074)	2.23	51	2.50	53
NMS HA 89 x (SAL x HA 410), F2 (1077)	3.94	82	3.67	89
NMS HA 89 x (OCC x HA 410), F2 (1047)	1.74	40	1.91	43
NMS HA 89 X (OCC X HA 410), F2 (1085)	3.68	84	1.61	41
NMS HA 89 X (OCC X HA 410), F2 (1097)	3.50	73	2.60	56
NMS HA 89 X (OCC X HA 410), F2 (1098)	4.59	95	3.24	79
<b>Recurrent parent HA 410</b>	<b>4.41</b>	<b>90</b>	<b>2.68</b>	<b>68</b>
<b>TEST 4 (New Selections)</b>				
NMS HA 89 x (SAL x HA 410), F2 (1120)	2.59	47	1.90	44
NMS HA 89 x (SAL x HA 410), F2 (1121)	2.00	52	0.69	21
NMS HA 89 x (SAL x HA 410), F2 (1137)	4.05	86	2.97	71
NMS HA 89 x (SAL x HA 410), F2 (1138)	4.12	98	2.13	68
NMS HA 89 x (SAL x HA 410), F2 (1139)	4.25	85	2.19	54
NMS HA 89 x (OCC x HA 410), F2 (1163)	2.39	54	2.18	48
NMS HA 89 x (OCC x HA 410), F2 (1195)	3.53	75	2.04	51
NMS HA 89 x (OCC x HA 410), F2 (1208)	1.92	58	0.68	51
<b>Recurrent parent HA 410</b>	<b>4.24</b>	<b>85</b>	<b>2.73</b>	<b>60</b>
<b>Checks</b>				
<b>Susceptible check HA 89 (S)</b>	<b>3.86</b>	<b>83</b>	<b>3.26</b>	<b>82</b>
<b>Susceptible check Cargill/Mycogen (270/272) (S)</b>	<b>2.13</b>	<b>54</b>	<b>4.04</b>	<b>82</b>
<b>Resistant check Croplan 305 (R)</b>	<b>1.86</b>	<b>41</b>	<b>1.95</b>	<b>47</b>
<b>Resistant check Croplan 343 (R)</b>	<b>0.87</b>	<b>3</b>	<b>0.95</b>	<b>27</b>

\*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. The number in parenthesis at the end of each pedigree is the family ID of the selected family.

**Table 2.** Replicated Sclerotinia head rot field evaluation of 2015 new interspecific crosses (Test 5) at Carrington, ND, and Staples, MN in 2016.

Pedigree*	Carrington, ND 2016		Staples, MN 2016	
	Disease Rating	Infected Plants	Disease Rating	Infected Plants
<b>TEST 5 (New Selections)</b>	0-5	%	0-5	%
[(OCC P1494594 x HA 410 <sup>5</sup> )](x) &/or x HA 89], BC4F3 (5085)	2.81	60	3.17	70
[(OCC P1494594 x HA 410 <sup>5</sup> ], x HA 89], BC4F2 (5086)	3.33	67	2.89	65
[(OCC P1494594 x HA 410 <sup>5</sup> ], x HA 89], BC4F2 (5087)	3.38	72	3.08	67
{(NMS HA89 x DIV) x HA 410 <sup>2</sup> }, (x), BC2F4 (5090)	3.03	68	2.12	55
{(NMS HA89 x DIV) x HA 410 <sup>2</sup> }, (x), BC2F4 (5091)	4.43	94	2.30	53
{(NMS HA89 x DIV), (x), BC2F4 (5092)}	5.00	100	5.00	100
{(NMS HA89 x DIV), (x), BC2F4 (5093)}	4.42	92	1.81	38
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5094)	4.18	88	2.93	63
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5095)	4.90	100	3.32	68
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5097)	4.21	88	4.32	89
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5098)	4.21	86	3.68	79
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5099)	4.75	100	4.17	83
{(NMS HA89 x DIV) x HA 410 <sup>3</sup> }, BC4F2 (5100)	4.31	94	2.71	68
<b>Recurrent parent HA 410</b>	<b>3.77</b>	<b>77</b>	<b>3.55</b>	<b>82</b>
<b>Checks</b>				
<b>Susceptible check HA 89 (S)</b>	<b>3.30</b>	<b>70</b>	<b>3.50</b>	<b>88</b>
<b>Susceptible check Cargill/Mycogen (270/272) (S)</b>	<b>2.43</b>	<b>57</b>	<b>3.18</b>	<b>64</b>
<b>Resistant check Croplan 305 (R)</b>	<b>1.67</b>	<b>33</b>	<b>2.45</b>	<b>60</b>
<b>Resistant check Croplan 343 (R)</b>	<b>0.69</b>	<b>23</b>	<b>0.22</b>	<b>6</b>

\*The first three letters of the *Helianthus* species are used to identify the species source: DIV=*H. divaricatus*; and OCC=*H. occidentalis*. (S)= Susceptible; (R)=Resistant. The number in parenthesis at the end of each pedigree is the family ID of the selected family.

### 2. QTL mapping populations development and entries from *H. californicus* for further characterization

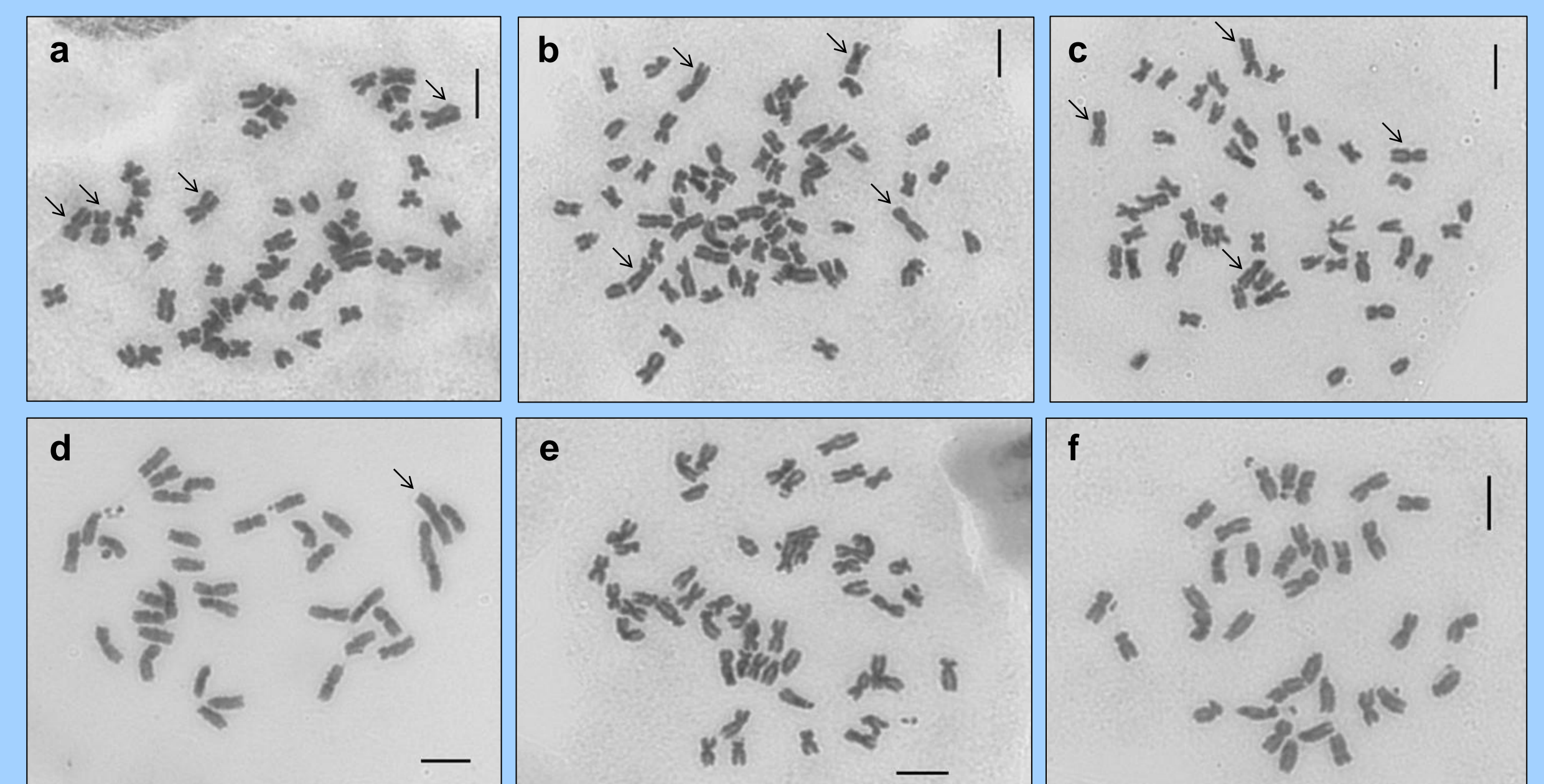
**Table 3.** Crosses between seven Sclerotinia sources and cultivated HA 234 to develop RIL populations for Sclerotinia resistance QTL mapping.

Entry #	Source	Pedigree	Selfed/Sib seed set (%)	Crossed seed set (%)
HR21	09/4008	(NMS HA 89 x 1018 (MAX)) x HA 441 <sup>3</sup> , BC3F3	43.3	55
HR92	09/4041	(NMS HA 89 x 1008 (NUT)) x HA 441, BC1F5	65	56.7
SR24	09/4011	(NMS HA 89 x 1018 (MAX)) x HA 441 <sup>3</sup> , BC3F3	15	26.9
SR99	10/4144	(NMS HA 89 x 1008 (NUT)) x HA 441, BC1F4	55	67
SR279	11/4484	{(DIV(68) x GRO (68)) x HA 410 <sup>3</sup> , BC2F2} x HA 410, BC3F2	43	65
SR288	11/4489	{STR (68) x HA 410 <sup>3</sup> , BC2F2} x HA 410, BC3F2	51	55
SR216	09/4271	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	-	20

**Table 4.** Five resistant and six susceptible entries to stalk rot (SR) derived from *H. californicus* (CAL) selected for further characterization by GISH and/or GBS techniques.

Entry #	Source	Pedigree	SR Score (%)	Selfed seed set (%)
28	09/4104	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0	25.00
216	10/4386	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0	67.5
235	11/4470	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0	61.25
258	11/4478	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0	27.54
304	09/4306	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0	18.33
29	09/4106	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	8-63	40.00
30	09/4110	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0-67	41.25
32	09/4114	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	0-56	45.00
41	09/4163	CAL 2376 x HA 410 <sup>5</sup> , BC4F5	0-50	47.50
228	11/4466	CAL 2376 x HA 410 <sup>5</sup> , BC4F4	33-45	34.21
317	11/4497	CAL 2376 x HA 410 <sup>5</sup> , BC4F5	7-46	72.50

### 3. Mitotic chromosome analysis in backcross progenies



**Figure 1.** Chromosome spreads of several backcross progenies derived from *H. atrorubens* (a-d) and *H. laevigatus* (e and f). (a) G95/72 x HA 89, BC<sub>1</sub>F<sub>1</sub>, 2n=51; (b) G16/1039 x HA 410, BC<sub>1</sub>F<sub>2</sub>, 2n=68; (c) G16/1039 x HA 410, BC<sub>2</sub>F<sub>1</sub>, 2n=49; (d) NMS HA 89 x G16/1036-46, BC<sub>2</sub>F<sub>1</sub>, 2n=35; (e) G16/1053 x HA410, BC<sub>2</sub>F<sub>1</sub>, 2n=50; (f) NMS HA 89 x G16/1053-64, BC<sub>2</sub>F<sub>1</sub>, 2n=34. Notice that there are large chromosomes (arrows) from *H. atrorubens* in the cultivated background in Figures a-d. Bars=5 µm.

## Summary

1. Replicated field evaluation in 2016 of 2015 retests for head rot resistance confirmed successful introgression of resistance genes.
2. Seed was increased in the field for more than 150 progeny families in 2016. The BC<sub>1</sub>F<sub>1</sub> progenies (2n=51) derived from four perennials were advanced to the BC<sub>2</sub>F<sub>1</sub> generation.
3. Seven Sclerotinia resistant sources were crossed with cultivated HA 234 in 2016 to develop the RIL populations for Sclerotinia resistance QTL mapping.

## References

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