

Novel Sources of Sclerotinia Basal Stalk 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. Sclerotinia basal stalk rot is one of the most serious diseases of sunflower. The genetics of resistance to basal stalk rot (BSR) is quantitative, requiring many genes, which complicates breeding efforts. There are 53 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 (GBS) 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 (LGs) 1, 3, 8, 9, 10, and 11. Additionally, 411 progeny families from crosses of amphiploid, hexaploid, and diploid perennials with cultivated lines were screened for BSR. 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. Twelve BSR germplasms are in the process of being released based on their higher levels of resistance. Populations of sunflower crop wild relatives from the USDA-ARS-NGS gene bank 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 basal stalk rot disease. Its mode of infection and the genetics of resistance are completely different from other forms of Sclerotinia infection. 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 53 species, 14 annual diploids (n=17) and 39 perennial species. Wild annual and perennial species have been an excellent source of resistance genes for economically important sunflower diseases (Seiler and Rieseberg, 1997). Basal stalk rot resistance was identified in wild annual species of *H. argophyllus*, *H. praecox*, and *H. petiolaris*, and several wild perennial species. Wild annual and perennial species were selected to transfer Sclerotinia BSR resistance into cultivated sunflower. In this poster we report the progress of transferring Sclerotinia BSR 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

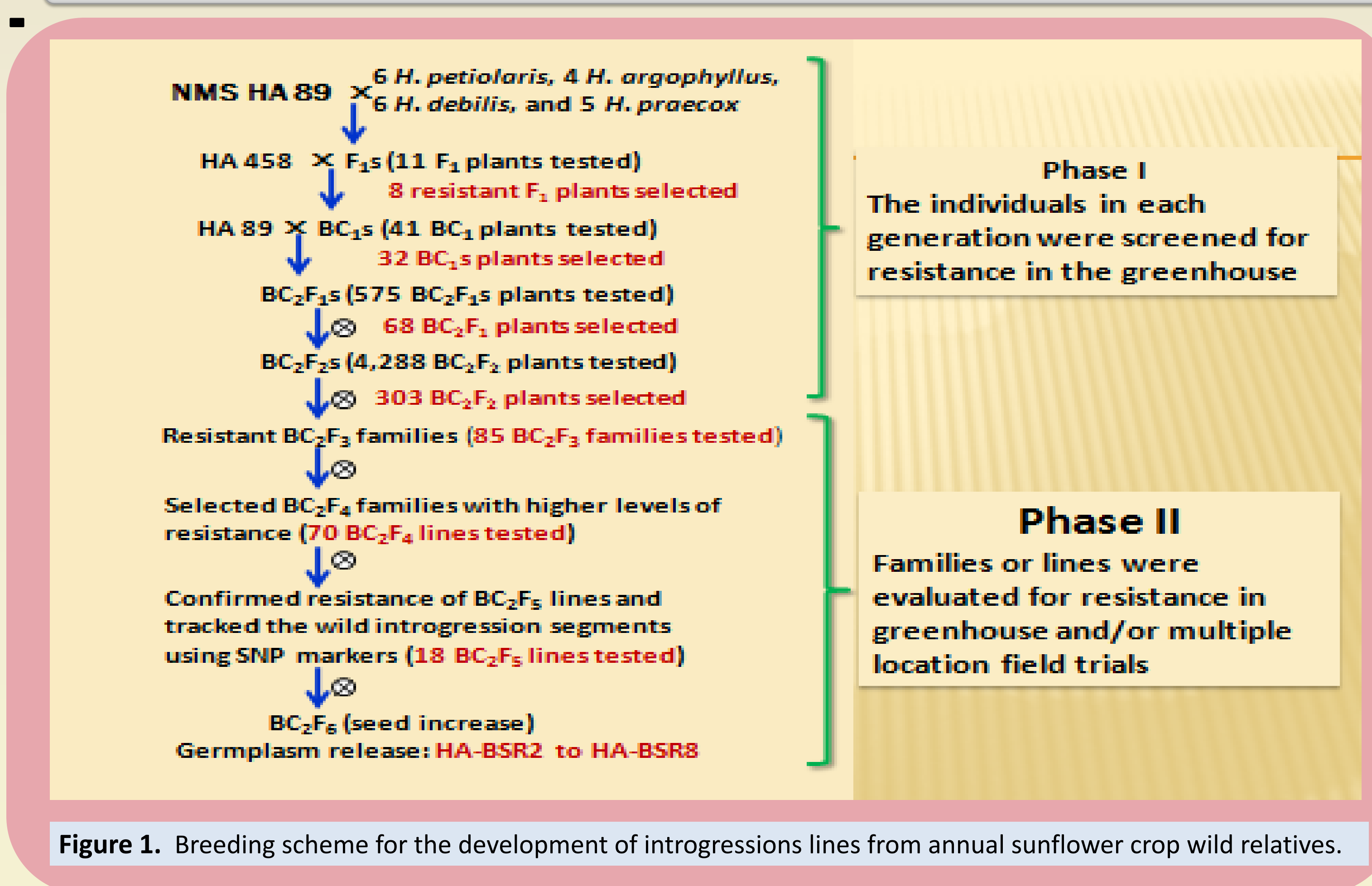


Figure 1. Breeding scheme for the development of introgression lines from annual sunflower crop wild relatives.

Results and Discussion

Transfer of Sclerotinia BSR resistance from wild annual species into cultivated sunflower is moving forward (Fig. 1). Wild introgressed families consistently showed superior Sclerotinia BSR resistance in the field evaluations tested over the years (Table 1). In 2014, 23 BC₂F₄ families derived from *H. argophyllus*, *H. petiolaris*, and *H. praecox* were evaluated for BSR resistance in multi-location field trials (Fig. 2). Across environments, all the introgressed BC₂F₄ families showed significantly higher Sclerotinia BSR resistance than both the susceptible checks, Cargill 270 and HA 89 (36% DI). The level of resistance in the introgressed families was similar to the resistant check, Croplan 305 (9.6% DI). However, ten BC₂F₄ 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 | | 2013 | | 2014 |
| Pedigree | BC ₂ F ₃ | Pedigree | BC ₂ F ₄ | BC ₂ F ₄ | BC ₂ F ₄ |
| <i>H. petiolaris</i> ssp. <i>fallax</i> | 11-256-049 | 0 | 12F-3405-2 | 4.0 | 8.4 |
| PI 435843 | 11-256-053 | 0 | 12F-3406-5 | 5.6 | - |
| <i>H. argophyllus</i> | 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 |
| | 11-291-45 | 5.3 | 12F-3451-4 | 3.9 | 7.9 |
| <i>H. praecox</i> ssp. <i>runyonii</i> | 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 |

Table 1. Evaluation of the 13 most resistant BC₂F₃ families and derived BC₂F₄ lines for Sclerotinia basal stalk rot resistance in the field from 2012 to 2014.

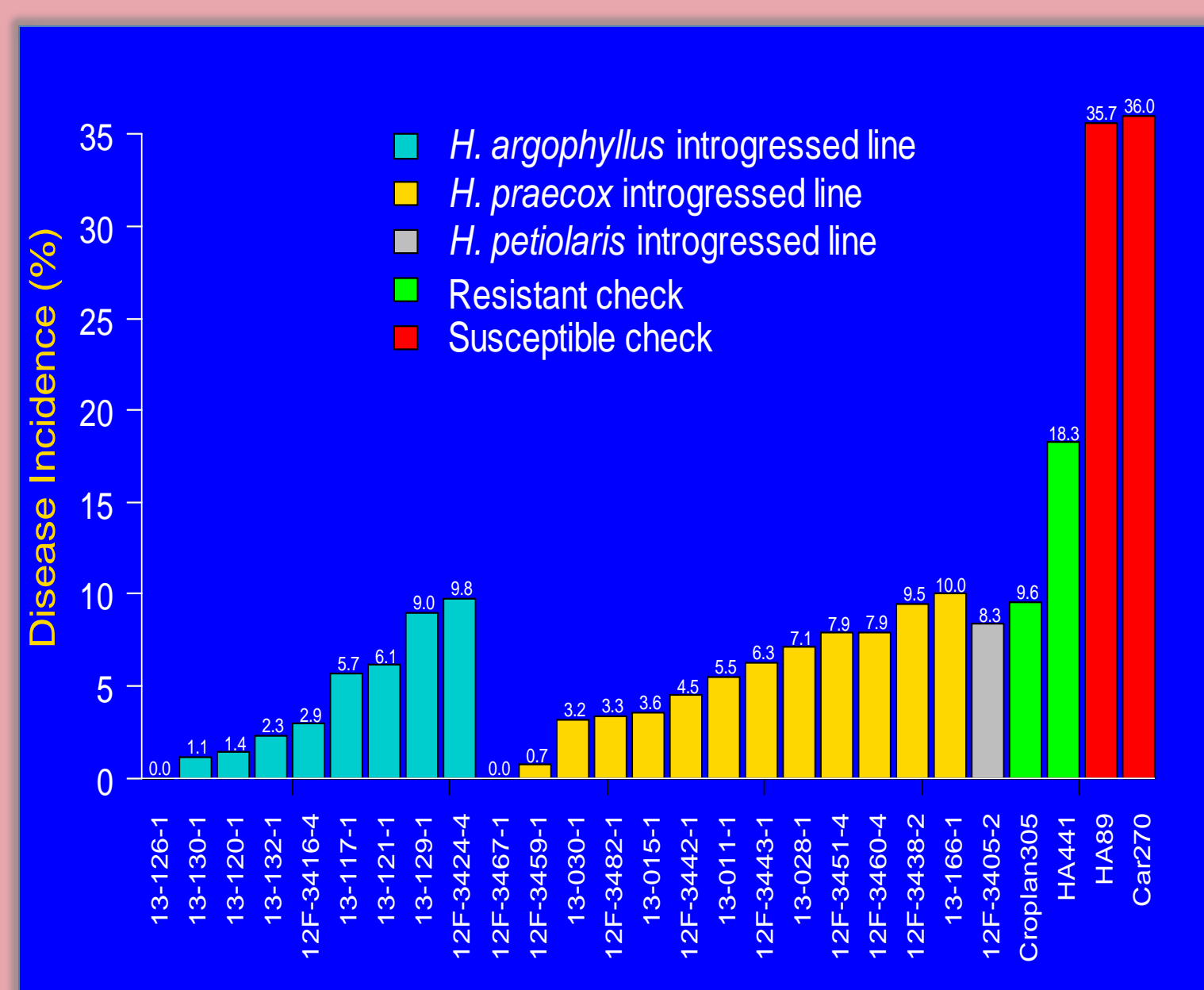


Figure 2. Mean performance of 23 BC₂F₄ families for Sclerotinia basal stalk rot resistance at two locations in North Dakota during 2014.

In 2015, six *H. argophyllus*, two *H. petiolaris*, and eight *H. praecox* derived BC₂F₅ 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 BC₂F₅ introgression lines showed significantly higher BSR resistance than the resistant inbred check, HA 441 (Fig. 3). Most of the introgression lines either showed significantly higher or similar levels of BSR resistance than the resistant 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 LGs 1, 3, 8, 9, 10, and 11. Some of these introgressed segments might be associated with BSR resistance. (Fig. 4).

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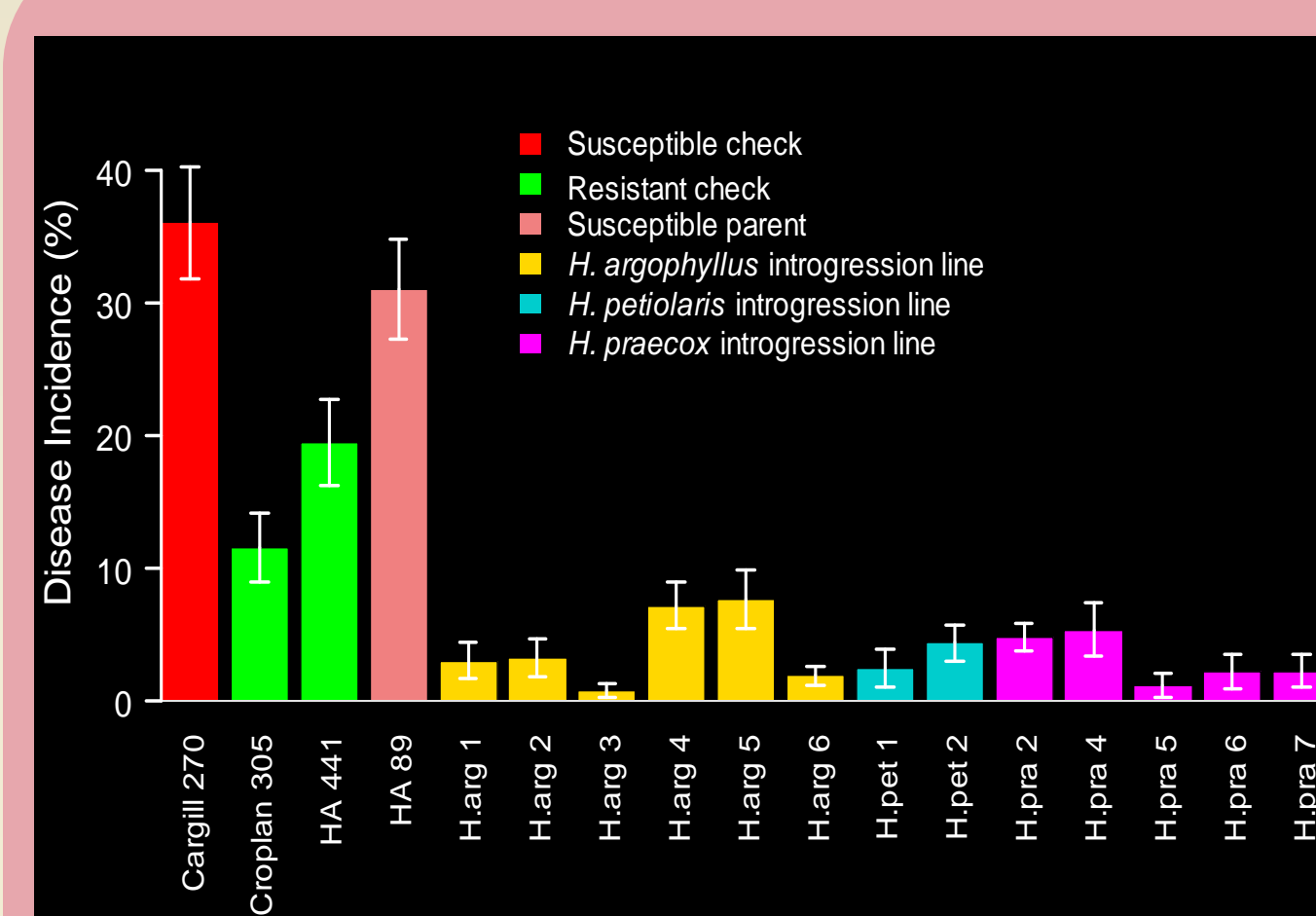


Figure 3. Mean BSR disease incidence (DI) of the most resistant wild sunflower introgression lines evaluated in seven environments during 2012-2015.

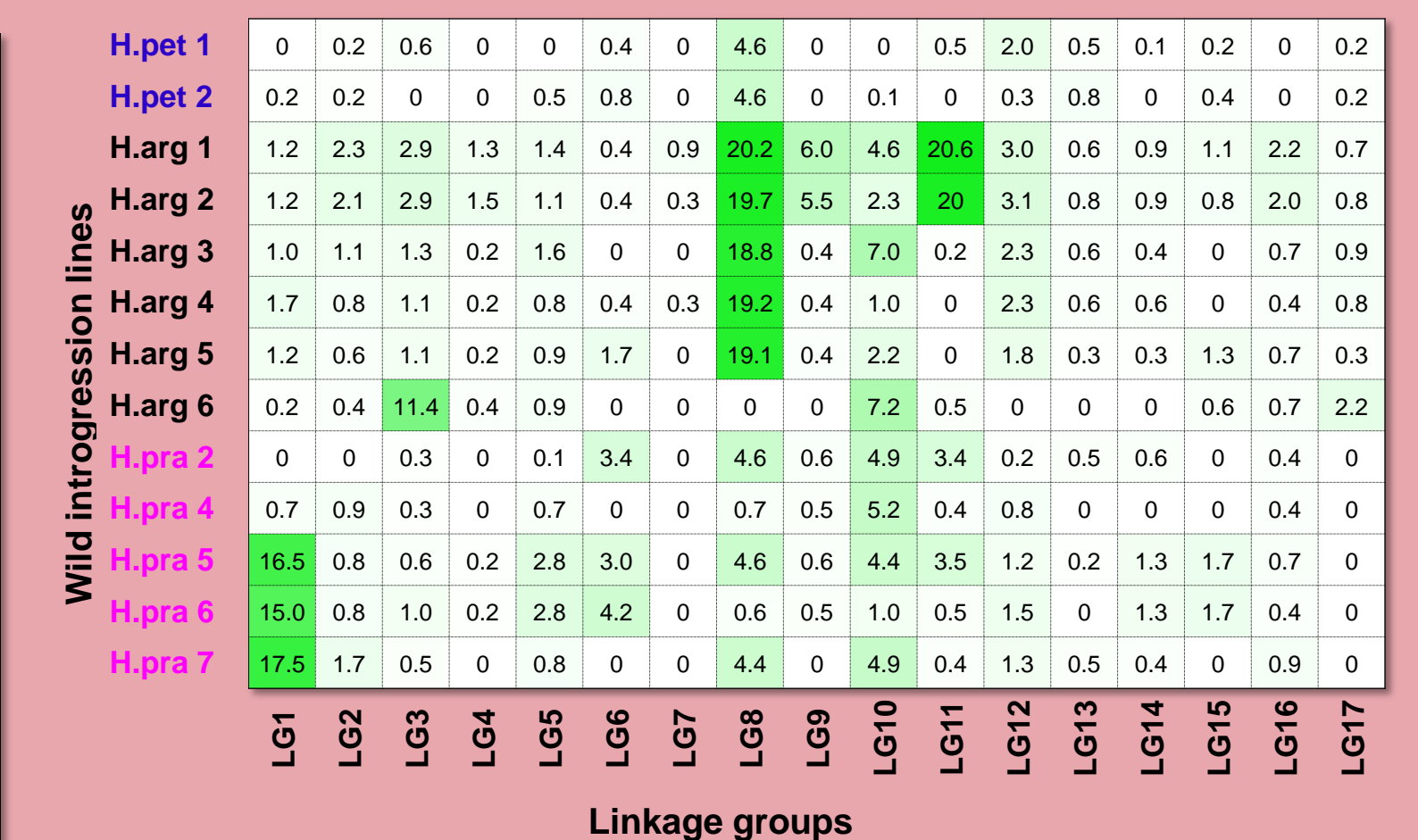


Figure 4. Tracking of *H. argophyllus*, *H. petiolaris*, and *H. praecox* chromosome segments in highly BSR resistant wild sunflower introgression lines using SNP markers. Intensity of the green color indicates the percentage of polymorphism between the parents and the introgressed lines.

Introgression of Sclerotinia resistance from perennial crop wild relatives

Several of the interspecific lines derived from wild perennial species showed very good resistance to BSR. 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 and the checks were identified (Table 2). 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 2). Seven Sclerotinia BSR tolerant germplasms from wild annual and five from perennial species field evaluated from 2009-2015 are scheduled for release in 2016-2017 (Table 3).

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

Summary

- Replicated field evaluations in 2009-2015 confirmed the successful introgression of Sclerotinia BSR resistance genes from annual and perennial sunflower crop wild relatives.
- GBS revealed the presence of *H. argophyllus* segments in LGs 3, 8, 9, 10, and 11 of the sunflower genome with LG 8 having the highest frequency.
- Progeny families with increased resistance for BSR will be selected for the development of QTL mapping populations.
- Twelve germplasm lines with increased levels of resistance for Sclerotinia BSR will be released in the near future.

References

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