Transferring Sclerotinia Stalk Rot Resistance Genes from Wild *Helianthus* Species into Cultivated Sunflower

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Abstract

Replicated field tests of 313 progeny families screened for stalk rot resistance at Carrington, ND in 2009 showed good introgression of resistance genes. These materials were planted again in 2010 for a second year of field evaluation, as well as the new families with seed increased in 2009. In 2010, replicated field tests with a total of 413 progeny families were screened for stalk rot resistance. Two years of data for the 313 entries for stalk rot resistance are reported. We have decided to eliminate the heavily infected families from both years, and further evaluate only the lightly infected families in 2011. Molecular tracking using SSR markers suggested a higher frequency of gene introgression when perennial diploids species were used. In 2010, eight accessions from three diploid and one tetraploid perennial species were established in the greenhouse for crossing with HA 410 and HA 451. The new crosses will provide more diverse resistance genes for developing Sclerotinia resistant germplasm.

Introduction

The necrotrophic 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). Wild perennial *Helianthus* species have been identified to contain an abundance of resistance genes to this fungus (Feng et al., 2007a, b; Feng et al., 2008; Feng et al., 2009). Genetic analysis has indicated that Sclerotinia resistance is multigenic (Gentzbittel et al., 1998), and that resistance to basal stalk and head rot are not related.

The objectives of this study were to: (1) transfer Sclerotinia stalk rot resistance from resistant wild perennial hexaploid and diploid *Helianthus* accessions, and interspecific amphiploids into cultivated sunflower, via the traditional backcross method; and (2) evaluate stalk rot resistance via field testing to identify progenies with higher levels of resistance.

Materials and Methods

Field test

Three hundred and thirteen progeny families in various backcross (BC) generations were screened for stalk rot resistance in replicated field trials at Carrington, ND in 2009 and 2010. All

BC progenies were obtained from eight accessions of four diploid species (*H. maximiliani*, *H. nuttallii*, *H. giganteus*, and *H. grosseserratus*), five amphiploids (*H. strumosus* × P21, *H. grosseserratus* × P21, *H. maximiliani* × P21, *H. nuttallii* × P21, and (*H. divaricatus* × P21) × (*H. grosseserratus* × P21), and one hexaploid (*H. californicus*) species. Stalk rot was rated as percent of plants infected on a 0 to 100 scale.

Polymorphic marker screening

Using 401 SSR primers, polymorphic markers were screened among wild perennial species, NMS HA89, and HA 410. The co-dominant markers in the F_1 progeny were selected for the analysis of the plants in BC generations with 2n=34 chromosomes.

Molecular tracking

In total, 292 plants with 2n=34 chromosomes were analyzed with polymorphic SSR markers, including 187 BC_1F_2 and BC_2F_1 plants from the crosses involving *H. maximiliani* (29 individuals), *H. giganteus* (27 individuals), and *H. grosseserratus* (131 individuals), 15 BC_2F_1 plants from the crosses involving amphiploids, and 90 BC_4F_2 plants from the crosses involving *H. californicus*. For each polymorphic primer, the percentages of wild segments derived from different backcrosses were calculated from the percentage of the plant with banding patterns different from the cultivated sunflower in different populations. The number of wild segments per plant and the percentage of progeny with wild segments were calculated based on the average data of 20 to 22 SSR markers for each cross, respectively.

Results and Discussion

Field evaluations for stalk rot resistance in 2009 and 2010

A two-year field evaluation for stalk rot resistance was conducted in 2009 and 2010 at Carrington, ND, for the 313 entries derived from wild perennial species backcrossed with HA 410 and HA 441, including the entries originally planned for a head rot resistance study. Table 1 shows the percentages of the infected plants and the entries tested for the two-year evaluation. Although the susceptible and resistant checks, as well as the recurrent parent HA 410 showed lower infection rates in 2010 than in 2009, the relative resistant and susceptible entries from the two-year evaluation were identified from different backcrosses. One amphiploid bulk had 5% in 2009, which showed 0% of infection in 2010. Three amphiploids involving wild perennials had 0% of infection in both years. It was clearly shown that the amphiploids have high levels of resistance to stalk rot. Also, some entries derived from head rot resistance sources also showed good stalk-rot resistance. The lines with high susceptibility to stalk rot will be eliminated from further evaluation and those with low infection rate will be evaluated further in 2011.

Table 1. Field screening for Sclerotinia stalk rot resistance at Carrington, ND in 2009 (Black- upper line) and 2010 (Turquoise- lower line) with 313 entries replicated twice, plus two susceptible checks, HA 89 and Cargill 270, two resistant checks, Croplan 343 and Croplan 305, and the recurrent parent HA 410. The average percentages of infected plants of entries derived from the same source were used to evaluate the Sclerotinia stalk rot resistance.

Pedigree	Percent Infected Plants (%) (No. of Entries)						
*(DIV x P21, Amp)(GRO x P21, Amp) x HA410, BC2F2	7 (4) 2 (4)	25 (2) 3 (2)	8 (5) 3 (5)	9 (2) 0 (2)			
(GRO x P21, Amp) x HA 410, BC3F2	6 (1) 0 (1)	10 (1) 8 (1)	0 (1) 4 (1)	13 (3) 20 (3)	10 (1) 8 (1)		
(MAX x P21, Amp) x HA 410, BC2F2	0 (1) 0 (1)	15 (1) 20 (1)	2 (1) 0 (1)				
(NUT x P21, Amp) x HA 410, BC2F2	21 (2) 0 (2)	0 (2) 2 (2)	11 (3) 2 (3)	18 (6) 1 (6)			
(STR x P21, Amp) x HA 410, BC2F2	15 (2) 9 (2)	16 (11) 2 (11)	4 (5) 3 (5)	25 (1) 0 (1)	14 (2) 7 (2)		
CAL 2376 x HA 410, BC4F2-BC5F3	10 (5) 1 (5)	7 (28) 1 (28)	6 (26) 1 (26)	5 (14) 1 (14)	6 (4) 2 (4)		
(NMS HA 89 x NUT 1008) x HA 441, BC1F4-BC2F3	5 (2) 10 (2)	0 (1) 5 (1)	10 (8) 9 (8)	8 (7) 7 (7)	20 (3) 9 (3)	4 (12) 3 (12)	
(NMS HA 89 x NUT 1324) x HA 441, BC1F4-BC2F4	17 (14) 11 (14)	10 (9) 4 (9)	6 (2) 3 (2)	6 (1) 14 (1)	5 (9) 9 (9)	8 (3) 17 (3)	4 (3) 6 (3)
(NMS HA 89 x MAX 1018) x HA 441, BC1F5-BC2F4	12 (7) 18 (7)	25 (7) 9 (7)	5 (4) 10 (4)	9 (1) 0 (1)	32 (1) 4 (1)	8 (3) 13 (3)	4 (2) 6 (2)
“	7 (15) 5 (15)	14 (3) 6 (3)					
(NMS HA 89 x MAX 1323) x HA 441, BC1F4-BC3F3	3 (9) 3 (9)	3 (8) 5 (8)	9 (4) 1 (4)	5 (3) 1 (3)			
(NMS HA 89 x MAX 1314) x HA 441, BC2F2-BC2F4	5 (2) 3 (2)	9 (6) 7 (6)	0 (1) 0 (1)	2 (1) 0 (1)	4 (1) 0 (1)	3 (1) 0 (1)	
(NMS HA 89 x GIG = PI547182) x HA 410, BC2F2	12 (3) 5 (3)						
(NMS HA 89 x GRO = PI613793) x HA 410, BC1F3	17 (3) 12 (3)	6 (6) 4 (6)	20 (2) 12 (2)	14 (9) 15 (9)			
(NMS HA 89 x MAX= PI586892) x HA 410, BC1F3-BC2F2	4 (3) 4 (3)	27 (2) 6 (2)	16 (1) 15 (1)				
Amphiploids (DIV, GRO, HIR, MAX, NUT, STR)	0 (1) 0 (1)	0 (1) 0 (1)	0 (1) 0 (1)	0 (1) 4 (1)	8 (1) 1 (1)		
Amiphloid bulk	5 (1) 0 (2)						
CROPLAN 343 (Resistant check)	14 (12) 5 (21)						
CROPLAN 305 (Resistant check)	5 (12) 1 (21)						
CARGILL 270 (Susceptible check)	22 (12) 4 (39)						
HA 89 (Susceptible check)	21 (10) 9 (17)						
HA 410 (Recurrent parent)	28 (12) 10 (16)						

* The first three letters of the *Helianthus* species are used, followed by an accession number.

Molecular tracking of 2n=34 individuals with polymorphic primers

Representative gel analysis for molecular tracking is shown in Figure 1. The data using 11 polymorphic markers from 11 linkage groups showed the percentages of wild segments in the whole population derived from different backcrosses ranged from 0 to 39.13% for different markers (Figure 2). Using 20 to 22 SSR markers for each cross, the average number of wild segments per plant was 0.31 to 4.42, and the percentage of progeny with wild segments were 1.56 to 21.91%. Molecular tracking of perennial species-specific markers in progeny families derived from the crosses involving *H. maximiliani*, *H. giganteus*, and *H. grosseserratus*, amphiploids, and hexaploid *H. californicus* utilizing co-dominant SSR markers indicated a higher frequency of introgression from diploid perennials than from hexaploid or interspecific amphiploids. The five sources ordered from high to low are: *H. grosseserratus*, *H. maximiliani*, *H. giganteus*, amphiploids, and *H. californicus* (Figure 3). The data suggested that there is an advantage in using diploid perennials for gene introgression.

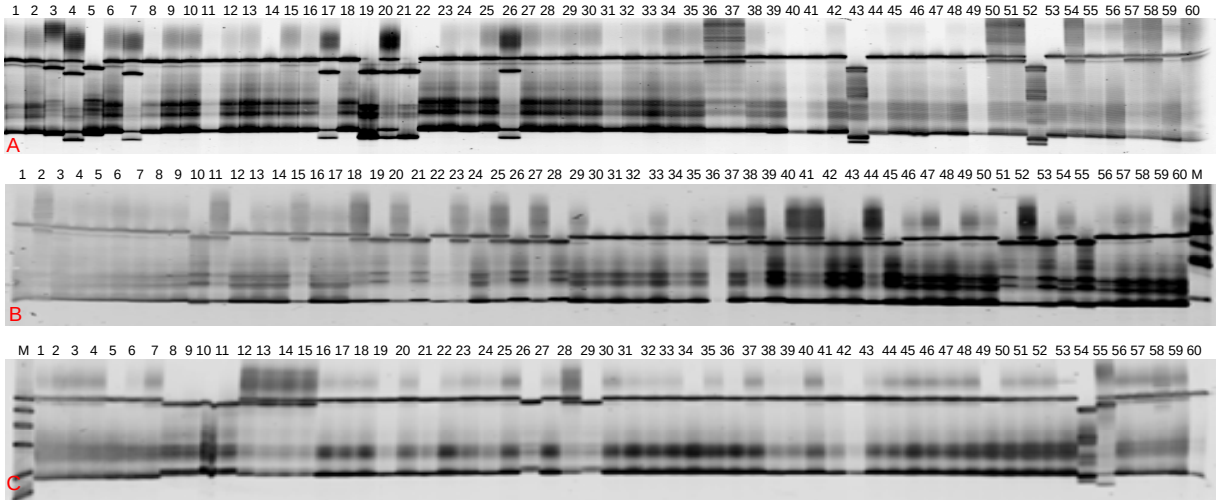

Figure 1. A gel of 162 individuals with 2n=34 chromosomes from the BC_1F_2 and BC_2F_1 generations of crosses involving diploids and amphiploids with the primer ORS541. Patterns representing the wild species were recorded in the progenies. In Figure 1A, 1, 2, 35: NMS HA 89; 3,4: F_1 of NMS HA 89 x *H. giganteus* (PI 547182); 5: *H. giganteus* (PI 547182); 6, 38: HA 410; 7: *H. giganteus* (PI 547177) x HA410. 8-34: Progenies of NMS HA89 x *H. giganteus* (PI 547182); 36, 37, 50, 51: NMS HA89 x *H. grosseserratus* (PI 613793); 43, 52: *H. grosseserratus* (PI 613793); 39-42, 44-49, and 53-60: Progenies of NMS HA89 x *H. grosseserratus* (PI 613793). Figure 1B (1-60) and Figure 1C (1-53): Progenies of NMS HA89 x *H. grosseserratus* (PI 613793). Figure 1C: 54: *H. grosseserratus* (PI 613793); 55: P21; 56: HA 410; 57-58: (Amphiploid of *H. grosseserratus* × P21) x HA410, BC_2F_1; 59-60: (Amphiploid of *H. nuttallii* × P21) x HA410, BC_1F_1.

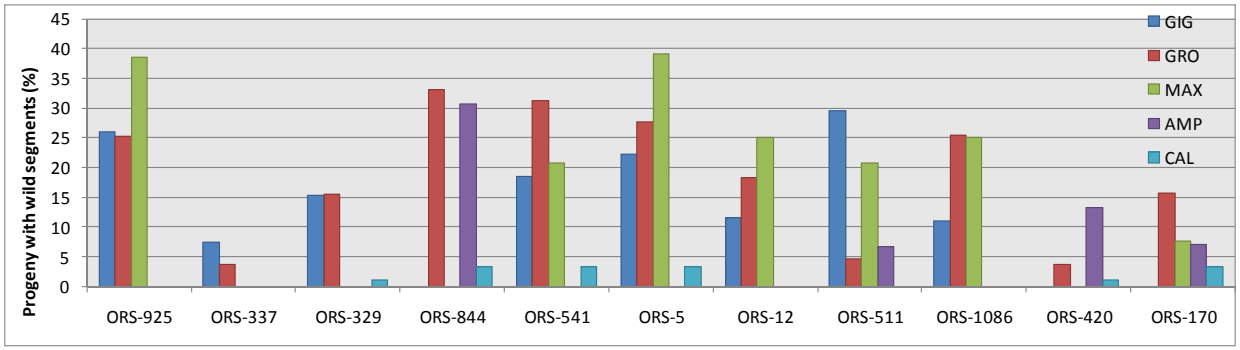

Figure 2. Progenies with different percentages of wild segments derived from different backcrosses were detected using 11 SSR markers from 11 linkage groups, suggesting that the progenies from the diploid wild species, *H. giganteus*, *H. grosseserratus*, and *H. maximiliani*, contained many more wild segments than those from amphiploids or hexaploid *H. californicus*.

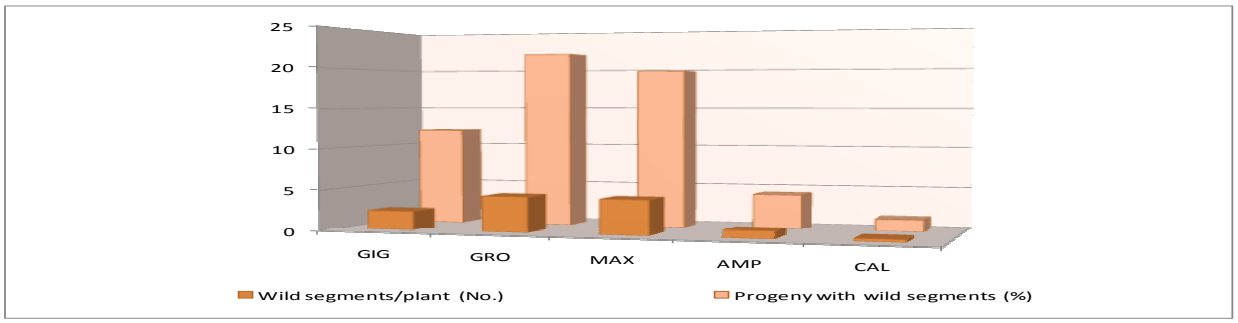

Figure 3. Comparison of the number of wild segments per plant and the percentage of progeny with wild segments among the different backcrosses detected by 20 to 22 SSR markers showed that the order of the five sources range from high to low is: *H. grosseserratus*, *H. maximiliani*, *H. giganteus*, amphiploids, and *H. californicus*.

Summary

A two-year field evaluation for stalk rot resistance showed progeny families derived from all the stalk rot resistant sources were performing well, and the lines originally derived from head rot resistant sources also showed good stalk rot resistance. The lines highly susceptible to stalk rot will be eliminated from further evaluation, and the lines with low infection rate will be evaluated further in 2011. Molecular tracking of perennial species-specific markers in progeny families utilizing SSR markers indicated a higher frequency of gene introgression from diploid perennials than from interspecific amphiploids or hexaploid, suggesting an advantage in using diploid perennials. Detailed analysis for the stalk rot resistance of each progeny family and the possible introgression of the wild segments will be studied in future experiments. In 2010, eight accessions from three diploid and one tetraploid perennial species were established in the

greenhouse. These new sources will be crossed with HA 410 and HA 451. Inbred line HA 451 is tolerant to both head and stalk rot. The backcross method will be used to transfer more diverse Sclerotinia resistance genes into cultivated sunflower.

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