Advancement of Pyramiding New *Sclerotinia* Stem Rot Resistance Genes from *H. californicus* and *H. schweinitzii* into Cultivated Sunflower

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Abstract

Interspecific hybridization of *H. californicus* and *H. schweinitzii* with HA 410 was conducted in 2006 based on the BC_1F_1s obtained in 2005. In total, 109 BC_1F_1 plants were grown in the greenhouse for backcrossing. Because of unbalanced chromosome numbers in the triploid BC_1F_1, the BC_1F_1 pollen fertility ranged from 2.45% to 4.63%, and seed set was very low. In total, 59 BC_2F_1 seeds were obtained from 136,220 pollinated florets. Based on these results, we are re-crossing the BC_1F_1 with HA 410 to produce more BC_2F_1 seeds to screen for stem rot resistance.

Introduction

Sclerotinia is a major disease in cultivated sunflower (*H. annuus*) across the world and present-day sunflower hybrids lack high resistance to *Sclerotinia* (Gulya et al., 1997). The USDA-ARS sunflower breeding program at Fargo has released some inbred lines with moderate tolerance to Sclerotinia head rot (HA 441) and stalk rot (HA 410) (Miller and Gulya, 1999). In general, wild *Helianthus* species possess more variability for Sclerotinia stem rot resistance than cultivated sunflower. An abundance of wild *Helianthus* accessions have been evaluated for Sclerotinia stem rot resistance with the perennial species having been found to be highly resistant (Tom Gulya, personal communication). A program of transferring resistance from two hexaploid perennials, *H. californicus* and *H. schweinitzii*, into tolerant line HA 410 was initiated in 2005. Results of crossing *H. californicus* and *H. schweinitzii* with HA 410, hybrid embryo formation and embryo rescue, and the performance of F_1 plants were reported by Feng et al. (2006). In total, 388 F_1 seedlings of *H. californicus* and 107 of *H. schweinitzii* crossed with HA 410 survived. Greenhouse evaluation indicated that these interspecific F_1 progeny possessed excellent stem rot resistance. Resistant F_1 plants were backcrossed with HA 410 in late 2006, and the cytological characteristics of the BC_1F_1 and BC_2F_1 progeny are reported here.

Materials and Methods

Resistant BC_1F_1 progenies obtained in 2005 from the crosses of *H. californicus* × HA 410 and *H. schweinitzii* × HA 410 (Feng et al., 2006) were further backcrossed with HA 410 to produce the BC_2F_1 progenies in the greenhouse. Backcross seed set was the number of seed divided by the number of florets pollinated. Pollen stainability and size of all BC_1F_1 and BC_2F_1 progenies were examined (Alexander, 1969). The chromosome numbers of individual plant progeny were determined by root tip chromosome counts using the standard Feulgen staining method.

Results and Discussion

The interspecific F_1 hybrids between resistant hexaploid accessions of *H. californicus* (2n=6x=102) or *H. schweinitzii* (2n=6x=102) with diploid cultivated HA 410 (2n=2x=34) were tetraploids (2n=4x=68) (Fig. 1), with relatively good pollen fertility, 32 to 54%, and backcross seed set of 2 to 11% (Feng et al., 2006). Their backcross BC_1F_1 progenies were triploids with approximately 2n=51 chromosomes (Fig. 2) (Table 1). Because of the unbalanced chromosome numbers in triploids, pollen stainability of the BC_1F_1 was reduced to 2.45% to 4.63% (Fig. 3) (Table 1). As a result, the BC_1F_1 plants produced very few BC_2F_1 seeds. For example, the cross of *H. californicus* 2376/2* HA 410 produced only 48 seeds after pollinating 99,000 florets, and *H. schweinitzii* 2415/2* HA 410 did not set any seed from 3260 pollinated florets. In total, 59 BC_2F_1 seeds including some partially filled seeds were obtained.

Only 24 BC_2F_1 plants (Fig. 4) with chromosomes 2n=40-49 (Fig. 5) were obtained with a pollen stainablity of 34.2% (Fig. 6). Further backcrossing of the BC_2F_1 plants with HA 410 is currently underway.

Our results demonstrated that the major obstacle in interspecific gene transfer between hexaploid perennials and cultivated sunflower is the BC_1F_1 generation, with the chromosome number of 2n=51. We would strongly suggest using embryo rescue to improve the establishment of the BC_2F_1 seedlings. Presently, we are crossing additional BC_1F_1 plants with HA 410 and culturing the BC_2F_1 embryos. We expect to produce a large population of BC_2F_1 progenies to provide a sound foundation for further backcrosses as we approach the base 2n=34 chromosome of the recurrent parent HA 410. Identification of resistant progenies will be initiated as early as the BC_3F_1 or BC_4F_1 generation.

Table 1. Chromosome number, pollen stainability, and seed set of BC_1F_1 or BC_2F_1 of *H. californicus* (2n=102) and *H. schweinitzii* (2n=102) backcrossed with HA 410 (2n=34).

Crosses	BC_1F_1				BC_2F_1	
	2n chromosome	Pollen stainability %	Number of seeds	Total flowers	2n chromosome	Number of plants
H. californicus 2376 × HA 410	50-53	4.63	48	99,900	40-49	22
H. schweinitzii 2404 × HA 410	48-52	4.53	5	21,780	-	-
H. schweinitzii 2405 × HA 410	49-52	2.64	6	11,280	46	2
H. schweinitzii 2415 × HA 410	-	2.45	0	3,260	-	-
Total			59			24

References

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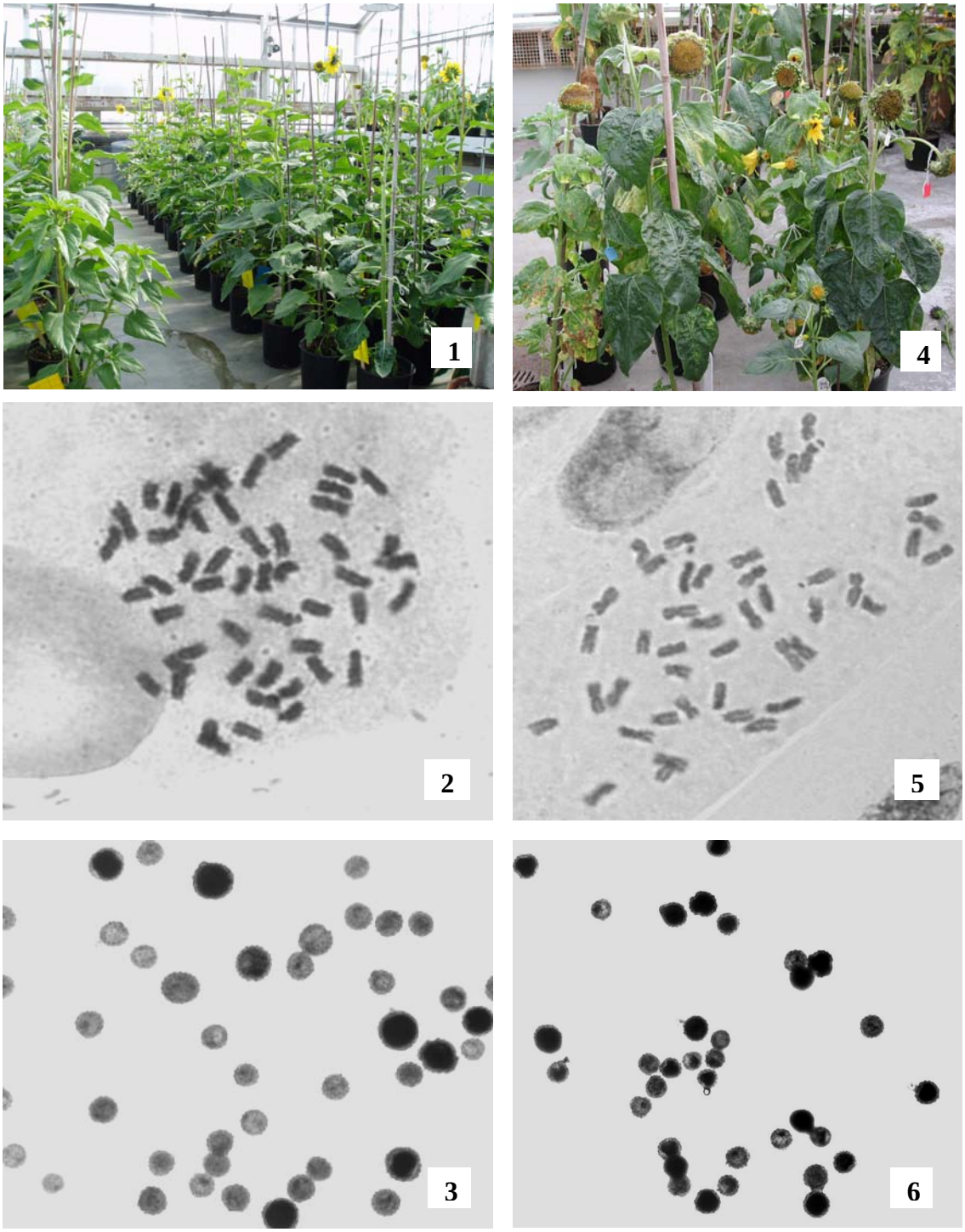

Figure.1. BC_1F_1 plants in greenhouse in April, 2006
Figure 2. BC_1F_1 chromosomes 2n=51(G05/662-1)
Figure 3. BC_1F_1 pollen fertility. Dark color indicates fertile pollen (G06/103)
Figure 4. BC_2F_1 plants in the greenhouse in December, 2006
Figure 5. BC_2F_1 chromosomes 2n=45 (G06/143)
Figure 6. BC_2F_1 pollen fertility. Dark color indicates fertile pollen (G06/211)