Development of Sclerotinia stem rot resistant germplasm utilizing hexaploid *Helianthus* species

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Summary

Sclerotinia sclerotiorum (Lib.) de Bary is a major disease problem in the world's sunflower production. A total of 409 and 120 progenies of interspecific hybrids between cultivated sunflower HA 410 and stalk rot tolerant wild perennial *Helianthus* species *H. californicus* and *H. schweinitzii*, respectively, were produced via an embryo rescue technique. Greenhouse evaluation indicated excellent stem rot resistance for interspecific F1 progeny and HA 410. Pollen stainability of crosses *Californicus* 2376 × HA 410, *Schweinitzii* 2404 × HA 410, *Schweinitzii* 2405× HA 410, and *Schweinitzii* 2415× HA 410 were 37.8%, 45.1%, 54.2% and 31.6%, respectively, suggesting good F1 fertility. Most F1 progenies had good backcross seed set, but with much lower sib-pollinated seed set.

Introduction

Cultivated sunflower and present-day hybrids lack an acceptable level of resistance to *Sclerotinia*. However, an abundance of wild *Helianthus* species are potential sources of genes for disease resistance. Among USDA-released tolerant lines, HA 410 had the lowest percentage of *Sclerotinia*-infected plants among the eight released *Sclerotinia*-tolerant lines for stem rot (Miller and Gulya, 1999). The hexaploid perennials *Helianthus californicus* DC. and *H. schweinitzii* T and G were identified to be highly resistant to stem rot in greenhouse tests at Fargo using artificial inoculation. Therefore, the main objectives of this project were to transfer new resistance genes from these two perennial species into HA 410 and to develop new germplasm lines superior to HA 410.

Materials and Methods

HA 410 is currently the most tolerant line for *Sclerotinia* stem rot. Perennial wild hexaploid *Helianthus* species, *H. californicus* 2376, and *H. schweinitzii* 2404, 2405, and 2415 were identified to possess a high level of resistance to *Sclerotinia* stem rot. All interspecific hybrids were produced using an embryo rescue technique (Chandler and Beard, 1983).

Five-day-old embryos were first cultured on a solid growth medium in Petri dishes with Gamborg's B5 salts (Gamborg *et al.*, 1968) supplemented with vitamins, amino acids, NAA (α-naphthalene acetic acid), and 120 g/kg sucrose. The enlarged embryos were transferred to a germination medium in test tubes with B5 salts plus 20 g/kg sucrose and 0.7% agar.

Stem rot field evaluations were conducted with inoculum consisting of *Sclerotinia* mycelium grown on millet grains, which was then deposited in the soil beside each plant at the V-10 to R 5.1 stage (Gulya *et al.*, 2004). Modifications of stem rot greenhouse evaluations include placing 5 g of millet seed infected with *Sclerotinia* mycelium (no sclerotia) in a 1-cm diameter hole, 10 cm away from the stem and 10 cm deep, followed by plant inspection every two days.

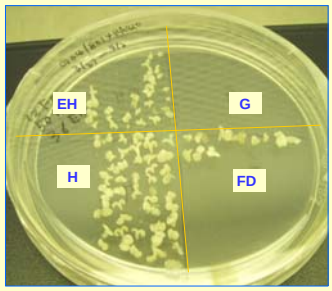

Figure 1. Four different embryo stage (G, EH, H and FD stage) after 7 days of embryo rescue.

Figure 2. Seedlings transferred from test tubes into greenhouse.

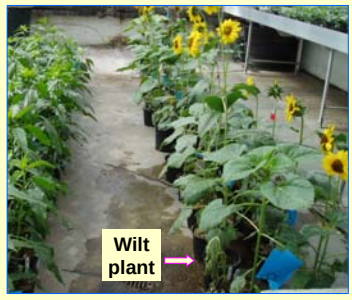

Figure 3. F1 stem rot greenhouse evaluation.

Figure 4. The F1 hybrids sib-pollinated and the backcrosses with HA 410

Results and Discussion

All four crosses *H. californicus* 2376 × HA 410, *H. schweinitzii* 2404 × HA 410, *H. schweinitzii* 2405 × HA 410, and *H. schweinitzii* 2415 × HA 410 produced many good quality embryos of different developmental stages for embryo rescue (Fig. 1). The success of the embryo rescue was high with the seedling survival rate of 56%, 36%, 41%, and 26%, respectively (Table 1, Fig. 2).

Our 2005 greenhouse evaluation indicated excellent stem rot resistance for interspecific F1 progeny between *H. californicus* × HA410 and *H. schweinitzii* × HA 410 (Table 2, Fig. 3). The pollen

and *H. schweinitzii* × HA 410 (Table 2, Fig. 3). The pollen stainability ranged from 31.6 to 54.2 %, suggesting good fertility. As a result, result, most F1 plants with resistance to stem rot produced ed acceptable backcross seed set for further evaluation and for further evaluation and backcrosses (Table 2, Fig. 4). r further evaluation and backcrosses (Table 2, Fig. 4).

BC1F1 progenies are presently being established in the house, and are expected to have 2n chromosome number of 51, and eir BC progenies with 2n=34 to 51. We expect to select the earliest est plants with 2n=34 chromosome number in the BC2F1, which will be followed with intercrossing and selection moving toward our objective

Table 1. The embryo rescue of interspecific F1 between *H. californicus* and *H. schweinitzii* with HA 410.

Species	No. seeds / flowers	Embryo developmental stages					Transferred enlarged embryos	Survival seedling %
		Globular	Early heart	Heart	Fully developed	Total		
Californicus 2376 × HA410	981/ 2269	32	305	336	62	735	409	56
Schweinitzii 2404 × HA410	91/ 560	3	5	34	42	84	30	36
Schweinitzii 2405 × HA410	175/ 2041	-	1	43	96	140	57	41
Schweinitzii 2415 × HA410	156/ 630	7	26	63	33	129	33	26

Table 2. Sclerotinia resistance and pollen stainability of *H. californicus, H. schweinitzii* and their F1 hybrids with HA 410, and the backcross and sib-pollinated seed set of the F1 plants.

Species or F_1	No. resistant plants	No. susceptible plants	Pollen stainability %	Backcross		Sib-pollination	
				Seed set %	Total heads	Seed set %	Total heads
*Californicus*2376 × HA410	69	0	37.8	2.71	280	0.19	96
Schweinitzii 2404 × HA410	11	0	45.1	11.24	39	0.57	37
Schweinitzii 2405 × HA410	12	0	54.2	1.74	35	0.58	25
Schweinitzii 2415 × HA410	9	0	31.6	4.44	25	1.42	23
Californicus 2376 (2n=102)	18	1	99.6	-	-	42.9	50
Schweinitzii 2404 (2n=102)	7	1	96.8	-	-	1.31	16
Schweinitzii 2405 (2n=102)	7	2	96.0	-	-	12.3	25
Schweinitzii 2415 (2n=102)	9	3	85.4	-	-	9.79	10

References

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