Development of Sunflower Germplasm with Resistance to *Sclerotinia* Stalk Rot

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Abstract

Sunflower (*Helianthus annuus* L.) inbred lines with higher levels of resistance to *Sclerotinia* stalk rot or wilt were selected from germplasm and recurrent phenotypic selection populations. Total immunity to *Sclerotinia* was not found. Resistance in the inbred lines appears to be polygenic, necessitating population improvement methods, such as recurrent phenotypic selection, effective in combining many genes to create genotypes with higher levels of resistance. Natural infection, supplemented with artificial infection, appears to be an effective screening method in the recurrent phenotypic selection procedure.

Key words - Sunflower, *Helianthus annuus, Sclerotinia sclerotiorum,* breeding, genetics

Introduction

Sclerotinia stalk rot or wilt [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] has been noted to be one of the most serious diseases of sunflower (Masirevic and Gulya, 1992). In the U.S., losses are mostly due to the basal stem rot form of *Sclerotinia.* These losses may be severe, approaching 100% in some fields. Plants infected one week after anthesis may lose up to 98% of their potential yield compared to 12% for plants infected 8 weeks after flowering (Dorrell and Huang, 1978). Factors associated with late infection are decreased oil content and quality, and lower seed and test weight.

The USDA-ARS genetics project located at Fargo, ND, USA, began a project to identify and develop germplasm with increased resistance to *Sclerotinia* wilt. The project was designed in three phases: 1) identify and select germplasm with increased resistance, 2) develop recurrent phenotypic selection procedures and populations by intermating resistant germplasm, and 3) cross highly resistant germplasms to develop new, improved germplasm with increased resistance.

Materials and Methods

Screening germplasm for resistance to *Sclerotinia*

Several accessions originating from Russia and Ukraine were obtained from the North Central Plant Introduction Station, Ames, IA. The accessions were planted in 1985 in a *Sclerotinia* screening nursery, Moorhead, MN, containing naturally and artificially incorporated sclerotia in the soil. Three to four plants of each accession

were selected and self-pollinated. Progeny of those accessions showing resistance were screened in 1986, 1987, and 1988 in the screening nursery at Moorhead.

Inbred lines developed from these germplasms and an inbred line derived from the Romanian hybrid 'Select', obtained from the FAO Hybrid Sunflower Trial, were tested in *Sclerotinia* field trials at Moorhead, MN (1990), Carrington Research and Extension Center, Carrington, ND (1990 and 1991), and Grandin, ND (1991), using two replications, and single-row plots, 6.1 m long with 75 cm between rows. Plants were thinned at the V4 stage (Schneiter and Miller, 1981) to 30 cm apart. The number of susceptible and resistant plants was recorded approximately 28 days after flowering.

The inbred lines were crossed in an intermating scheme to produce six hybrid combinations. CMS HA 124 and RHA 801 were also utilized as parents based on their performance in the inbred line screening trial and previous tests (Fick et al., 1983; Gulya, 1985). The hybrids were planted at six locations over three years, 1989-1991. The locations were Moorhead, MN (1989-1990), Grandin, ND (1991), Carrington, ND (1989-1991), Sigco Research (1990-1991), Dahlgren & Co. (1989-1991), and Northrup King & Co. (1989). The hybrids NK 285 and Hybrid 894 were used as checks at all locations.

Recurrent phenotypic selection

S_2-derived S_4 selections from plants of three recurrent selection populations were planted in a *Sclerotinia* screening nursery at Moorhead, MN, in 1990. Surviving plants were self-pollinated and planted in a *Sclerotinia* screening nursery near Grandin, ND, in 1991. Three to four plants of each line were selected and self-pollinated, and progeny screened for resistance in a *Sclerotinia* screening nursery near Grandin, ND, in 1992, and near Glyndon, MN, in 1993. Two experimental restorer lines were crossed with the cytoplasmic male sterile lines, CMS HA 390 and CMS HA 124 to produce hybrids. Three experimental maintainer lines were crossed with NMS RHA 801 to produce hybrids.

The experimental hybrids were evaluated in a *Sclerotinia* infested fields near Glyndon, MN, in 1993 and 1994, Woodland, CA, in 1993, Carrington, ND, in 1993, and near Camet, Argentina, during the 1993-1994 growing season. The field near Woodland, CA, was in cooperation with Pioneer Hi-Bred Intl., and the field near Camet, Argentina, was in cooperation with Dekalb Argentina S.A. The Carrington, ND, experiment was conducted in cooperation with the Carrington Research and Extension Center, North Dakota Agricultural Experiment Station. All hybrids were compared with the checks, Hybrid 894 and NK 285, using two replications, and single-row plots, 6.1 m long, with 75 cm between rows.

Results and Discussion

Screening germplasm for resistance to *Sclerotinia*

Five open-pollinated cultivars, Armavirskij 50, Harkovskij 100, Salyut, Sputnik, and Start had plants with resistance to *Sclerotinia* infection (Table 1). Additional screening revealed that selections from Armavirskij 50, Start, and Select had a greater degree of resistance than selections from the other cultivars. The selection from Armavirskij 50 was determined to be a maintainer line that was converted to the PET1 cytoplasmic male sterility (CMS), and was designated CMS HA 390. The selections from Start and Select were determined to have fertility restoration factors for the PET1 CMS and were designated RHA 391 and RHA 392.

The female lines HA 124 and HA 390 were significantly more resistant than the check line HA 89 over the three years of testing (Table 2). The restorer lines RHA 801, RHA 391, and RHA 392, were significantly more resistant than the check line RHA 274. The genotype X environment interaction was not significant indicating that the lines performed similarly across environments.

Hybrids between CMS 124 and CMS 390 and the restorer lines RHA 801, RHA 391, and RHA 392 were significantly more resistant to *Sclerotinia* wilt than NK 285, and four of the six hybrids were significantly more resistant to *Sclerotinia* than Hybrid 894, averaged over years and locations (Table 3). The restorer lines RHA 391 and RHA 392, in combination with CMS HA 390, and the restorer line RHA 392, in combination with CMS HA 124, produced hybrids with the highest resistance to *Sclerotinia*. Yield and oil percentage (Table 4) of the hybrids were generally lower than Hybrid 894 and commercial check lines, except for CMS HA 390/RHA 391. The HA 390 hybrids were later and taller than most check hybrids. Also hybrids with the restorer line RHA 391 were taller than the check hybrids.

In conclusion, inbred lines were identified with higher levels of *Sclerotinia* stalk rot resistance than the check inbreds HA 89 and RHA 274, and resistance was consistent over environments. The resistance appeared to be expressed in hybrid combinations. Agronomic performance of the hybrids was generally lower than the check Hybrid 894 and other commercial hybrids.

Recurrent phenotypic selection

Recurrent phenotypic selection was implemented on three populations. The first population, Romania R-Line *Sclerotinia* Resistant Population-1, was obtained in 1986 through an Office of International Cooperation and Development (OICD) germplasm exchange with I.C.C.P.T., Fundulea, Romania. The second population, USDA B-line *Sclerotinia* Recurrent Selection Population, was comprised by random mating a composite of maintainer lines screened for *Sclerotinia* resistance in 1987, near Moorhead, MN. The third population, USDA B/SCL B-Line population, was created by crossing HA 821 with selections derived from the open-pollinated variety

Armavirskij 50, HA 338, S.A. PTC composite, and HA 89*2/*H. pauciflorus (rigidus)* ssp. *subrhomboideus*. HA 821 was released by USDA and the North Dakota Agricultural Experiment Station in 1986. Armavirskij 50 was obtained from VNIIMK, Krasnodar, Russia. HA 338 is a maintainer line released by the USDA and the North Dakota Agricultural Experiment Station in 1988 and was derived from the cross HA 89*3/*H. praecox* 419. S.A. PTC Composite was obtained from a germplasm exchange with the Grain Crops Research Institute, Potchefstroom, Republic of South Africa, in 1986. HA 89*2/*H. pauciflorus (rigidus)* ssp. *subrhomboideus* was a cross made by USDA in 1982 in Fargo, ND. All of the lines were screened in 1986 and 1987 for *Sclerotinia* resistance on an infested field near Moorhead, MN. The F_1 hybrids of the four lines crossed with HA 821 were intermated to form the USDA B/SCL B-Line population.

The procedures utilized in the recurrent phenotypic selection were as follows. The original populations were planted on a *Sclerotinia* infested field using 25 6.1 m long rows, with 75 cm between rows. Plants were thinned at the V4 stage to 30 cm apart. Each population was represented by 500 plants. Plants were covered with cotton cloth bags before anthesis to insure self-pollination. At the end of the growing season, all plants with visible signs of *Sclerotinia* infection were discarded. Some plants were infected very late in the season with a lesion at the base of the stem only 3 to 4 cm in length. Self-pollinated heads were threshed and cleaned, and any plant with less than 50 seeds was discarded to select for higher self-fertility.

Seed from the S_0 plants were planted as S_1 rows on a *Sclerotinia* infested field the following year, using 6.1 m long rows, with 75 cm between rows. Plants were thinned at the V4 stage to 30 cm apart. Ten plants were selected for artificial infection. The ten plants were selected based on uniformity of plant stage and marked with paint that remained on the plant throughout the entire season. The plant stage at which artificial infection was applied was R2 or when the bud was approximately 4 cm in diameter. The method utilized for artificial inoculation was developed by Mancl and Shein (1982). *Sclerotinia* was grown on autoclaved, moist oats in canning jars for 3 to 4 weeks at 18 to 22°C in the dark. The jars were shaken each week to mix the oats thoroughly. After the *Sclerotinia* mycelium uniformly infected the oats, the oat kernels were dried and stored in plastic bags at 3°C. Approximately 5 ml of the dried inoculum was placed 1.5 to 2.0 cm from the base of the plant at a depth of 2.5 to 5 cm, using a "jab planter" often used to plant hills of maize (*Zea mays* L.). Disease evaluation on each plant was made three times; two weeks after anthesis, four weeks after anthesis, and at physiological maturity (R9 stage). Four non-artificially infested plants were selected for bagging with cotton bags.

Based on number of plants not infected, compared within the population and with the checks, HA 124 and RHA 801, a 20% selection intensity was used to identify the S_1 lines with highest resistance. The best plant among the four bagged plants was selected for random mating in the next generation.

Selected S_1 plants were random mated in the greenhouse during the winter season at Fargo, ND. Seeds of selected S_1 plants were planted in a 20 cm diameter plastic pot. Pots were thinned to two plants. At anthesis, one plant was hand-emasculated and pollen from the other plant was mixed equally with pollen from all other S_1-derived S_2 plants. The mixed pollen was used to pollinate all emasculated plants of each population. The resultant random-mated seed was designated as Cycle 2 and was planted the following year in the field as described above. Lines with the highest level of resistance were tested further, as well as being included in creating the next cycle of selection. Three cycles have been completed in each population.

Five inbred lines derived from the recurrent phenotypic selection populations were identified with increased resistance to *Sclerotinia* infection. Two restorer lines, RHA 408 and RHA 409, were derived from the Romania R-Line *Sclerotinia* Resistant Population Cycle 1 and Cycle 2, respectively (Table 5). Two maintainer lines, HA 410 and HA 411 were derived from the USDA B-Line *Sclerotinia* Recurrent Selection Population Cycle 2 and Cycle 3, respectively. The maintainer line, HA 412, was derived from the USDA B/SCL B-line Population Cycle 1.

Hybrids with RHA 408, RHA 409, HA 410, HA 411, and HA 412 had significantly lower *Sclerotinia* infection than the check hybrids, Hybrid 894 and NK 285. Hybrids with RHA 408, RHA 409, and HA 411 were highest yielding in the tests. Oil percentage of hybrid seed (0% moisture basis) was highest with the three maintainer lines. In 1994, head rot infections of *Sclerotinia* were also prevalent at the Glyndon, MN, site, significantly infecting the two check hybrids. Even though no selection had been made for head rot resistance, hybrids with RHA 408 and HA 411 showed good resistance to this disease. RHA 408 and RHA 409 produced the tallest heights hybrids compared with the two checks.

In conclusion, inbred lines selected from germplasm and recurrent phenotypic selection populations were identified with higher levels of resistance to *Sclerotinia* than the check inbreds. Total immunity to wilt was not found in any selection. The resistance observed in the lines appears to be polygenic, with population improvement methods, such as recurrent phenotypic selection, effective in combining many genes to create genotypes with higher levels of resistance. Crossing high yielding, high oil cultivars with *Sclerotinia* resistant germplasm and creating populations was effective in selecting lines with improved agronomic characteristics and equal levels of *Sclerotinia* resistance. Natural infection, supplemented with artificial infection, appears to be an effective screening method in recurrent phenotypic selection.

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Table 1. Preliminary screening of open-pollinated cultivars of sunflower for resistance to *Sclerotinia*, 1985, Moorhead, MN (USA).

Cultivar	No. of Plants	
	Resistant	Susceptible
Armavirec	14	10
Armavirskij 14	8	12
Armavirskij 50	16	5
Armavirskij 3497	9	8
Avangard	10	6
Chernianka 66	5	12
Cjakinski 269	11	9
Harkovskij 50	8	6
Harkovskij 100	14	3
Majak	9	12
Odesskij 63	10	12
Peredovik	3	13
Progress	6	12
Rassvet	11	9
Rennespelij 38	8	12
Salyut	12	2
Smena	8	6
Sputnik	11	5
Start	9	0
Tombrovsky	8	10
VNIIMK 6540	1	25
VNIIMK 8931	9	6
Voshod	7	8
Yugovostockny	7	14
Zaria	13	9
Select (Hybrid)	16	0

Table 2. Screening of inbred lines for *Sclerotinia* tolerance over two years, 1990-1991, and three locations, Moorhead, MN (1990), Carrington, ND (1990-1991), and Grandin, ND (1991).

Inbred Line	Year			Mean
	1989	1990	1991	
	% Resistant			
HA 89	73	78	60	72
HA 124	95	93	83	92
HA 390	95	100	62	90
RHA 274	83	78	35	71
RHA 801	93	98	99	96
RHA 391	98	94	94	95
RHA 392	98	85	88	91
Mean				87
LSD 0.05				13

Table 3. Performance of hybrids grown in *Sclerotinia* nurseries over three years, 1989-1991.

Hybrid	Year			Mean
	1989	1990	1991	
	% Resistant			
CMS HA 124/RHA 801	64	76	92	77
CMS HA 124/RAH 391	62	83	89	78
CMS HA 124/RHA 392	62	92	88	81
CMS HA 390/RHA 801	50	74	82	69
CMS HA 390/RHA 391	74	93	80	82
CMS HA 390/RHA 392	70	89	83	81
NK 285	52	57	66	58
HYBRID 894	53	70	75	66
Mean				74
LSD 0.05				11

Table 4. Hybrid sunflower trial planted at Casselton, ND, 1991.

Hybrid	Yield	% Oil	Days to Flowering	Height	200 Seed Weight
	kg/ha			m	g
CMS HA 124/RHA 801	1708	40.0	68	1.8	10.0
CMS HA 124/RHA 391	1634	39.6	69	1.9	10.7
CMS HA 124/RHA 392	1750	39.4	68	1.9	9.9
CMS HA 390/RHA 801	1665	42.5	71	1.9	9.8
CMS HA 390/RHA 391	1853	44.7	72	2.0	11.7
CMS HA 390/RHA 392	1796	42.3	71	1.8	10.0
Hybrid 894	1834	40.7	68	1.7	10.5
Cargill 207	2024	40.7	69	1.9	12.3
NK 277	1889	45.6	68	1.8	10.6
Pioneer 6440	1755	46.1	69	1.8	10.0
Cargill 187	1954	41.8	66	1.6	10.0
SeedTec 317	1814	46.0	68	1.8	11.8
Hysun 354	1884	44.9	68	1.7	8.8
Mean	1807	42.1	69	1.8	10.6
LSD 0.05	256	3.2	3	.1	1.4

Table 5. Restorer and maintainer lines developed and released in 1995 for tolerance to *Sclerotinia sclerotiorum* stalk rot, utilizing recurrent phenotypic selection.

Line	Source Population and Cycle
RHA 408	Romania R-Line Sclerotinia Tolerant Population C1
RHA 409	Romania R-Line Sclerotinia Tolerant Population C2
HA 410	USDA B-Line Sclerotinia Recurrent Selection Population C2
HA 411	USDA B-Line Sclerotinia Recurrent Selection Population C3
HA 412	USDA B/SCL B-line Population C1