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EVALUATION OF HELIANTHUS SPECIES FOR DISEASE RESISTANCE AND OIL CONTENT AND QUALITY[1]

By

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Abstract

Most species and subspecies of Helianthus have not been collected, and we are in the process of evaluating this diverse germplasm for useful genetic traits. Achene oil content ranged from less than 20 to 40.2 percent for the 30 species (297 accessions) evaluated. It appears that H. niveus and H. salicifolius are potential sources of genetic variability to increase oil content in cultivated sunflower. Much variability in fatty acid composition was also observed.

Twenty-one species (268 accessions) have been initially screened for resistance to Sclerotinia sclerotiorum. The reaction of accessions varied from highly susceptible (90-100% disease) to moderately resistant (70-79% disease), with the majority of test samples in the former category. Such differences were found among and within species.

Some Texas collections have also been screened for resistance to Puccinia helianthi (races 1 and 3) and Plasmopara halstedii. Some plants of H. praecox, H. argophyllus, and H. annuus were found to be resistant to both diseases. Whether this resistance to downy mildew is due to the presently used Pl_2 gene or a novel gene is unknown.

Introduction

Wild Helianthus germplasm, besides contributing the basic stock from which cultivated sunflower originated, continues to contribute specific characteristics for sunflower improvement. Since it is obvious that we must look to this material for insect and disease resistance in the future, one of our objectives

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has been to amass a complete live collection of the 50 or so Helianthus species and most subspecies. Initial screening has been for resistance to Sclerotinia (Sclerotinia sclerotiorum (Lib.) de Bary), rust (Puccinia helianthi Schw.), and downy mildew (Plasmopara halstedii (Farl.) Berl. and de T.), as well as for high achene oil content and novel oil fatty acid composition. Results of preliminary screening are presented here.

Materials and Methods

Seed used in this screening work was collected from natural growing sites in 1976 and 1977. Species location and identification were largely determined from Heiser's sunflower monograph (5). Plant specimens were also colelcted and identities were later confirmed by Dr. Charles B. Heiser, Jr., Indiana University, Bloomington. Seed of most accessions were colelcted and dried, and are being maintained under cold room conditions at Bushland, Texas. Where collection of seed of perennial species was not possible, root parts were collected and transplanted to the field or greenhouses at Bushland.

Plants were screened for Sclerotinia resistance at Morden, Manitoba using 226 accessions of wild Helianthus collected in the U.S. and 42 collected in Canada. Species included were H. niveus (Benth.) Brandegee, H. debilis Nutt., H. praecox Engelm. and Gray, H. petiolaris Nutt., H. neglectus Heiser, H. annuus L., H. rigidus (Cass.) Desf., H. x laetiflorus Pers., H. giganteus L., H. grosseserratus Martens, H. maximiliani Schrader, H. salicifolius A. Dietr., H. simulans E.E. Wats., H. heterophyllus Nutt., and H. radula (Pursh) Torr. and Gray. In preparation for screening, seed of each accession were pretreated with fungicide (captan) and placed between wet filter papers in petri dishes. They were then maintained at 2°C for 8 to 10 weeks to break dormancy. Twenty seeds in each U.S. collection and 70 to 120 seeds of each Canadian collection were used. Germination rate varied greatly among accessions, ranging from 0 to 100%.

The screening tests were conducted in the greenhouse using wooden flats. Each flat contained soil which was infested with 500 g of S. sclerotiorum grown on autoclaved seeds of barley, rye, and sunflower (1:1:1 V/V/V). Germinated seed of each accession were planted approximately 3 cm deep. Disease rate was determined 3 weeks after planting by counting the number of surviving plants.

Screening for downy mildew and rust (races 1 and 3) resistance was accomplished at Fargo using 136 accessions consisting of H. debilis, H. praecox, H. petiolaris, H. neglectus, H. annuus, and H. argophyllus. Most of this material was collected in Texas. In preparation for the downy mildew test, seed were germinated on blotter paper, dehulled when the radicle lengths were 1-2 cm long, and placed in 5 cm petri dishes. Fifteen ml of distilled water, containing at least 10^4 zoosporangia/ml, were added to each dish. Plants were then placed in a dark cabinet at 20 C for 18 hours. The seedlines were then transplanted into 12.7 cm pots containing sterile soil, and maintained on greenhouse benches for 14 days at 20-25 C. Disease reaction was ascertained by placing the seedlings in a saturated humidity chamber for 18 hours. Susceptibility was indicated by the sporulation on aboveground parts. Resistant plants were assumed to be those that showed no sporulation.

Resistance to races 1 and 3 of rust was determined on 6-week-old plants. Mixtures of urediospores and talc (1:10) were rubbed on individual leaves using folded cheesecloth squares. One fully expanded leaf per plant was used for each race. Plants were immediately placed in a saturated humidity chamber for 24 hours and then transferred to greenhouse benches and maintained at 20-25 C. Resistance or susceptibility was determined 12 days later by the presence or absence of urediospores on inoculated leaves.

Oil quantity and fatty acid composition were also determined at Fargo on seeds of H. niveus, H. debilis, H. praecox, H. petiolaris, H. neglectus, H. annuus, H. argophyllus, H. bolanderi A. Gray (H. exilis A. Gray according to Jain et al (6)), H. paradoxus, H. gracilentus A. Gray, H. pumilus Nutt., H. ciliaris, H. mollis, H. occidentalis, H. divaricatus L., H. eggertii Small, H. strumosus L., H. tuberosus, H. rigidus, H. nuttallii, T and G., H. maximiliani, H. salicifolius, H. californicus DC., H. resinosus Small, H. microcephalus, T and G., H. glaucophyllus D.M. Smith, H. longifolius Pursh, H. angustifolius L., H. radula, and H. carnosus Small. Oil content was determined on 2 ml of hand-picked seed (achenes) using a Newport[5] NMR analyzer (4). Seed samples were dried at 130 C for 3 hours, cooled, and analyzed. The precision of the NMR analyzer with the 2 min 11 s integration time was ± 0.1% oil. Fatty acid composition of the oil was determined by gas chromatography on a silar 9-CP column and a hydrogen flame detector. An electronic digital integrator was used to determine the composition of individual fatty acids.

Results and Discussion

Disease Resistance

Genetic resistance to Sclerotinia is badly needed in North America (7). As with resistance to other diseases, we look to wild relatives for this trait, and hope to find a single dominant gene for resistance. The reaction of different accessions of wild Helianthus to this disease in our tests varied from highly susceptible (over 90% of plants infected) to moderately resistant (70-79% of plants infected), with the majority of the test samples in the former category (Table 1). Such differences were found among accessions within a species and among species. It must be emphasized that these results are tentative, and it is possible that some plants classified as resistant, were escapes. Some of these results are being verified by retesting the most promising entries.

The existence of resistance to S. sclerotiorum has been previously reported in some species of wild Helianthus as well as in interspecific hybrids (7,8,9). Pustovoit and Pustovoit (9) found that H. tuberosus var. purpurellus L., H. rigidus, H. subcanescens, and H. macrophyllus were immune to Sclerotinia. Pustovoit et al (8) further developed interspecific hybrids possessing group immunity to the main sunflower pathogens, including Sclerotinia. A high degree of resistance was, however, not found in our collection which included H. tuberosus and H. rigidus.

Presently it appears that annual species (especially H. annuus) may be the best sources of resistance. Whether the more complicated use of perennials

[5] Mention of a trademark or proprietary product does not constitute a guarantee or waranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

(such as H. tuberosus) to develop resistance in a breeding program is warranted seems debatable.

The screening work for resistance to rust (both races 1 and 3) and downy mildew at Fargo identified four entries from H. praecox (ssp. runyonii stat. nov. and ssp. hirtus stat. nov.), one H. argophyllus entry, and 18 wild H. annuus entries as resistant to these diseases. Another gene for resistance to downy mildew is needed due to the specific reaction type presently induced by Pl_2, and the fact that this is the only gene imparting resistance to the North American race of this fungus (10). Complementary genetic tests will be conducted on the resistance mechanisms discovered here with traditional forms. The resistance found in many of the lines tested in this study is probably conditioned by the Pl_2 gene, due to the location of collections. Some were collected in Texas, and this is where Pl_2 resistant material was originally found (11).

Rust resistance is a fairly common trait in wild sunflowers (3) and new forms or genes should be available if ever needed in breeding programs. Some of the resistance sources identified here should contain novel genes for resistance. Their usefulness should also be fairly direct due to cross-compatibility of species containing these genes and cultivated material. Currently, complimentary genetic tests and screening for resistance to both Race 1 and Race 3 is in progress, and new germplasms resistant to both races are being developed.

Oil Content

Whether achene oil content of cultivated sunflower can be increased efficiently by introgressing wild germplasm, is debatable. All wild Helianthus collections we have screened have been lower in oil than some cultivated types (Table 2). Nonetheless, high oil genotypes of wild Helianthus should contribute new genes for oil content if utilized in a breeding program to increase this trait. Highly dominant and heterotic effects for oil content also appear to be absent (2,3). Of the 297 collections (representing 30 species) reported on here, one collection of H. niveus ssp. canescens had the highest oil percentage (Table 2). Again, this data is tentative since analysis was on a single seed sample from each collection, and since environmental effects probably were very high.

Fatty Acid Composition of Oil

Oleic and linoleic fatty acid contents of cultivated sunflower oil vary greatly, depending mainly upon the temperature during seed development (1). It would be advantageous if genetic stability for this trait could be developed. The possibility also exists of discovering genetic characteristics which allow the linoleic acid content of sunflower oil to be changed.

Differing environmental effects (especially temperature) probably affected fatty acid composition of seed samples analyzed here (Table 3) since they were collected from different locations. Nonetheless, implications from data can be derived. It appears that the wild annual species may be the best source of genes to utilize in a breeding program to alter fatty acid composition of sun-

flower oil. Some entries of both subspecies of H. petiolaris appear to be a good genetic resource to increase linoleic acid content, while lowering oleic. H. paradoxus, H. argophyllus, H. annuus, and all subspecies of H. praecox appear to be good sources of genetic variability to increase the level of palmitic acid if desired.

In order to determine the usefulness of these collections in a breeding program, it will be necessary to grow them in a common environment so that environmental effects on fatty acid composition and oil content will be uniform and comparable to standard germplasms.

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TABLE 1. Reaction of wild Helianthus collections to Sclerotinia sclerotiorum under greenhouse conditions. Numbers of accessions are given which had various percents of susceptible plants.

Species and Subspecies	Disease %		
	70-79	80-89	90-100
niveus			
ssp. canescens	0	0	3
debilis			
ssp. silvestris	0	0	2
praecox			
ssp. praecox	0	0	1
ssp. hirtus	0	1	3
ssp. runyonii	0	1	3
petiolaris			
ssp. petiolaris	2	1	24
ssp. fallax	0	0	4
neglectus	0	0	7
annuus	4	18	125
argophyllus	1	1	10
paradoxus	0	1	1
ciliaris	0	0	1
mollis	0	1	5
occidentalis			
ssp. plantagineus	0	0	1
hirsutus	0	0	1
tuberosus	0	1	4
rigidus			
ssp. rigidus	0	0	3
ssp. subrhomboideus	0	0	2
H. x laetiflorus	0	0	1
giganteus	2	2	8
grosseserratus	0	0	1
maximiliani	0	3	13
salicifolius	0	0	2
simulans	0	0	2
heterophyllus	0	0	1
radula	0	0	1

TABLE 2. Oil content of wild *Helianthus* collections.

Species and Subspecies	No. of Collections	Achene Oil % Ave.	Range
niveus			
ssp. *niveus*	1	33.48	--
ssp. *canescens*	6	34.19	24.8 - 40.17
ssp. *tephrodes*	1	37.36	--
debilis			
ssp. *debilis*	1	33.39	--
ssp. *cucumerifolius*	1	19.14	--
ssp. *silvestris*	3	32.25	30.48-34.87
ssp. *tardiflorus*	1	34.41	--
praecox			
ssp. *praecox*	1	34.80	--
ssp. *hirtus*	4	29.82	26.63-31.89
ssp. *runyonii*	4	29.31	25.12-31.06
petiolaris			
ssp. *petiolaris*	21	29.77	20.62-33.97
ssp. *fallax*	12	27.88	15.73-37.67
neglectus	7	25.76	16.86-33.05
annuus	187	25.40	8.22-35.93
argophyllus	11	22.52	16.08-30.25
bolanderi	2	25.38	23.2 -27.56
paradoxus	1	25.22	--
gracilentus	2	30.01	31.93-28.09
pumilus	1	22.05	--
ciliaris	1	18.54	--
mollis	4	28.14	27.46-29.29
occidentalis			
ssp. *plantagineus*	1	24.65	--
divaricatus	1	22.86	--
eggertii	1	22.24	--
strumosus	1	24.49	--
tuberosus	3	26.93	20.5 -31.41
rigidus			
ssp. *rigidus*	4	27.21	25.08-30.02
nuttallii			
ssp. *nuttallii*	2	26.06	21.93-30.18

Table 2 (con't.)

Species and Subspecies	No. of Collections	Achene Oil %	
		Ave.	Range
maximiliani	1	20.79	--
salicifolius	2	36.77	36.06-37.47
californicus	1	24.88	--
resinosus	2	19.48	11.97-26.98
microcephalus	1	14.92	--
glaucophyllus	1	24.90	--
longifolius	1	20.72	--
angustifolius	1	25.16	--
radula	1	23.30	--
carnosus	1	16.81	--

TABLE 3. Fatty acid composition of oil of wild Helianthus species.

Species and Subspecies	No. of Collections	Range in fatty acid content (% of oil)						
		Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Arachidic 20:0	Behenic 20:0
niveus								
ssp. canescens	6	7.2-9.4	--	3.5-8.0	17.1-26.0	54.7-68.4	2.7-3.8	0.3-3.4
debilis								
ssp. cucumerifolius	1	7.1	0.1	4.0	40.1	47.2	0	0.4
ssp. silvestris	3	7.2-7.7	0.1-0.3	4.6-5.5	19.0-22.6	64.4-67.3	0	0.1-0.2
praecox								
ssp. praecox	1	8.8	0.1	5.0	28.7	57.2	0	0.2
ssp. hirtus	4	8.5-9.6	0	6.2-7.0	26.4-33.0	49.6-57.3	4.6	0.1-0.2
ssp. runyonii	4	7.7-9.4	0	4.9-7.2	31.4-41.0	44.3-52.8	0	0.1-0.3
petiolaris								
ssp. petiolaris	17	6.1-8.6	0.06-0.2	3.3-5.8	13.8-33.9	52.9-70.5	3.5-5.7	0.3-0.6
ssp. fallax	4	6.2-7.3	0	3.8-5.2	12.2-31.9	55.1-74.0	2.2-5.7	0.3-0.6
neglectus	8	7.2-8.3	0.1	4.0-6.8	20.2-32.3	52.1-62.2	2.8-4.0	0.3-0.7
annuus	91	5.8-9.4	0.1-0.2	2.8-7.0	13.1-40.5	40.0-71.0	2.3-5.5	0.3-1.9
argophyllus	12	6.5-9.7	0	5.2-11.1	16.2-48.9	36.9-68.4	3.1	0.1-0.3
paradoxus	1	11.1	0.2	7.1	23.4	57.3	0	0.2
ciliaris	2	6.9-8.7	0	5.3-5.8	17.5-20.2	63.4-63.9	3.0-4.9	0.1-0.2
mollis	4	7.3-9.1	0.1	3.9-5.1	12.0-35.4	45.3-76.2	4.6	0.3-0.4
occidentalis								
ssp. occidentalis	1	7.3	0	4.4	29.2	56.1	0	0.2
strumosus	1	6.8	0	5.0	13.8	73.7	0	0.5
tuberosus	2	5.1-7.8	0.1	3.2-5.6	15.8-16.3	70.6-74.3	0	0.2-0.7
rigidus								
ssp. rigidus	3	6.1-7.3	0.1-0.2	3.9-5.5	13.0-18.1	67.7-76.0	0	0.3-0.7
maximiliani	1	9.3	0	5.6	16.7	67.2	6.1	0.4
salicifolius	2	5.5-5.7	0.2-0.3	4.3-4.4	17.4-29.4	58.4-72.4	0	0.1
resinosus	1	6.6	0	5.0	34.0	53.4	0	0.8