CHARACTERIZATION AND HOST RANGE OF SUNFLOWER MOSAIC POTYVIRUS

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ABSTRACT

Mosaic symptoms were observed on wild sunflower (*Helianthus annuus*) near Brownsville, Texas. The presence of long, flexuous rods in leaf dips and pinwheel inclusion bodies using TEM indicated that the causal agent was a potyvirus. All cultivated sunflower hybrids and inbred lines tested by mechanical inoculation were susceptible, with USDA lines Rha 271 and Rha 858 the most susceptible. Of eighty other species tested, only sunflower and zinnia were susceptible, developing systemic mosaic, while *Chenopodium quinoa* developed chlorotic local lesions. Diagnostic hosts for seven potyviruses known to infect sunflower were all negative. Transmission by the green peach aphid, *Myzus persicae* and the Russian olive aphid, *Capitophorus elaegni*, was successful. Virion length from purified preparations was 717 nm, with a capsid protein size of 33 kDa. Seed yield from infected, greenhouse grown plants was reduced 75%, and seed transmission was confirmed. Sequencing of the capsid protein confirmed that sunflower mosaic virus (SuMOV) is a distinct, new virus, with the closest relatives being tobacco etch and endive necrosis. SuMOV is also distinct from the new sunflower potyvirus identified in Argentina, termed sunflower mottle virus.

INTRODUCTION

Virus diseases of cultivated sunflower (*Helianthus annuus* L.) or other wild species of the Helianthus genus are very rare, and scientific documentation is similarly scarce.
In addition to these records on naturally occurring viruses on sunflower, the VIDE database (www.biology.anu.edu.au/Groups/MES/vide) lists 36 viruses shown to produce symptoms in artificial inoculations (2). In the United States, there are only three records of sunflower naturally infected by a virus: cucumber mosaic, on sunflower in research plots in Beltsville, MD (7), tobacco ringspot on wild sunflower in the Rio Grande Valley of Texas (4), and sunflower mosaic on wild *H. annuus* near Austin, Texas (1). The objectives of this study were to characterize the virus, study its host range, and look for sources of resistance in sunflower.

MATERIALS AND METHODS

Source and maintenance of the virus. Leaves of wild *Helianthus annuus* L. showing mosaic symptoms were collected from roadside ditches and fields near Brownsville, Texas.Infected leaves were macerated in 0.01M phosphate buffer, pH 7.0 and rubbed onto sunflower plants dusted with 600 mesh carborundum. Preliminary tests demonstrated the utility of adding sodium sulfite (1%) and PVP (0.25%) to enhance infection. Plants were inoculated 14 to18 days after planting, with symptoms manifesting themselves in 8-12 days.
Host range: In total, 81 species in ten families were inoculated with the sunflower mosaic virus. Plant species tested, grouped by family, included: **Amaranthaceae**: *Amaranthus caudatus* L. and *Gomphrena globosa* L.; **Asteraceae:** *Bidens cernua* L., *B. humilis* L., *Centaurea repens* L., *Cichorium endivia* L., *C. intybus* L., *Cirsium arvense* (L.) Scop., *Cynara cardunculus* L., *Lactuca sativa* L., *L. serriola* L., *Sonchus arvensis* L., and *Zinnia elegans* Jacq.; **Brassicaceae**: *Brassica napus* L.and *B. rapa* L.; **Chenopodaceae**: *Beta vulgaris* N., *Chenopodium album* L., *C. amaranticolor* Cost & Rey, *C. capitatum* L., *C. foliosum* L., *C. giganteum* L., and *C. quinoa* Wild; **Cucurbitaceae**: *Cucumis sativus* L., *Cucurbita maxima* Duch, *C. pepo* L and *Lagenaria siceraria* ; **Fabaceae**: *Desmodium tortuosum* L, *Phaseolus vulgaris* L., *Pisum sativum* L.,.*Vicia faba* L., *Vigna sinenesis* (L.) Endl, *V. unguiculata* L.; **Iridaceae**: *Belamcanda chinensis* (L.) DC; **Lamiaceae:** *Pogostemon cablin* (Blanco) Benth.; **Solanaceae**: *Capsicum annum* L., *C. fructescens* L., *Datura metel* L., *D. meteloides* L., *D. stramonium* L., *Lycopersicon esculentum* Mill.; *Nicandra physaloides* L., *Nicotiana bentamiana* L., *N. cleavlandii* L., *N. debneyi* L., *N. glutinosa* L., *N. rustica* L., *N. sylvestris* L., *N. tabacum* L., *Petunia* x hybrid Hort. Vilm.-Andr. and *Physalis pubescens* L.; **Tropaeolaceae:** *Tropaeolum nanum* L., and *T. majus* L.. Multiple cultivars of *Capsicum annum, C. fructescens, Cichorium intybus, C. endivia, Lactuca sativa, L. serriola, Nicotiana tabacum, Pisum sativum,* and *Zinnia elegans* were tested.

In addition to the above test plants an effort was made to examine a representative number of ornamental plants from different genera within the **Asteraceae.** Forty-six species within 32 genera were tested, including *Achillea ptarmica* L., *Ageratum houstonianum* Mill., *A. retroflexus* L., *Arcototis stoechadifolia* Bergius, *Aster lateriflorus* (L.) Britton, *A. nova-angeliae* L., *Bellis perenis* L, *Berlandiera lyrata* L., *Boltonia asteroides* (L.) L'Her., *Brachycome iberidifolia* L., *Bupthalmum salififolium* L., *Calendula officinalis* L, *Catananche caerulea* L. , *Centaurea macrocephala* Mussin-Puschk., *C. montana* L., *C. dealbata* L., *C. moschata* L., *C. cyanus* L., *Chrysanthemum leucanththemum* L, *C. maximum* Ramond, *C x morifolium* Ramat., *C. tenuiloba* L., *Coreopsis grandiflora* Hogg ex Sweet, *Cosmos*

bipinnnatus Cav., *Dahlia pinnata* Cav., *Dimporhotheca sinuata* DC., *Echinacea purpurea* (L.) Moench, *Echinops ritro* L., *Erigeron karvishshianus* L., *E. speciosus* (Lindl.) DC., *Gaillardia aristata* Pursh , *Gazania rigens* L., *Helenium autumnale* L., *Helichrysum cassinianum* L., *Heliopsis helianthoides* (L.) Sweet, *Liatris spicata* (L.) Willd., *L. pycnostachya* Michx., *Matricaria inodora* L., *Melampodium perfoliatum* (Cav.) Kunth., *Microseries ringens* L., *Pyrethrum roseum* Bieb., *Rudbeckia californica* Gray , *R. fulgida* Ait., *R. nitida* Nutt., *Senecio cineraria* DC., *Tagetes erecta* L., *Tithonia rotundifolia* (Mill.) S.F. Blake, *Xeranthemum anuum* L., and *Zinnea angustifolia* Kunth.

Sunflower host range. A total of 52 oilseed and confection hybrids from the United States, Europe and South Africa were tested by mechanical inoculation as described above. Released USDA inbred lines and germplasms, representing both oilseed and confection types, including 38 females and 19 male or restorer lines were tested. Additionally, 20 USDA interspecific releases tested, representing crosses between 14 different *Helianthus* species and cultivated sunflower, were tested. In total, 129 sunflower hybrids, inbreds or cultivars were evaluated.

Seed and aphid transmission. Seeds from greenhouse-grown, mosaic-infected plants of two sunflower hybrids (400 from each hybrid) were planted in an insect-free greenhouse and grown for six weeks to observe for virus symptoms. Leaves showing any symptoms were harvested , and used to inoculate susceptible line Rha 271 to complete Koch's postulates. Two different aphids were employed in the vector tranmission study: *Capitphorus elaegni* (Del Guerico) and *Myzus persicae* (Sulzer). A non-viruliferous colony of *C. elaegni* was maintained on sunflower, while *M. persicae* was maintained on Chinese cabbage. Aphids were removed from their host plants, transfered to petri dishes for a starvation period, and then allowed to feed on virus-infected sunflower plants. The aphids were then individually transferred to RHA 271 sunflower plants) and allowed to feed for 24 hrs, after which the plants were sprayed with insecticide to kill all aphids.

RESULTS

Maintenance of virus. Sunflowers inoculated with SuMOV were most susceptible 14-18 days after planting, at the V-1 stage when the first pair of leaves had just expanded. Susceptibility decreased with plant age, with 10-20% infection observed on 40-day-old plants. While all hybrids and inbred lines initially tested were susceptible, the USDA lines RHA 271 and RHA 858 were the most susceptible, and were chosen for viral increase and as "back-inoculation" hosts.

Host range. Of the 81 species in ten plant families tested, only sunflower and zinnia in the Asteraceae displayed mosaic symptoms, and the only host to develop local lesions was Chenopodium quinoa. No symptoms of any type were observed on host species diagnostic for any of the potyviruses previously known to infect sunflower (Table 1).

Sunflower Host Range. All of the cultivated sunflower germplasm tested was fully susceptible to SuMOV, developing typical mosaic symptoms in 10-20 days after inoculation. This included 52 oilseed and confection hybrids, 38 released USDA inbred lines and 20 USDA interspecific releases, totaling 129 sunflower entries. A cross section of cultivated germplasm from the USDA Plant Introduction Station collection of 210 accessions was tested and all were susceptible. Tests in progress with wild *H. annuus* accessions from Texas

appear to be the first sunflower germplasm with any resistance to SuMOV.

Seed and Aphid Transmission. Both the green peach aphid, *M. persicae*, and the Russian olive aphid, *C. elaegni*, successfully transmitted SuMOV from sunflower to sunflower, in 20% and 13% of plants tested, respectively. Symptoms often appeared in 6 to 7 days, several days sooner than observed with mechanical inoculations. In the seed transmission study, 12% of the 400 F-2 plants derived from virus-infected plants produced mosaic symptoms with one of the two hybrids. Seeds from the virus-infected plants of the second hybrid failed to demonstrate viral transmission.

Microscopy Electron and light microscopy. Examination of leaf dips from naturally-infected wild *H. annuus* leaves revealed the presence of long, flexuous rods, which confirmed the presence of a virus and prompted this study. Viral particles were infrequently observed in leaf dip preparations of artificially-inoculated cultivated sunflower, but were readily observed in high numbers in leaf-dip preparations of zinnia. Light microscopy and TEM observations of sunflower leaves showing mosaic symptoms revealed both pinwheel inclusion bodies and laminated aggregates in the cytoplasm. No inclusion bodies of any type were observed in nuclei in sunflower leaf sections. Viral particle size measured in leaf dip preparations and from purified virus was 717 nm, with a capsid protein size of 33kDa.

DISCUSSION

This is the first detailed characterization of what is probably the same virus reported on wild sunflower in Texas in 1967 (1), and is the first report of any virus on wild or cultivated sunflower in North America in the last 30 years. This virus is distinct from another potyvirus causing sunflower mottle in Argentina (4). SuMOV is also distinct from a newly observed unspecified virus in India (6), based on limited host range information. While SuMOV was initially discovered on wild sunflower, during the progress of this work, mosaic symptoms have been observed on confection sunflower fields in central Texas. Neither this virus nor any other virus, however, has been observed on cultivated or wild sunflower in the main sunflower production areas of the Central Great Plains (Kansas, Nebraska, Colorado) or the Northern Great Plains (North Dakota, South Dakota, Minnesota).

The host range of SuMOV is very restricted, causing mosaic only in *Helianthus annuus* and *Zinnia* species. Initial tests on some perennial *Helianthus* species indicates that SuMOV may infect annual *Helianthus* species preferentially. Finding local lesions hosts was similarly difficult, with only *Chenopodium quinoa* (of six species tested) producing necrotic local lesions

The decreasing susceptibility with plant age and the relative absence of aphid vectors early in the growing season may explain why this and other viruses have not been observed in northern growing areas of the United States. However, since SuMOV is transmitted by seed, phytosanitary inspections of Texas-produced seed should scrutinize for any virus symptom.

The failure to date find any resistance to SuMOV in cultivated sunflower germplasm highlights the potential significance of this virus. In tests in progress, we have documented that viral infection will kill seedlings of some susceptible varieties, and decrease seed yields up to 75% in the other varieties. While all tests of interspecific crosses with both annual and perennial *Helianthus* species have been susceptible to SuMOV to date, we have apparently found resistance in some wild H. Annuus accessions which were collected from the same

region in Texas in which the virus was observed. These wild *H. annuus* plants also appear to carry resistance to rust (*Puccinia helianthi*) and downy mildew (*Plasmopara halstedii*), and thus we hope to transfer resistance to rust, SuMOV and downy mildew into lines to be released shortly by the USDA.

Table 1. Reaction of diagnostic hosts for six potyviruses (*Artichoke Latent = ArLV, Clover Yellow Vein = ClYVV, Bidens mottle = BiMoV, Bidens mosaic = BiMV, Pepper veinal mottle = PVMV, Sunflower Chlorotic Mottle = SuCMV*) reported to naturally infect sunflower, in comparison with Sunflower Mosaic (SuMOV)

Hosts	**ArLV**	**ClYYV**	**BiMOV**	**BiMV**	**PVMW**	**SuCMV**	**SuMOV**
Capsicum annuum	NI	NI	NI	NI	MO	NT	NI
Chenopodium quinoa	LL	LL	LL	LL	LL	LL	LL
C. amaranticolor	LL	LL	LL	LL	LL	LL	NI
Gomphrena	LL	S	NI	NI	NI	CMO	NI
Lactuca sativa	NI	NI	MO	MO	S	NI	NI
Nicotiana spp.	MO	LL	LL	MO	MO	CMO	NI
Phaseolus vulgaris	NI	MO	NI	NI	NI	NI	NI
Pisum sativum	NI	S	NI	MO	NT	NI	NI
Zinnia elegans	NI	MO	MO	MO	MO	MO	MO

Symptom codes: NI= not infected, LL= local lesions, MO= mosaic, CMO= chlorotic mottle, S= susceptible, but symptom type not specified in literature, NT= not tested.

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