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Occurrence and transmission of sowbane mosaic virus in seed from naturally infected plants of spinach (*Spinacia oleracea*)

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Abstract

Sowbane mosaic virus was isolated as a single infectious component from seedlings and seeds from a seed-lot of spinach (*Spinacia oleracea*) propagated in Hungary, and was identified by experimental host-range, thermal-inactivation point, dilution end-point, electron microscopy and serology. *Chenopodium quinoa* was more susceptible and/or sensitive to infection than *C. amaranticolor* and was a better indicator host, but *C. amaranticolor* is of diagnostic value. This is the first report on natural infection of spinach by the virus and on its natural seed-transmission in spinach. Growing-on tests on whole seeds and infectivity tests on separate embryos and seed-coats showed that over 30% of the seeds' embryos were infected and c. 80% of the seed-coats contained the virus. The incidence of infection in the spinach crop from which the seed was obtained must have been high.

Introduction

Sowbane mosaic virus (SoMV) was first detected in 1952 in a single mottled plant of *Chenopodium murale* L. (sowbane or nettleleaf goosefoot) from a beet field near Riverside, California (Bennett and Costa, 1961). The highly contagious Sobemovirus (Kado, 1971) has a limited host range, is transmissible in sap and by insects and also in seed of *C. murale* (up to 70%), *Atriplex pacifica* and *C. album* (Bennett and Costa, 1961) and of *C. quinoa* (Bancroft and Tolin, 1967).

The virus has been of main concern to plant virologists as a contaminant, particularly from *Chenopodium* test or indicator plants (Kado, 1967; Engelbrecht and Van Regenmortel, 1968). Infection from seed may be semi-latent (Kado, 1966, 1967) or symptomless, especially during summer, and symptoms in plants infected from the seed may be provoked by rub-inoculation with water (Dias and Waterworth, 1967; Engelbrecht and Van Regenmortel, 1968). Hence, reported natural occurrence of the virus when *Chenopodium* spp. are used as indicator is questionable. The 'apple latent virus 2' detected with *C. amaranticolor* (Kirkpatrick et al., 1965), was soon reidentified as SoMV, possibly introduced via the test plants used (Bancroft and Tolin, 1967). Vector transmission, as possibly by a leafminer fly (Costa et al., 1958), a leafhopper, and a fleahopper (Bennett and Costa, 1961), may be merely mechanical (Costa et al., 1958). Pollen from diseased plants containing the virus (*Atriplex coulteri*; Bennett and Costa, 1961) or surface contaminated with it (*C. quinoa* and spinach; Francki and Miles, 1985) can act as a source of infection for mechanical introduction into superficially pollen-polluted plants by rubbing (Francki and Miles, 1985; Hardy and Teakle, 1992) and by *Thrips tabaci* (Hardy and Teakle, 1992).

Information on natural infection by the virus is limited. Most reports concern one or a few plants tested, as of *C. murale* in California, USA (Bennett and Costa, 1961) and in Yugoslavia (Juretić, 1976), and *C. trigonon* (fishweed) and *C. album* in Queensland, Australia (Teakle, 1968). Reports on natural infection of grapevine (Šarić and Juretić, 1980), sour cherry (Šarić, 1971), peach and plum (Šutić et al., 1971; Šutić and Juretić; 1976, Šarić and Juretić, 1980), sugarbeet (Buturović and Juretić, 1980) in Yugoslavia, and fig in Italy (Quacquarelli, 1971) have not convincingly shown that the virus identified did indeed originate

from the described source. However, actual presence of the virus in grapevine in Germany, as first suggested by frequent sap-transmissions to *C. quinoa*, was corroborated by direct serological testing of grapevine leaf samples (Bercks and Querfurt, 1969).

In 1989 we isolated the virus from a highly infested seed-lot of spinach (*Spinacia oleracea*) commercially produced in Hungary. Thereafter, Horváth et al. (1993) independently reported the virus from that country in symptom-bearing plants of *C. hybridum*, prevalent in potato fields. These two findings now suggest the virus to be widespread there in nature. This paper describes our investigations on the virus in relation to spinach.

Materials and methods

Virus isolate. In August 1989, four spinach seedlings were obtained from a seed company that had commercially propagated the seed in Hungary. During the company's seed-quality testing, several seedlings showed slight plant stunting, leaf rolling and mild mottling, suggestive of virus infection. We then tested a seedling sample for virus infection, and the isolate obtained (Sp44) was used for identification and further studies. The same virus was later directly isolated from the seedlings we grew (Figure 1) from a seed sample from the lot tested by the company.

Virus transmission and maintenance. The virus was transmitted mechanically, using 0.03 M K-Na phosphate buffer (pH 7.3) for grinding and carborundum as an abrasive. Disinfection of hands and utensils was with special care in a 10% trisodiumphosphate solution to prevent unwanted cross contamination. The virus isolate was maintained in living plants of *C. quinoa* and stored in leaf material of such plants desiccated and kept over $CaCl_2$ at 4 °C. Testing of plant tissue for the virus was by inoculation onto *C. amaranticolor* and/or *C. quinoa.*

Serology and electron microscopy. The antisera used were provided by Dr. G. Morvan, Montfavet, France (produced against an undocumented isolate) and by Dr. H.E. Waterworth, Beltsville, Maryland, USA (produced against a Canadian isolate: Dias and Waterworth, 1967), and testing was by gel diffusion and by decoration electron microscopy. Other antisera cited were from D.Z. Maat's IPO-DLO collection.

Seed testing. Seed was tested for infection by planting in steam-sterilised potting soil and visual ispection of seedlings for symptoms, and by soaking seeds individually in 1 ml of phosphate buffer for 12 h and grinding whole seeds, or seed-coats and embryos separately, and inoculating the homogenates onto the indicator plants.

Results

Identification

Host range and symptoms. Virus was readily transmitted from the seedlings originally obtained and from seedlings raised from the seed sample received, as well as directly from the seeds. Observations on visible reactions and results of back inoculations are summarized in Table 1. *Chenopodium amaranticolor* showed many to numerous tiny diffuse, sometimes pin-point chlorotic to necrotic local lesions about a week after inoculation, mostly followed two weeks later by large, diffuse chlorotic, sometimes irregularly star-shaped, systemic lesions, often associated with leaf malformation in, and stunting of, plant tips, and with growth reduction of affected plants. *C. quinoa* reacted after four days with numerous diffuse chlorotic to yellowish flecks and spots on inoculated leaves, followed about one week later by progressive chlorotic spotting to overall interveinal leaf chlorosis, reduced size of tip leaves, severe stunting of plant tips, growth reduction of plants and often tip necrosis. *Nicotiana* spp. often showed diffuse, often inconspicuous systemic chlorotic spots or flecks (Table 1). *Spinacia oleracea* 'Mazurka' and 'Symphonie' both reacted with diffuse chlorotic flecks on inoculated leaves some six days after inoculation. After a further $1^1/_2$ weeks this was followed by diffuse systemic chlorotic flecking, rolling and distortion of young heart leaves.

Persistence of infectivity in expressed sap. Inoculation with sap diluted $\times 10^7$ still yielded numerous local lesions and systemic symptoms in *C. quinoa*, whereas in *C. amaranticolor* no symptoms were seen beyond a dilution of 10^4. In *C. quinoa*, local symptoms were still obtained after heat treatment of sap for 10 min at 90 °C. For *C. amaranticolor* the highest temperature treatment still allowing symptom development was 65 °C.

Serology and electron microscopy. Gel-diffusion tests with antisera to a number of soil-borne, mostly seed-

Figure 1. Spinach seedlings with infection from the seed, 23 days after sowing. Right, healthy control.

transmitted viruses (the nepoviruses *Arabis* mosaic virus, cherry leafroll virus, raspberry ringspot virus, strawberry latent ringspot virus, tobacco ringspot virus, and tomato black ring virus, and the dianthovirus carnation ringspot virus) and with an antiserum to the prevalent seed-borne spinach latent virus (Bos et al., 1980) were all negative. However, the antiserum to SoMV provided by Dr. Morvan yielded a clear reaction line. When desiccated infected leaf material of *C. quinoa* was retested recently after grinding in buffer, this antiserum and the one from Dr. Waterworth both produced clear precipitin lines. Spherical particles c. 30 nm in diameter were easily observed in plant sap negatively stained, and such particles reacted with antibodies to SoMV in electron microscope decoration tests.[1]

Seed-transmission tests

Occurrence of the virus in plants from growing-on tests performed by the seed company already pointed to seed transmission of the virus. Our own tests were meant to prove that infection had not resulted from contamination from an outside source, to quantify the degree of seed-lot infestation, and to study the location of virus within the seed. The results of two growing-on tests and of three infectivity tests on *C. quinoa* of individual whole spinach seeds (one test) and separated individual seed-coats and embryos (two tests) are summarized in Table 2. The whole seeds were also tested on *C. amaranticolor*. Then, infection was detected in 18 whole seeds only instead of 23 as when tested with *C. quinoa* (Table 2). In growing-on tests the abnormal seedlings showed slight growth reduction, leaf narrowing and rolling, and the leaves of some of them were diffusely mottled (Figure 1).

Discussion

In artificial host range and symptoms, and in the extremely high dilution end-point and thermal-inacti-

[1] After submission of this paper for publication, Dr. D.-E. Lesemann, Braunschweig, Germany, found a strong decoration of particles of our virus with the German antiserum to the grapevine isolate of SoMV, and the trapping efficiency of electron microscope grids coated with the antiserum was high when tested with our virus (Lesemann, pers. comm. 20 Febr. 1996).

Table 1. Reactions of test plant species and results of back inoculations

Test species	Reaction[1]	Reaction of *C. quinoa*[2] after back inoculation from	
		Inoculated leaves	Uninoculated leaves
Amaranthus caudatus	L –	nt	nt
Beta vulgaris	– S	nt	nt
Celosia plumosa	L S	nt	++
Chenopodium amaranticolor	L (S)	nt	++
C. quinoa	L S	nt	++
Cucumis sativus 'Gele Tros'	– s	nt	++
Gomphrena globosa	– –	nt	nt
Lactuca sativa 'Patty'	– s	nt	++
Nicotiana glutinosa	l s	++	++
N. megalosiphon P1	l s(S)	++	++
N. rustica	l s	++	++
N. tabacum 'White Burley'	l s(S)	++	++
Phaseolus vulgaris 'Bataaf'	l s	++	++
Pisum sativum 'Koroza'	– s	nt	++
'Rondo'	– s	nt	++
Solanum lycopersicum 'Moneymaker'	– (S)	nt	+
Solanum villosum	– (S)	nt	(+)
Spinacia oleracea 'Mazurka'	L S	nt	nt
'Symphonie'	L S	nt	nt
Vicia faba 'Kompakta'	– (–)	nt	(+)

[1] L local reaction; l symptomless local infection; S systemic symptoms; s symptomless systemic infection; ( ) reaction indistinct; – no reaction, but not tested for infection by back inoculation.
[2] + positive reaction; ++ clearly positive reaction; nt not tested by back inoculation.

Table 2. Results of seed testing

Type of test	Nr. of seeds or seedlings	Nr. abnormal or infected	Proportion abnormal or infected (in %)
Growing-on test and visual observation			
A	55	18	32.7
B	130	43	33.1
Testing of individual seeds or seed parts on C. quinoa			
A whole seeds	36	23	63.9
B seed-coats	24	19	79.2
embryos	19	7	36.8
C seed-coats	18	16	88.9
embryos	16	5	31.3

vation point, the virus isolate from spinach (Sp44) undoubtedly reacted as SoMV. This diagnosis was confirmed by particle size, and gel-diffusion and decoration serology. The biotests (host-range experiments and back inoculations, and dilution and heat treatment series) did not reveal the single or combined involvement of any other familiar virus (e.g., cucumber mosaic virus, also known to occur in seed of spinach; Bos, unpublished results 1977) or possibly new virus. Also, no reaction was obtained with antisera to a number of spherical seed- and soil-borne viruses that produce systemic symptoms in *C. quinoa*, such as some nepoviruses and spinach latent ilarvirus (seed-transmitted in spinach: Bos et al., 1980).

C. amaranticolor and *C. quinoa* were good indicator plants for the virus. When testing spinach seeds for infection and determining the persistence of infectivity in expressed sap, we confirmed Hardy and Teakle's (1992) finding that *C. amaranticolor* is less readily infected than *C. quinoa*. Systemic symptoms in *C. amaranticolor* were often star-shaped chlorotic spots highly characteristic of the virus, earlier also descriptively named '*Chenopodium* star mottle virus' (Kado, 1966). This diagnostic symptom has a number of times promptly warned us for the presence of SoMV as a contaminant.

Spinach was already known as a suscept to infection by SoMV (Bennett and Costa, 1961; Dias and

Waterworth, 1967; Kado, 1966, 1967; Juretić, 1976; Francki and Miles, 1985), and as a good propagation host for purification of the virus (Kado, 1967). Our paper is the first report on natural infection of spinach by SoMV.

Seed transmission of SoMV in spinach could earlier not be detected in 251 seedlings from inoculated spinach plants (Bennett and Costa, 1961), but contamination of pollen from inoculated spinach plants has been reported (Francki and Miles, 1985). Our experiments have clearly shown that the virus infects the embryo and is thus internally carried in the seed from where it gets into the seedling. More seeds carry the virus in their seed-coats than in the embryo. Survival of SoMV in the seed-coat, as with some other highly stable and contagious viruses, could lead to mechanical transfer to originally virus-free seedlings at transplanting. However, in our tests, the percentages of embryo infection (37 and 31) detected corresponded well with the percentages of seedlings found visually infected in the growing-on test (33). Therefore, testing whole seeds for infectivity by inoculation to indicator plants or for the presence of antigen by serology would not be a good way to predict true natural transmission via seeds.

The high percentage of embryo infection (over 30%) and the very high percentage of seed-coat infection (c. 80%) observed suggest an extremely high incidence of virus-infected plants in the crop grown for seed in Hungary since the spinach seed sample we have tested was a random sample from a commercial lot. This conclusion, together with the recently reported massive natural infection of *C. hybridum* in potato-weed communities in Hungary (Horváth et al., 1993), implies that the virus is widespread and prevalent there. Transmissibility of SoMV in commercial seed is of great potential economic significance. Seed infection in the embryo makes disinfection by heat or chemical treatment of infested seed-lots impracticable. That is why the infested seed-lot was immediately destroyed when the virus was detected in it.

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