Berseem Mosaic, a Seed-Transmitted Virus Disease

M. D. MISHRA, S. P. RAYCHAUDHURI, A. GHOSH, and ROY D. WILCOXSON, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India

ABSTRACT

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A mosaic disease observed in berseem plants in India was sap-inoculable and transmitted by *Aphis gossypii* but not by *A. rumicis* or *A. craccivora*. Mechanical inoculation produced chlorotic lesions on leaves of *Phaseolus vulgaris, Chenopodium amaranticolor,* and *C. album*. Systemic mottling was produced on *Petunia* sp.; *Solanum melongena; Lycopersicon esculentum; Nicotiana tabacum* 'White Burley,' 'Harrison's Special,' and 'Xanthi'; *N. rustica; N. glutinosa; Trifolium pratense; Medicago sativa;* and *Melilotus alba*. Cowpea (*Vigna sinensis*) was a symptomless carrier. The virus in crude sap of infected tobacco plants had a thermal inactivation point between 51 and 54 C, a dilution end point between 1:140 and 1:180, and longevity in vitro between 4 and 5 hr at room temperature (25–30 C). Shadow-coated leaf-dip preparations from diseased berseem and tobacco plants showed bacilliform particles 45 × 18 nm. The virus, serologically related to alfalfa mosaic virus (AMV) and probably a new strain of that virus, was transmitted by 60–70% of berseem seeds.

Berseem, or Egyptian clover (*Trifolium alexandrinum*), introduced into India from Egypt in 1940, is cultivated as fodder from October to February in steadily increasing areas of northern India. A mosaic disease was observed in 1967 on berseem plants grown in pot culture at the Indian Agricultural Research Institute (IARI), New Delhi, and later in berseem fields at the IARI and the Indian Veterinary Research Institute at Izatnagar and in cultivators' fields in Delhi and Uttar Pradesh. This paper reports on the virus associated with the disease.

MATERIALS AND METHODS

The virus culture was isolated originally from naturally infected berseem plants grown in pots at the IARI, and later from plants obtained from cultivators' fields, by mechanical inoculation of a number of test plants of berseem, *Nicotiana glutinosa*, and *N. tabacum* 'White Burley.' A pure culture of the virus was obtained by single-lesion isolations from *Chenopodium amaranticolor* leaves. Because berseem plants do not survive the summer in New Delhi, the pure virus culture was maintained in *N. glutinosa* and *Solanum melongena* and in seeds from infected plants of berseem.

Mechanical inoculations were made by rubbing test plants with phosphate-buffered homogenates (pH 7.0, 0.1 M) of diseased berseem leaves. The leaves had been soaked in 0.5% Na_2SO_3 aqueous solution for 1 hr at 10 C. The fresh leaves of the test plants were dusted with Celite before inoculation. For host range studies, 28 plant species belonging to six families were inoculated in a similar manner. Each plant was back-inoculated on tobacco to confirm virus infection of the species.

Insect transmission studies were made with *Aphis gossypii* Glov., *A. craccivora,* and *A. rumicis* collected from berseem and tobacco plants. Apterous adults of these species were starved 3 hr, allowed an aquisition feeding of 30 min on leaves of diseased berseem plants, and then released on healthy berseem or tobacco plants for 18 hr (overnight) for inoculation feeding. On an average, 10 viruliferous aphids were fed on a single test plant and 10 sets of test plants were used for a single aphid species. The insects were killed at the end of the inoculation feeding period, and the test plants were kept for further observations in an insectproof greenhouse.

The seeds collected from diseased berseem plants were sown the next season under insectproof conditions, and the resulting plants were observed for symptoms. The seedlings showing symptoms were tested by bioassaying on *C. amaranticolor* and by direct observation of bacilliform particles in the leaf-dip preparations with the electron microscope. The experiment was repeated for 3 yr.

The biologic and physical properties of the virus were studied by standard procedures with the inoculum prepared from infected tobacco plants.

For electron microscopy, leaf-dip preparations (2,3) made on collodion-coated grids were shadow-casted with gold and examined under a Philips (EM 50) electron microscope. To determine average dimensions, 50 virus particles that appeared intact were measured.

Clarified extracts from diseased leaves of berseem and tobacco plants, prepared in normal saline, were tested for serologic relationship with antisera of cucumber mosaic virus, tobacco mosaic virus, tobacco ringspot virus, cowpea mosaic virus, and alfalfa mosaic virus. These tests were by microprecipitin reaction as outlined by van Slogteren (13).

Present address of fourth author, who was visiting professor at the Indian Agricultural Research Institute: Department of Plant Pathology 304 Stakman Hall, University of Minnesota, St. Paul, MN 55108.

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RESULTS

Host range and symptomatology. The symptoms on berseem in the fields appeared during January and February, when the crop was growing profusely. They became progressively more prominent in the fresh growth after each cutting. Distinct light green mottle, which appeared on the leaves along veins and veinlets, was in the form of either patches or chlorotic streaks, especially as the season warmed up. Sometimes, the pattern of light green mottling turned into a slightly yellow-green area.

The symptoms seen in the naturally infected berseem plants were reproduced in artificially inoculated berseem plants. The first symptom was a clearing of veins. Systemic symptoms required a long time to develop, sometimes more than 20 days. The symptoms were diffused or masked in summer when the temperature was very high in the glasshouse.

The virus was transmitted to 16 species belonging to three families by inoculation with an extract prepared in phosphate buffer (pH 7.0, 0.1 M) from young leaves of berseem showing symptoms of the disease (Table 1).

Melilotus alba was systemically infected. Symptoms were light green mottling, chlorotic patches, and streaks along the veins and veinlets, as in berseem.

Inoculated leaves of *N. tabacum* 'Harrison's Special' developed oak-leaf patterns, whereas leaves of 'Xanthi' had chlorotic or necrotic local lesions or ringspots. Systemic symptoms on *Nicotiana* spp. varied from mild mottle to bright mottle or chlorotic vein banding. Symptoms were very prominent on *N. rustica,* which at times also developed necrotic spots. Initial symptoms on *N. glutinosa* were mild chlorotic spots or vein clearing. A systemic mottling developed within a week on all these hosts, as the leaves became older.

Symptoms on *Phaseolus vulgaris* were necrotic local lesions that often coalesced. No systemic symptoms were observed. On cowpea (*Vigna sinensis*), symptoms were not evident but back-inoculation to tobacco gave a positive reaction, indicating the systemic nature of the infection.

Pinpoint chlorotic local lesions appeared on *C. amaranticolor, C. murale,* and *C. album* 2–4 days after inoculation. A chlorotic halo occasionally formed when the weather was warmer, and lesions sometimes became necrotic.

In warmer weather from May to September, the incubation period in host plants was increased, and symptoms on some hosts were masked or diffused.

The following plant species were not infected by the virus as indicated by negative back-inoculation tests from inoculated plants: *Amaranticolor viridis, Beta vulgaris, Lactuca sativa, Brassica* sp., *Cucurbita pepo, Cucumis sativus, Lathyrus odoratus, Pisum sativum, Solanum nigrum, Datura stramonium, Capsicum annuum,* and *Physalis flori dana.*

Insect transmission. *A. gossypii* transmitted the virus from berseem to berseem but not from *N. glutinosa* to *N. glutinosa, N. tabacum* 'White Burley' and 'Harrison's Special,' *N. rustica, Lycopersicon esculentum,* or berseem. *A. rumicis* and *A. craccivora* failed to transmit the virus from any host.

Seed transmission. On an average, 60–70% of berseem seedlings showed a very mild systemic mosaic mottling on freshly emerging leaves and sometimes on the cotyledons. The symptoms were clearer on seedlings at the six-to-eight leaf stage and became progressively more prominent on freshly emerging leaves after each harvesting. These symptoms are similar to those seen in naturally infected berseem plants in the field. Bioassaying of some randomly selected apparently diseased seedlings on *C. amaranticolor* and direct observations by means of the electron microscope of bacilliform particles in these seedlings indicated infection of the seedlings with AMV.

Physical properties. Tests on physical properties of the virus were done and young plants of *N. glutinosa* were inoculated to determine the effects of treatments on the virus. However, infected *N. tabacum* 'White Burley' leaves were invariably used for preparing the infectious crude extract. The virus in the undiluted leaf extract tolerated exposure at 51 C for 10 min but was inactivated at 54 C. The virus in the extract was infective at a dilution of 1:140 but not of 1:180. The virus was viable in undiluted crude extract for 4–5 hr at room temperature (25–30 C).

Electron microscopy. The shadow-coated leaf-dip preparations both from artificially inoculated berseem and tobacco plants and from naturally diseased berseem plants revealed only bacilliform virus particles (Fig. 1) averaging 45 × 18 nm.

Serologic reaction. The microprecipitin reactions were negative between clarified extract from diseased berseem and tobacco leaves and antiserum of cucumber mosaic virus, tobacco mosaic virus, cowpea mosaic virus, and tobacco ringspot virus but positive with antiserum of alfalfa mosaic virus.

DISCUSSION

Berseem is grown in the cooler regions of northern India for cattle feed and green

Fig. 1. Bacilliform particles of berseem mosaic virus in leaf-dip preparations from diseased berseem and tobacco plants.

Table 1. Reaction of plant species inoculated with berseem mosaic virus

Plant species	Symptoms[a]	Infected/inoculated (no.)	Incubation period (days)
Solanaceae			
Solanum melongena L.	mM	3/5	10–12
Lycopersicon esculentum Mill.	mM	3/6	10–12
Nicotiana tabacum L.			
'White Burley'	M	8/10	10–12 (up to 15)
'Harrison's Special'	M	6/8	10–12 (up to 15)
'Xanthi'	M	2/4	10–12 (up to 15)
N. rustica L.	M	8/10	10–12 (up to 15)
N. glutinosa L.	M	9/10	10–12 (up to 15)
Petunia hybrida Vilm.	mM	2/2	10–12 (up to 15)
Leguminosae			
Phaseolus vulgaris L.	Ch LL	2/2	15
Vigna sinensis Endl.	S	1/2	15
Dolichos lablab L.	Ch LL	1/2	15
Melilotus alba Lam	mM	1/2	15
Medicago sativa L.	M	1/2	15
Trifolium pratense L.	M	2/2	15
Trigonella foenum-graecum L.	M	2/2	15
Chenopodiaecae			
Chenopodium amaranticolor Coste and Reyn.	Ch LL	5/5	2–4 (up to 7)
C. album L.	nLL	2/2	2–4 (up to 7)
C. murale L.	nLL	2/2	2–4 (up to 7)

[a] mM = mild mosaic, M = mosaic, Ch LL = chlorotic local lesions, nLL = necrotic local lesions, S = symptomless.

manure. The only report of a virus disease affecting it in nature is by Verma and Mishra (14), of an enation disease. Kreitlow and Price (8) infected *T. alexandrinum* with alfalfa mosaic virus (AMV) by artificial inoculation. The virus disease we observed in nature in berseem closely resembled AMV in physical properties, transmission, symptomatology, host range, serology, and particle morphology.

The virus isolated from berseem, however, differed from all the typical strains of AMV in that it was not transmitted by *A. craccivora* and *A. rumicis,* produced no symptoms on cowpea, did not infect *Capsicum annuum, Cucumis sativum, Pisum sativum,* and *Datura stramonium,* and was readily transmitted by seed of berseem. We therefore propose naming the isolate the berseem strain of AMV.

Our studies also indicated the seedborne nature of the virus in berseem seeds (60–70% transmission). AMV is recorded as seedborne in *Capsicum* (12) and in lucerne (1,6) but not in red clover (11) or white clover (7). The percentage of infected seeds in berseem seems to be the highest recorded.

In India, AMV was first recorded on Commonwealth Mycological Institute maps (4). The disease reported in this study was noticed in 1967 in berseem by Mishra (9). Nagaich and Giri (10) recorded a strain of AMV from *Primula* and potato at Simla that differed from the berseem isolate in being transmitted by *A. rumicis* and not infecting tomato plants. They did not inoculate cucumber, however. Ekbote and Mali (5) also described an isolate of AMV from alfalfa fields at Parbhani in Maharashtra that resembled the type strain and the yellow mosaic strain of the virus and infected berseem mechanically.

LITERATURE CITED

1. BECZNER, L., and S. MANNINGER. 1975. The epidemiology, aphids and seed transmission of alfalfa mosaic virus. Novenyvedelmi Kutato Intez. Evko. 13:167-176.
2. BRANDES, J. 1957. Eine electronenmikroskopische Schneilmethode zum Nachweisfadenund stabchenformiger Viren, insbesondere in Kartoffeldunkelkaimen. Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschweig) 9:151-152.
3. BRANDES, J. 1964. Identifiziering von gestrecklen pflanzenpathogenen Viren auf morphologischer Grundlage. Mitt. Biol. Bundesanst. Land. Forstwirtsch. Berlin-Dahlem. 110:1-130.
4. COMMONWEALTH MYCOLOGICAL INSTITUTE. 1961. Distribution Map of Plant Diseases. No. 388, ed. 1. Kew, Surrey, England.
5. EKBOTE, A. U., and V. R. MALI. 1977. Studies on alfalfa mosaic virus. Indian Phytopathol. 30:152.
6. FROSHEISER, F. I. 1974. Alfalfa mosaic virus transmission to seed through alfalfa gametes and longevity in alfalfa seed. Phytopathology 64:102-105.
7. KREITLOW, K. W., and O. J. HUNT. 1958. Effect of alfalfa mosaic virus on flowering and seed production of ladino white clover. Phytopathology 48:320-321.
8. KREITLOW, K. W., and W. C. PRICE. 1949. A new virus disease of ladino clover. Phytopathology 39:517-528.
9. MISHRA, M. D. 1977. Progress and trends of virus research in India. Trop. Agric. Res. Ser. 10:13-35.
10. NAGAICH, B. B., and B. K. GIRI. 1968. A strain of alfalfa mosaic virus from *Primula* and potato. Indian Phytopathol. 21:446-447.
11. STUTEVILLE, D. L. 1964. Virus diseases of red clover. Diss. Abstr. 25:2162.
12. SUTIE, D. 1959. The role of cayenne pepper seed in virus transmission. Phytopathol. Z. 36:84-93.
13. VAN SLOGTEREN, D. H. M. 1955. Serological micro reactions with plant viruses under paraffin oil. Pages 45-50 in: Proc. 2nd Conf. Potato Virus Diseases, Lisse-Waganengin, 1954.
14. VERMA, V. S., and M. D. MISHRA. 1975. Bersim enation disease. Zentralbl. Bakteriol. Abt. 2. 1305:709-710.