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IDENTIFICATION OF WHEAT STREAK MOSAIC VIRUS IN IRAN[1]

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ABSTRACT

The virus causing wheat mite-borne mosaic previously reported from the Fars Province of Iran was identified as wheat streak mosaic virus (WSMV) on the basis of host range, morphology and serological properties. The virus was infectious to many plant species in the gramineous family. The filamentous virus particles had a normal length of about 700 nm. Immunosorbent electron microscopy showed a positive reaction between the Iranian isolate and antisera to the American isolates of WSMV. The virus was widely distributed in the Fars Province although not economically important at the present.

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تشخیص ویروس موزائیک مخطط گندم در ایران

پروانه فولادوند و کرامت اله ایزدپناه
بترتیب دانشجوی سابق فوق لیسانس و استادبخش گیاهپزشکی دانشکده کشاورزی دانشگاه شیراز

خلاصه

ویروس موزائیک کنه زا دگندم که قبلا" از مزارع فارس گزارش شده بود ، براساس دامنه میزبانی ، مورفولوژی پیکره ها و صفات سرولوژیکی ، ویروس موزائیک مخطط گندم (wheat streak mosaic virus = WSMV) تشخیص داده شد . ویروس مزبور توانست در تعداد قابل توجهی از گیاهان خانواده گرامینه تولید آلودگی کند . طول نرمال پیکره های رشته ای ویروس حدود ۷۰۰ نانومتر بود . تلفیق سرولوژی والکترون میکروسکپی (immunosorbent electron microscopy) نشان داد که ویروس مزبور با آنتی سرمهای WSMV از آمریکا واکنش مثبت دارد . این ویروس دارای پراکندگی وسیعی در نقاط مختلف

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INTRODUCTION

Plants in the gramineous family can be infected by a number of viruses among which wheat streak mosaic virus (WSMV), agropyron mosaic virus (AMV) and ryegrass mosaic virus (RMV) are vectored by eriophyid mites (10). WSMV is transmitted by *Eriophyes (Aceria) tulipae* Keifer, while AMV and RMV are transmitted by *Abacarus hystrix* Nal. The three viruses have filamentous particles of about 700 nm length (1, 9, 13). Hordeum mosaic virus is similar to the above viruses with respect to morphology, mechanical transmissibility and host range but attempts to transmit it with eriophyid mites have not been successful. On the other hand, the agent of wheat spot mosaic is transmitted by *E. tulipae* but no information is available about its nature (11).

WSMV, AMV and RMV occur in North America and certain European countries. In addition WSMV has been reported from the Soviet Union, Jordan and Turkey (2, 10, 14) but attempts to find the virus in Australia, New Zealand, India, Pakistan, Egypt and Iran were unsuccessful (12). However, a virus with morphological and biological similarity to WSMV was found in wheat fields in the Bajgah Experiment Station by Izadpanah (6). The virus, tentatively called wheat mite-borne mosaic virus (WMBMV), was transmitted by a mite similar to *E. tulipae* but not by cereal aphids common in the area. The purpose of the present study was to identify WMBMV and determine its distribution in the Fars Province. A preliminary report on the host range of the virus has been published (5).

MATERIALS AND METHODS

WMBMV used in this study was isolated from wheat (*Triticum aestivum* L., cv. Roshan) plants with mosaic symptoms in the Bajgah Experiment Station 15 km north of Shiraz. It was transferred to the same wheat cv. first by mechanical inocu-

lation and subsequently by eriophyid mite (6). A single plant showing typical disease symptoms after mite inoculation was selected and used as the source of the virus. The virus was propagated and maintained in wheat. Mechanical inoculation was used in all experiments. Infected leaves were harvested three weeks after inoculation and used either fresh or kept at -20°C until used.

Seeds of various plant species used in host range studies were obtained locally and planted in a greenhouse. Infection of the inoculated plants was ascertained by back inoculation and reproduction of typical symptoms in wheat.

A concentrated virus preparation for rabbit injection was obtained by homogenizing 300 g frozen tissue in 2 vol. of 0.1 m acetate buffer, pH 4.8, containing 0.5% 2-mercapto-ethonol (2-ME) and 0.1% sodium diethyl dithiocarbamate (DIECA). The sap was squeezed through cheesecloth, the pH adjusted to 4.8 for a few minutes and the extract centrifuged at 1900 *g* for 10 min. The clarified extract was made 20% in sucrose and subjected to three cycles of high (100000 *g*) and low (1900 *g*) speed centrifugation with resuspension in water containing 0.5% 2-ME and 0.1% DIECA. The final pellet was resuspended in 3 ml 0.02 M phosphate buffer, pH 7, which was then emulsified with equal volume of Freund's incomplete adjuvant and injected subcutaneously behind the neck of a rabbit. Four injections were made at weekly intervals. A week after the last injection, the rabbit was bled and the serum collected.

The locally prepared antiserum and two WSMV antisera obtained from M.K. Brakke (University of Nebraska, Lincoln, Nebraska, U.S.A.) and J.K. Uyemoto (Kansas State University, Manhattan, Kansas, U.S.A.) were used in immunosorbent electron microscopy (3, 8). Agar-gel-diffusion was performed in 0.75% Ionagar (Difco Laboratories, Detroit, Michigan, U.S.A.).

Electron microscopy was carried out in a Philips EM-300 electron microscope (Biology Dept., Shiraz University).

Particle length was determined on electron micrographs of trapped particles. A diffraction grating grid with 2160 lines per mm was used as standard.

Wheat fields in various regions of the Fars Province were visited in Spring 1986 to determine if the virus and/or the eriophyid mites were present. Suspected samples were taken to the laboratory and inspected under a dissecting microscope for the presence of mites. Attempts were made to transmit the virus from each specimen by mechanical inoculation to wheat. Leaf dip preparation in 2% potassium phosphotungstate was used to determine presence or absence of filamentous virus particles in each specimen.

RESULTS

Host Range and Symptomatology

The following gramineous plants were infected with WMBMV by mechanical inoculation:
Avena sativa L. (oat), *Bouteloua curtipendula* (Michx.) Torr., *Elymus canadensis* L., *E. junceus*, *Hordeum vulgare* L. (cvs. Himalaya and black barley), *Poa* sp., *Secale cereale* L. (rye), *Triticum aestivum* L. (wheat cv. Roshan), *T. durum* Desf. (durum wheat), *T. monococcum* L. (einkorn), *Zea mays* L. (maize local hybrids SC4, SC46A, SC704, and ZP8C704). Symptoms on these plants developed one to three weeks after inoculation. In *Triticum* spp. the symptoms consisted of chlorotic streaks and stripes which gradually spread over the leaves (Fig. 1). In later stages of the disease, the whole plant appeared chlorotic and some leaves became necrotic. In barley, oat, rye, maize and *B. curtipendula*, mottling was the main symptom. No symptoms appeared on *E. canadensis*, *E. junceus* and *Poa* sp. but the virus was recovered from these plants by back inoculation to wheat.

The following plants were not infected:
Agropyron elongatum (Host.) Beauv., *A. intermedium* (Host.)

Beauv., *Bromus inermis* Leyss, *Cenchrus ciliaris* L., *Dactylis glomerata* L., *Echinochloa* sp., *Festuca arundinacea* Schreb., *Hordeum violaceum* Boiss. et Huet., *Lolium perenne* L., *Oryzopsis holciformis* (M.B.) Hack., *Panicum antidotale* Retz. and *Zea mays* (maize local hybrids SC501, SC702 and SC707).

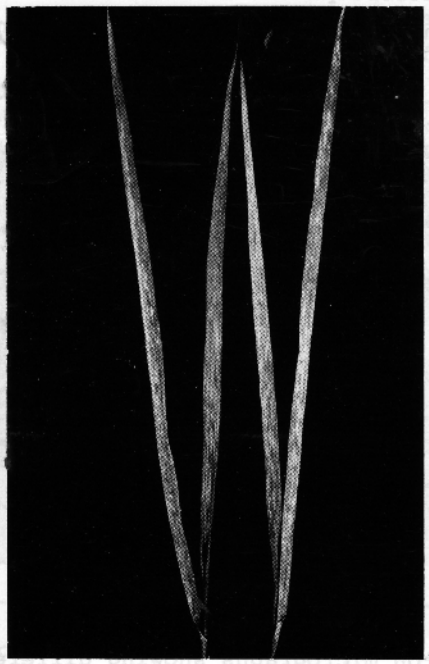

Fig. 1. Streaking and mottling of wheat (cv. Roshan) three weeks after mechanical inoculation with WMBMV.

Serology

No virus-specific reaction was observed between locally produced antiserum and crude or concentrated extracts of infected wheat tissue in agar-gel diffusion. In immunosorbent electron microscopy, positive results were obtained with both WSMV antisera and locally produced WMBMV antiserum. In trapping experiments with the three antisera, the number of particles per electron microscope field increased 14-15 times compared to normal serum (Fig. 2 and Table 1). All antisera decorated

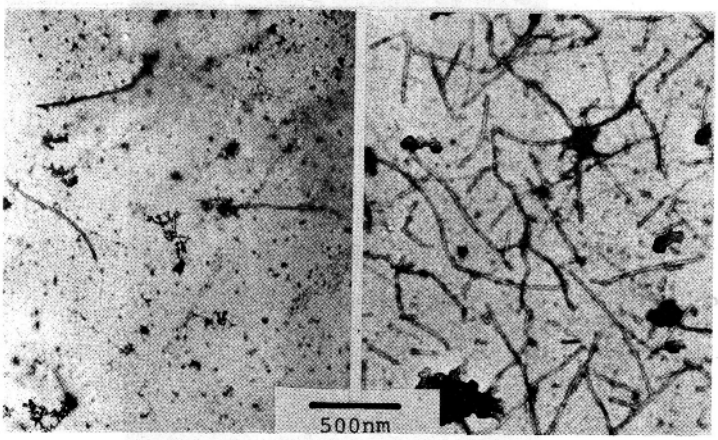

Fig. 2. Electron micrographs showing difference in the number of WMBMV particles adsorbed to electron microscope grids pretreated with (left) normal serum or (right) wheat streak mosaic virus antiserum from the United States (J.K. Uyemoto).

Table 1. Relative number of WMBMV particles adsorbed to electron microscope grids pretreated with two wheat streak mosaic virus antisera, antiserum to WMBMV or normal serum. All sera were used at 1/50 dilution. Virus source was crude sap of infected wheat leaves.

Type of serum	Ave. No. particles per E.M. field†
WSMV-antiserum (M.K. Brakke)	88.8
WSMV-antiserum (J.K. Uyemoto)	83.0
WMBMV-antiserum (produced locally)	83.6
Normal serum	5.9

†Average of 5 random electron microscope fields.

the virus particles (Fig. 3).

Particle Length

A total of 263 particles present in eight electron micrographs were used in length determination. The length of particles varied between 63 and 1050 nm. However, based on the length of 31% of the particles forming a major group between 600 and

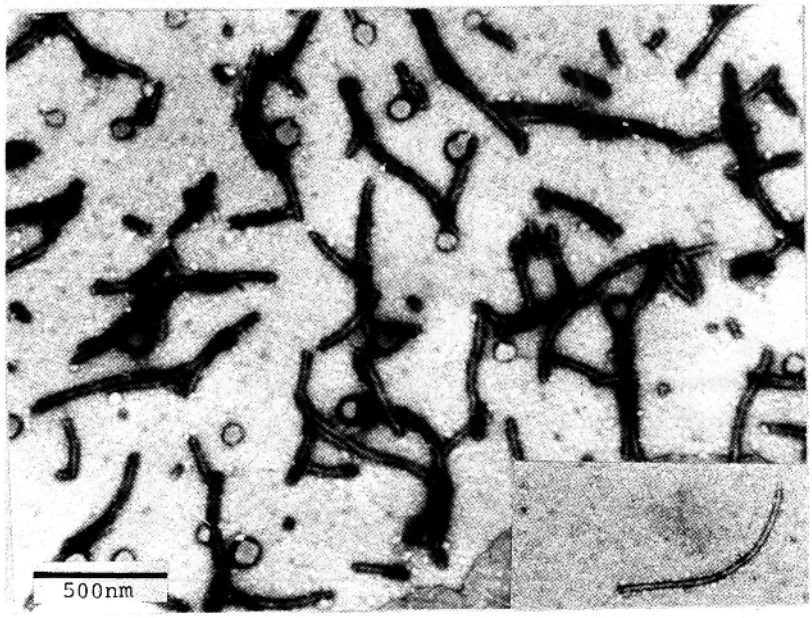

Fig. 3. Electron micrograph showing decoration of WMBMV particles with wheat streak mosaic virus antiserum from the United States (M.K. Brakke). Inset shows a WMBMV particle treated with normal serum.

800 nm, the calculated normal particle length was 704.5 nm.

Distribution

Wheat plants with typical symptoms of mite-borne mosaic were found in Eqlid, Fassa, Neyriz and Shiraz vicinity. They contained a mechanically transmissible virus similar to WSMV. Eriophyid mites were collected in Eqlid, Neyriz, Darab and Shiraz vicinity. The virus and the eriophyid mite were often found together in the same plant. However, in some instances, the virus was present in the field but the mite could not be located. Occasionally, plants were infested with the mite but there was no indication of virus infection.

Severely stunted plants with yellow mosaic symptoms in various locations often contained a rhabdovirus (6).

DISCUSSION

The Iranian WMBMV resembles WSMV, AMV, and RMV in mite and mechanical transmissibility, general host range and particle morphology. It is similar to WSMV in its stability in immunosorbent electron microscopy (7). Both trapping and decoration results indicate that WMBMV is closely related to, if not identical with, WSMV occurring in the United States. Moreover, natural infection of wheat and lack of infectivity to *D. glomerata* excludes RMV as the cause of the disease in Iran. Likewise, infection of oat and lack of infectivity to *Agropyron* spp. shows that WMBMV is different from AMV (1, 9, 13). Nevertheless, the host range of WMBMV is somewhat different from that of WSMV, which may be due to the differences in virus strain or the experimental conditions.

Numerous attempts to purify WMBMV during the course of this study were unsuccessful (4). The antiserum produced by injection of "concentrated extracts" into rabbits had a titer too low to be useful in agar-gel diffusion. However, it was comparable to WSMV antisera from the United States in immunosorbent electron microscopy.

The identity of the mite vector of WMBMV in Iran is still pending. However, the vector is similar to *E. tulipae* not only in gross morphology but also in biology as it causes inrolling and "trapping" of wheat leaves and transmits WMBMV which is closely related to WSMV (Slykhuis, personal communication with second author). The mite has been found naturally infesting not only wheat (6) but also Johnson grass (*Sorghum halepense* (L.) Pers.) and Bermuda grass (*Cynodon dactylon* (L.) Pers.) (Izadpanah, unpublished). In addition, the mite breeds well on barley, maize, and leek (*Allium porrum* L.) in the greenhouse. Johnson grass and Bermuda grass are ubiquitous weeds which may serve as the mite reservoir in this country.

Although the mite vector of WMBMV is present in widely separated areas in the Fars Province, the present agricultural regime involving summer fallow together with high temperature and low humidity in the summer prevent its population increase. As a result, the mite and the virus are not economically important at the present.

NOTE: The identity of the vector mite as *Eriophyes tulipae* Keifer was recently confirmed by Dr. H. Daneshvar, Acarologist, Plant Pest and Disease Research Institute, Ministry of Agriculture, Tehran, Iran.

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