Eggplant Mottled Dwarf Virus in Potato in Iran

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ABSTRACT

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A previously unrecorded disease of potatoes in west-central Iran, characterized by severe stunting, chlorosis, apical necrosis, and wilting, was found to be due to infection by a mechanically transmissible tuber-borne plant rhabdovirus. The virus had bullet-shaped particles measuring 76 × 190 nm in leaf-dip preparations and was biologically and serologically similar to previously described isolates of eggplant mottled dwarf virus (EMDV). This is the first report of natural occurrence of EMDV in potato.

In 1984 and subsequent years, a previously unreported disease occurred in potato (*Solanum tuberosum* L.) in Chahar Mahall-Bakhtiari Province in west-central Iran. Symptoms of the disease included severe stunting, chlorosis, apical necrosis, and wilt (Fig. 1). The disease was found to be caused by a mechanically transmitted plant rhabdovirus that was subsequently identified as eggplant mottled dwarf virus (EMDV) and designated EMDV-P/Ir (potato/Iran).

MATERIALS AND METHODS

Virus source. Virus isolates were obtained from naturally infected potato cvs. Cosima, Draga, Aula, and Pashandi. Leaf extracts from plants showing typical disease symptoms were inoculated to healthy test plants of *Nicotiana glutinosa* L. and *N. rustica* L. Virus isolates were maintained in *N. glutinosa, N. rustica,* and *N. benthamiana* Domin. and were assayed by indicator plant indexing, electron microscopy, and serology for the presence of viruses other than the rhabdovirus associated with the disease syndrome.

Mechanical inoculation. Test plants were inoculated mechanically with leaf extracts of systemically infected *N. glutinosa, N. rustica,* or *N. benthamiana* prepared by grinding leaf tissue in 0.1 M phosphate buffer, pH 7.2, containing 1% (w/v) Celite as an abrasive.

Electron microscopy. Leaf dip preparations from infected potato, *N. benthamiana, N. glutinosa,* and *N. rustica* were negatively stained with 2% ammonium molybdate, pH 6.8, (AM) without prior fixation or with 2% sodium phosphotungstate, pH 7.0, (PTA) after fixation with 5% glutaraldehyde in 0.1 M sodium phosphate, pH 7.0. For particle size measurement, the electron microscope was calibrated using the lattice spacing of stained catalase crystals (11).

Serology. Serological tests were done by double immunodiffusion in 0.8% agarose gels prepared in distilled water containing 0.2% (w/v) NaN_3. Antigens consisted of untreated leaf sap of *N. benthamiana* infected with the Iranian potato rhabdovirus, an Italian eggplant isolate of EMDV (EMDV-E/It) (9), and a Moroccan tomato isolate of EMDV (EMDV-T/Mo) (4). Leaf sap from healthy *N. benthamiana* was used as the control. Antigens were tested against antisera to EMDV-T/Mo, the constricta isolate of potato yellow dwarf virus (PYDV-C), and the sanguinolenta isolate of PYDV (PYDV-S), which were prepared previously as described (4).

Tuber transmission. Tubers of eight Aula potato plants with primary disease symptoms were collected and stored at 5–10 C for 6 mo. The tubers were then planted in pots in a greenhouse at 10–25 C, and plants that grew from the tubers were indexed for virus presence.

RESULTS

Symptoms, mechanical transmission, and host range. Symptoms of primary infection by EMDV-P/Ir in potato consisted of severe stunting, adaxial folding of the young leaflets, leaf epinasty, chlorosis, and wilting of the entire plant, starting at the stem apex and progressing downward (Fig. 1). Purple coloration developed at leaflet bases in purple-flowered cultivars such as Cosima. Secondary symptoms in plants grown from infected tubers consisted of retarded growth, adaxial folding of the young leaflets, chlorosis, and lethal systemic necrosis (Fig. 2).

EMDV-P/Ir produced local lesions without systemic infection in mechanically inoculated *Datura metel* L., *D. stramonium* L., *Gomphrena globosa* L., and *Phaseolus vulgaris* L. 'Red Kidney.' Local and/or systemic symptoms were produced in potato cultivars Aula, Cosima, and Pashandi and in eggplant (*S. melongena* L.), *S. nigrum* L., *Lycopersicon esculentum* Mill. 'Rutgers,' *N. benthamiana, N. debneyi* Domin., *N. glutinosa, N. rustica, N. tabacum* L., *Petunia hybrida* Vilm., and *Physalis floridana* Rydb. The symptoms in inoculated potato were identical to those observed in the field, and symptoms in solanaceous test plants were similar to those caused by other EMDV isolates (2,4,5,9). These symptoms included vein yellowing, chlorosis, and chlorotic spotting. No local or systemic infection occurred in *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., or *Capsicum annuum* L. The presence of EMDV-P/Ir in test plants showing symptoms was confirmed by electron microscopy and serological testing. There was no evidence of the presence of other viruses in the inoculum source plants used in these studies.

Electron microscopy. Plants infected naturally or experimentally with EMDV-P/Ir contained typical rhabdovirus particles (Figs. 3–9). In unfixed leaf dip preparations negatively stained with AM, the majority of particles were broken and were similar in appearance to AM-stained particles of EMDV-T/Mo (4). Particle measurements of EMDV-P/Ir were made from leaf-dip preparations from systemically infected *N. benthamiana.* Leaf extracts were fixed with 5% glutaraldehyde before negative staining with PTA. In these preparations, the majority of particles were bullet-shaped (Figs. 4–7), measuring 76 × 190 nm (average of 50 measurements). Longer bacilliform particles, measuring 76 × 390 nm (Fig. 9), were also observed. No other viruslike particles were seen in infected plants.

Serology. In double immunodiffusion tests, EMDV-P/Ir did not react with

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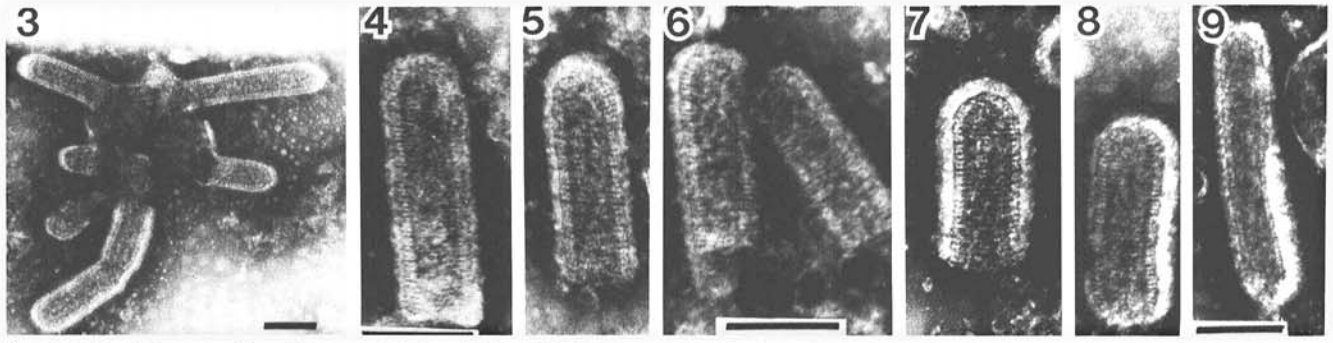
Figs. 1 and 2. Symptoms of Iranian potato isolate of eggplant mottled dwarf virus (EMDV-P/Ir) on naturally and artificially infected potato. **(1)** Severe stunting and inward rolling of young leaflets in mechanically inoculated plant of potato cv. Pashandi (left) compared with healthy plant of same cultivar and same age (right). **(2)** Chlorosis, stunting, and leafrolling resulting from natural tuber infection of potato cv. Aula.



Figs. 3–9. Particle types of eggplant mottled dwarf virus (EMDV-P/Ir) occurring in leaf dip preparations from infected *Nicotiana benthamiana.* **(3–7)** Bullet-shaped particles and **(8 and 9)** bacilliform particles. Crude leaf extracts were fixed with 5% glutaraldehyde before negative staining with 2% neutral sodium phosphotungstate. Scale bars = 100 nm (magnification is identical in Figs. 4–8).

antisera to either PYDV-C or PYDV-S. However, a positive reaction was obtained with antiserum to EMDV-T/Mo, and the precipitin lines of EMDV-E/It and EMDV-P/Ir were confluent (Fig. 10).

Tuber transmission. Thirteen of 20 tubers collected from diseased mother plants gave rise to progeny plants showing typical rhabdovirus disease symptoms, including stunting, chlorosis, chlorotic spotting, and inward rolling of leaflets. Biological and serological indexing confirmed that all 13 diseased progeny plants were infected by EMDV. The remaining seven apparently healthy progeny plants were found to be virus-free.

DISCUSSION

The results of these inoculation experiments indicate clearly that the rhabdovirus originally found in field-infected potatoes in Iran is the causal agent of the disease characterized by stunting, chlorosis, necrosis, and wilting. The morphological, biological, and serological properties of the virus identify it as an isolate of EMDV (EMDV-P/Ir) that did not differ in any significant respect from previously described isolates of the same virus (2,4,5,9).

EMDV has been reported from eggplant in Italy (6), Morocco (4), Tunisia (3), Algeria (7), and Turkey (10); from tomato in Morocco (4) and Italy (2); from hibiscus in Morocco, the Canary Islands, and Greece (5); and from honeysuckle (*Lonicera* sp.) in Tunisia (6) and possibly Afghanistan (8). However, there have been no previous reports of natural infection of potato by EMDV. In Iran, EMDV causes severe symptoms both in imported cultivars such as Cosima, Draga, and Aula and in the local cultivar Pashandi. EMDV is not known to be seed-transmitted (4), and infection in tomato, eggplant, and other seed-propagated crops would therefore depend on spread of the virus from sources outside the crop. In potato, EMDV-infected tubers constitute a source of vegetatively propagated infection within the crop. In addition to direct potential losses due to EMDV infection, the occurrence of this virus in potato introduces the need to monitor its occurrence in areas of seed potato production. Although EMDV has now been reported from several locations around the Mediterranean basin (2–10) and has been found in weed hosts such as *S. nigrum* (5) and *S. sodomeum* L. (4), no vectors of the virus have been identified. The severity of EMDV symptoms in potato and the potential importance of EMDV infection on this widely cultivated crop warrant further research on vector identification and virus epidemiology.

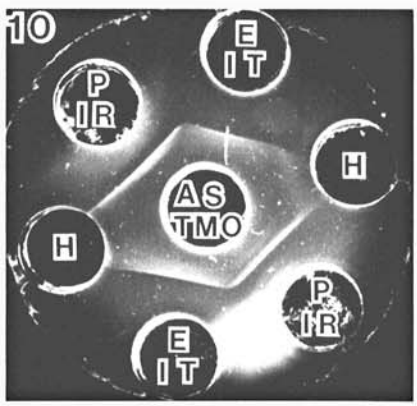


Fig. 10. Immunodiffusion reaction of eggplant mottled dwarf virus isolates from eggplant (E/It) and potato (P/Ir) with antiserum to the Moroccan tomato isolate (AS T/Mo). Antigens in all cases consisted of crude untreated leaf sap from infected *Nicotiana benthamiana.* H = healthy *N. benthamiana* sap. Immunodiffusion medium was 0.8% agarose in distilled water containing 0.2% (w/v) NaN_3.

Although minor differences have been reported between some isolates of EMDV (1), no serological differences were detectable among the three isolates of EMDV used in this study. This indicates that similar isolates of EMDV are widely distributed from the Mediterranean basin to the Middle East and that the virus may be more economically important than previously thought.

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