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Vector Transmission of Eggplant Mottled Dwarf Virus in Iran

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Abstract

Eggplant mottled dwarf virus (EMDV) is a plant rhabdovirus whose natural means of transmission has remained unknown. In the present study various arthropods including two mite, one psyllid, one thrips, five aphid, four planthopper and 14 leafhopper species were examined for their competence to vector EMDV. Healthy eggplant seedlings were inoculated by arthropods either from naturally infested EMDV infected plants or after having access to source plants and subsequent incubation on rearing host. Transmission was achieved only by the agallian leafhopper *Agallia vorobjevi*. Symptoms began to appear 20 days after inoculation. Viruliferous leafhoppers were also captured in the field. Symptomatic plants and vector leafhoppers reacted positively with EMDV antiserum in enzyme linked immunosorbent assay. This is the first report on the identification of EMDV vector in Iran.

Introduction

Eggplant mottled dwarf virus (EMDV) is a rhabdovirus known in the Mediterranean region since 1969 (Martelli, 1969). The virus has been reported from eggplant (Martelli, 1969), tomato (Ladipo, 1977; El Maatoui et al., 1985; Lockhart, 1987), tobacco (Polveraki et al., 1996), pepper (Roggero et al., 1995), potato (Danesh et al., 1989), cucumber (Roggero et al., 1995), muskmelon (Ciffuo et al., 1999), *Pittosporum tobira* (Plavsic et al., 1976), *Hibiscus rosa-sinensis* (Lockhart, 1987), and honeysuckle (Martelli and Cherif, 1987). It occurs in northern Africa, southern Europe and the Middle East (Martelli and Russo, 1973; Ladipo, 1977; Plavsic et al., 1978; El Maatoui et al., 1985; Martelli and Hamadi, 1986; Danesh et al., 1989; Al-Musa and Lockhart, 1990; Ghorbani, 1995; Ciffuo et al., 1999). In Iran, EMDV was identified first in potato in which it caused chlorotic stunt (Danesh et al., 1989; Danesh and Lockhart, 1989) and later in various crops including eggplant, tomato, tobacco, pepper, nightshade, cucumber, and snakemelon (Ghorbani, 1995; Babaie, 2000). The rate of infection varies from place to place and from crop to crop. Higher rates of incidence are found in cooler regions. Incidence rates of up to 35% have been reported in eggplant fields in Shahrekord in central Iran (Babaie, 2000).

EMDV is among the few plant rhabdoviruses which are mechanically transmissible. Although the virus clearly spreads in the fields, the means of its natural transmission has remained unknown. Mechanically transmissible rhabdoviruses are mostly vectored by aphids (e.g. *Lettuce necrotic yellows virus*, *Sonchus yellow net virus*) but some are transmitted either by mites (e.g. *Coffee ring spot virus*) or leafhoppers (e.g. *Potato yellow dwarf virus*) (Peters, 1981). Other rhabdovirus vectors are among planthoppers and lace bugs (Francki et al., 1985; Murphy et al., 1995). However, several insect species previously tested failed to transmit EMDV. These insects included the aphids *Myzus persicae* (Martelli and Russo, 1973; El Maatoui et al., 1985; Al-Musa and Lockhart, 1990), *Macrosiphum euphorbiae* (Martelli and Russo, 1973), *Aphis gossypii* and *A. fabae* (El Maatoui et al., 1985) and the planthopper *Laodelphax striatellus* (Martelli and Russo, 1973). Because of low rates of disease incidence, a sluggish aerial arthropod has been suggested as the vector of this virus. In the present study a number of insects and mites were tested for their ability to transmit EMDV. This study led us to the identification of the EMDV vector in central provinces of Iran.

Materials and Methods

Source of the virus

A naturally infected eggplant from Shahrekord in central Iran was used as the source of the virus for transmission studies. The plant showed severe type of symptoms. The virus isolate was characterized serologically and physicochemically (Babaie, 2000). The virus was maintained in the greenhouse by repeated mechanical or graft inoculation to eggplant or tobacco (*Nicotiana tabacum* cv. Turkish).

Table 1
Arthropods used in EMDV transmission trials, their rearing hosts and inoculation results on eggplant seedlings

Arthropod	Rearing host	Number per plant	Number of plants: positive/inoculated
Aphids			
Aphis fabae Scop.	Broad bean	30	0/50
A. fabae subsp. *solanella* Theob.	Nightshade	30	0/50
A. frangulae subsp.*gossypii* Glov.	Eggplant	30	0/50
A. craccivora Koch	Alfalfa	30	0/50
Myzus persicae Sulz.	Eggplant	30	0/50
Planthoppers			
Laodelphax striatellus (Fallen)	Wheat	20	0/10
Unkanodes tanasijevici Dl.	Wheat	20	0/10
Sogatella vibix (Haupt)	Wheat	20	0/10
Toya propinqua (Fieber)	Bermuda grass	10	0/20
Leafhoppers			
Psammotettix striatus Linnaeus	Wheat	20	0/10
Macrosteles laevis (Ribaut)	Wheat	20	0/10
M. quadripunctulatus Kirschbaum	Wheat	10–15	0/20
Chiasmus conspurcatus Perris	Wheat	10–15	0/20
Balclutha punctata (Fabricius)	Wheat	10–15	0/15
Exitianus fasciolatus Melichar	Bermuda grass	5–10	0/15
Circulifer tenellus Baker	Sugar beet	5	0/20
C. haematoceps (M. R.)	Sugar beet	5	0/20
Austroagallia sinuata (M. R.)	Alfalfa	5	0/20
Anaceratagallia laevis Ribaut	Alfalfa	5	0/30
Agallia vorobjevi Dlab.	Alfalfa	2–3	58/75
E. alsius Ribaut	Clover	5	0/20
Cicadula divaricata Ribaut	Barley	10	0/20
Phelepsius intricatus (H.- S.)	Celery	10	0/10
Thrips			
Thrips tabaci Lindeman	Eggplant, potato, tobacco	20–30	0/45
Psyllids			
Trioza sp.	Eggplant	10	0/20
Mites			
Aculops lycopersici Massee	Eggplant	40–50	0/30
Tetranychus turkestani (Ugar. aeiouNik)	Eggplant	40–50	0/50

Virus acquisition and inoculation by arthropods

Arthropods used in transmission studies (Table 1) were collected in or around EMDV infected fields. Colonies of non-viruliferous aphid species were reared from nymphs, born on filter paper in a petri plate from a single viviparous female. Nymphs of various instars were given access of 24 or 48 h to EMDV-infected eggplant. After an incubation period of 3 weeks on corresponding rearing host (Table 1), they were transferred to healthy eggplant seedlings using 30 aphids per plant for 4–5 days. Colonies of leafhoppers and planthoppers were developed by caging a female and a male of each species on a suitable host. Second and third instar nymphs were given access for 48 h on an infected host, kept on rearing host for 30 days and caged on test seedlings for 3 days. Variable numbers of hoppers were used per cage covering one to three seedlings (Table 1). Alternatively, certain hoppers were collected in high EMDV incidence fields and tested directly by caging individual insects on a test seedling.

Adult psyllids were collected in potato or eggplant fields and caged on EMDV source plants. After 20 days, groups of 10 insects were transferred to eggplant seedlings for inoculation feeding access of 10 days. The nymphs of Thrips (*Thrips tabaci*), eriophyid mites and tetranychid mites were transferred directly from field infested, EMDV infected eggplants to the test seedlings with a hairbrush. Each test seedling received 20–30 thrips or 40–50 mites for 20 days.

Inoculated test plants were sprayed with an insecticide at the end of inoculation feeding period and kept in the greenhouse for a period of 45 days for possible symptom development.

Assay of inoculated plants and arthropods

Inoculated plants were examined periodically for symptom appearance and assessed by enzyme-linked immunosorbent assay (ELISA) using antiserum to a Moroccan isolate of EMDV (courtesy of B.E.L. Lockhart, University of Minnesota, MN, USA) 30 days after inoculation. Samples of all aphid, planthopper and leafhopper species were also tested by ELISA after laps of incubation period. Insects that fed on healthy plants were used as control (Gera et al., 1978; Hibino and Kimura, 1982). Each ELISA sample consisted of two to three hoppers or 20 aphids which were crushed with glass rods in 400 μl of specimen buffer. Double antibody sandwich (DAS) ELISA was performed according to Clark and Adams (1977).

Results and Discussion

All mite, psyllid, thrips, aphid, planthopper and 13 of 14 leafhopper species tested failed to transmit EMDV (Table 1) as evidenced by lack of symptoms in inocula-

Fig. 1 Typical symptoms of EMDV in eggplant 4 weeks after inoculation of the virus by *Agallia vorobjevi*. Arrows show vein clearing in young leaves

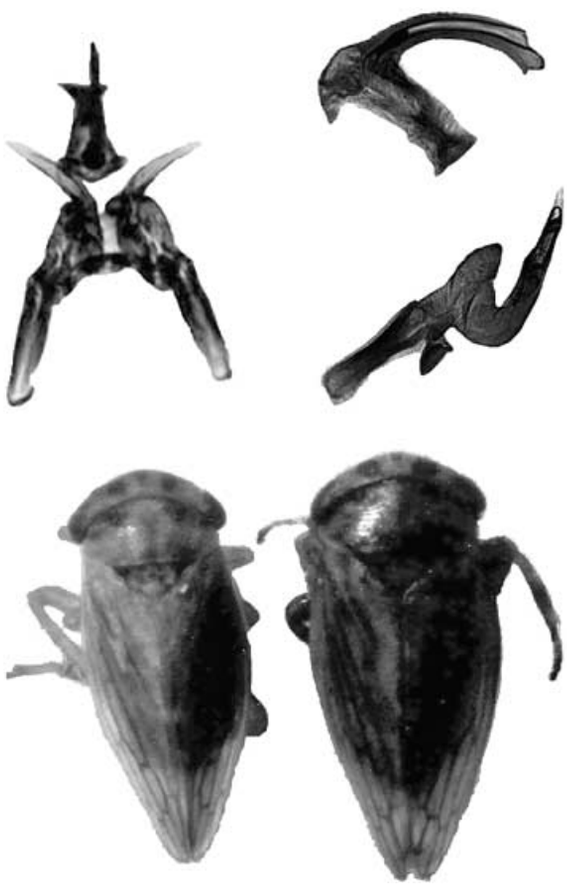

Fig. 2 *Agallia vorobjevi* (vector of EMDV): male (left), female (right) and male genitalia (top)

ted test plants and negative ELISA results. Only *Agallia vorobjevi* Dl. (Cicadellidae) (Fig. 2) was capable of transmitting the virus. Inoculated plants developed vein clearing 20 days and typical yellow vein symptoms (Fig. 1) 30 days after inoculation. Symptomatic plants were positive for EMDV when tested by ELISA. ELISA also detected EMDV in viruliferous *A. vorobjevi* but not in other insects.

Table 2 shows the results of two experiments. When two to three leafhoppers were caged per pot containing

Table 2
Rate of EMDV transmission to eggplant by *A. vorobjevi* under experimental conditions

	Experiment 1	Experiment 2	Average
Number of pots inoculated	12	26	
Total number of plants inoculated	27	48	
Percentage of pots with infection	100	57.7	78.8
Percentage of infected plants	92.6	68.7	80.7

two to three seedlings, up to 100% of pots showed at least one infected plant. In another experiment, adult *A. vorobjevi* were collected in potato and eggplant fields and caged singly on eggplant seedlings. Three of 32 leafhoppers transmitted EMDV. This showed that *A. vorobjevi* is the natural vector of EMDV.

Agallia vorobjevi is a light brown leafhopper with a pair of black spots near the anterior margin of pronotum and a triangular spot on scutellum. Aedeagus has a ventral subapical denticle and the process of the anal tube has a forked apex (Fig. 2) (Emel'yanov, 1967). The leafhopper breeds well on alfalfa and clover but has a low population in potato and eggplant fields.

Results of the present study confirm earlier reports on non-transmissibility of EMDV by certain aphids and planthoppers (Martelli and Russo, 1973; El Maatoui et al., 1985; Al-Musa and Lockhart, 1990). We also report that mites, psyllids, thrips, planthoppers and most leafhoppers commonly found in potato and eggplant fields are not capable of transmitting EMDV. The low rates of EMDV incidence, however, is not due to sluggish behaviour of the vector as suggested earlier (Ladipo, 1977; Ciffuo et al., 1999); it is rather due to low population of the vector in the fields with solanaceous crops.

EMDV resembles *Potato yellow dwarf virus* (PYDV) (Black, 1970) in mechanical transmissibility and vector type. Both viruses are vectored by species of Agallinae. EMDV, however, appears to have a wider host range and confined to the Old World while PYDV is known only from North America. It would be of interest to study if other Agallinae species are involved in EMDV transmission as is the case for PYDV. Another point of interest would be identification of the main EMDV reservoir for crops such as potato in which the virus causes economic loss in certain areas. Alfalfa has already been reported as a symptomless host of EMDV (Danesh et al., 1989).

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