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BROADBEAN WILT VIRUS – IDENTIFICATION, HOST RANGE AND DISTRIBUTION IN THE FARS PROVINCE OF IRAN[1]

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Abstract – A virus isolated from petunia with mosaic symptoms was identified as broadbean wilt virus (BWV) on the basis of host range, mode of transmission, morphology and serology. The virus was similar to the Australian strain of BWV with respect to the symptoms produced in pea, tomato, petunia, *Chenopodium* spp. and snapdragon and its failure to infect nasturtium and parsley. Some reported hosts of the Australian and other strains, however, were not infected by our isolate. Mungbean was found to be a local lesion host for the virus. Surveys of broadbean crops indicated rare occurrence of the virus in the Fars province of Iran.

INTRODUCTION

Broadbean wilt virus (BWV) was initially studied by Stubbs [17, 18] who described the symptoms of the disease and certain properties of the virus in Australia. The virus produced severe necrosis of terminal leaves and stem followed by wilting and often death of broadbean plants. Similar symptoms in broadbeans were also produced by a necrotic strain of petunia ringspot virus [11] which was found to be related to BWV [4, 9, 10]. Several other viruses from widely different sources have been shown to be related to BWV. These include nasturtium ringspot virus [2, 4, 6, 12, 16], parsley virus 3 [5, 6], *Digitalis* mosaic virus [15], P.O. pea streak virus [7], a virus inducing blight of spinach [14], two viruses from pepper [12] and a virus found in catalpa and carrot [13]. More recently, Uyemoto and Provvidenti [21] were able to recognize two distinct serological types (serotypes) among 7 isolates of BWV. Serotype I included isolates from pea, spinach, broadbean, nasturtium and *Plantago* I, whereas isolates from lettuce and *Plantago* II were members of serotype II.

Strains of BWV have been reported from various parts of the world [20]. This paper is the first to report on the isolation and characterization of a BWV strain in Iran.

MATERIALS AND METHODS

The virus was originally isolated from petunia (*Petunia hybrida* Hort.) plants with

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1. Contribution from the Department of Plant Protection, College of Agriculture, Pahlavi University, Shiraz, Iran. Part of M.S. thesis by the senior author.

2. Former graduate student and Professor, respectively.

chlorotic spots in a greenhouse in Shiraz, Iran. It was sap-transmitted and passed through two successive single-lesion isolations in *Chenopodium quinoa* L. and maintained and propagated in broadbean (*Vicia faba* L.).

Plants were grown in a greenhouse at temperatures ranging from 15 to 25°C. During the summer, the light intensity was reduced by partial shading and in the winter, supplementary light was provided. The test plants were grown in plastic pots containing a mixture of peat moss and sterilized field soil.

Inocula for host range studies were prepared by grinding infected tissues with an approximately equal amount (w/v) of 0.01 M potassium phosphate buffer, pH 7.6. Assays were usually made by determining the dilution end point of preparations on broadbean. Test plants were uniformly dusted with 500-mesh carborundum powder and rubbed with a forefinger dipped in the inoculum. Control plants were rubbed with phosphate buffer alone.

A modification of the method of Taylor *et al.* [19] was used for purification of the virus. All operations took place at 4°C. Terminal leaves and stems of infected broadbean plants were collected 6–10 days after inoculation (just before wilting) and homogenized in 0.1 M potassium phosphate buffer, pH 7.6, containing 0.1 M sodium diethyldithiocarbamate and 0.01% thioglycollic acid. One millilitre of buffer was used per g of tissue. The sap was filtered through cheesecloth, left overnight, emulsified with 0.25 volume chloroform and stirred for 20 min with a magnetic stirrer. Differential centrifugation was used to separate the virus from normal host components using a Sorvall RC-2B centrifuge for 10–20 min at 7000 *g* and a Spinco Model L3-50 centrifuge for 1.5–2 hr at 78,000 or 100,000 *g*. The pellets of high speed centrifugation were stirred in 0.01 M phosphate buffer with a tube shaker for 3–18 hr. The virus preparation was further purified by centrifuging in sucrose density gradients [3] prepared in the same buffer. Gradients were made by layering 4, 7, 7 and 7 ml of 10, 20, 30 and 40% (w/v), respectively, sucrose solutions in cellulose nitrate tubes of Spinco SW 25.1 rotor. 1–1.5 ml of virus were layered on top of the sucrose columns and centrifuged for 2.5–3.5 hr at 24,000 rev/min. The virus bands were monitored through and recovered with an ISCO density-gradient fractionator (Instrumentation Specialties Co., Lincoln, Nebraska). Sucrose was removed from the samples by dialysis for 24 hr against 0.01 M phosphate buffer and the virus suspensions were concentrated by high-speed centrifugation.

For electron microscopy, the dialyzed virus was negatively stained in 2% potassium phosphotungstate, pH 7, and the grids were examined and photographed in a Phillips EM 300 electron microscope operated at 60 kV.

An antiserum to BWV was prepared by giving a rabbit one intramuscular injection in the hip and two subcutaneous injections behind the neck. The final high-speed centrifugation pellets were resuspended in 0.01 M phosphate buffer, pH 7.6, emulsified in Freund's incomplete adjuvant (Difco Laboratories, Detroit, Mich.) and used for injections made at one-week intervals. Two millilitres of the virus-adjuvant emulsion were used in each injection but the amount of virus was not determined.

For comparative purposes, an antiserum against type II of BWV, kindly provided by Professor R.M. Gilmer of Cornell University, New York, was also used in this study. Tube and microprecipitin [1] and agar gel diffusion [8] tests were used for various serological studies.

RESULTS AND DISCUSSION

Host range studies

The virus produced tip necrosis in broadbean plants 5–6 days after inoculation. The tips of unfolded leaves turned black and necrosis later extended down to the stem and root followed by wilting and death of the plants (Fig. 1). Tip necrosis was used as a diagnostic symptom for the virus in this study. Plants which survived the initial symptoms, showed systemic mosaic, reduction in size, and inward rolling of the margins of young leaves (Fig. 2).

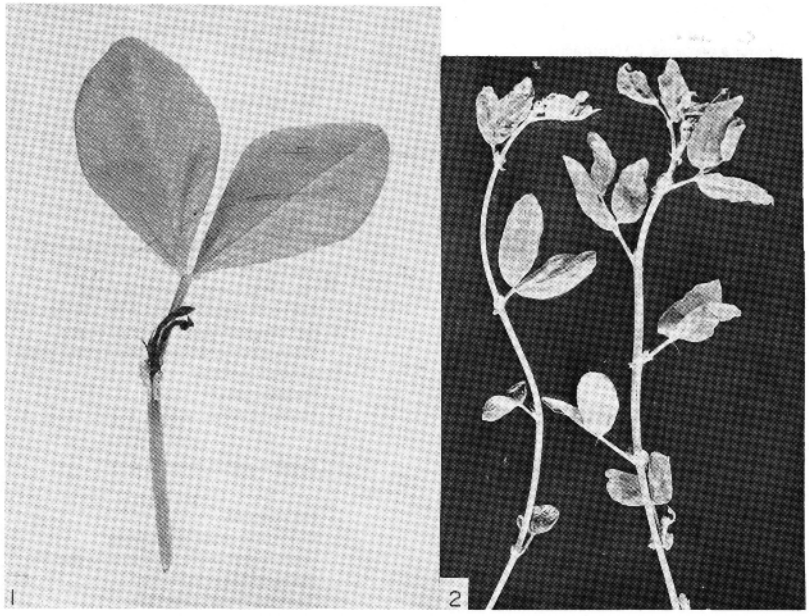

Fig. 1. Tip necrosis in broadbean 7 days after inoculation with BWV.
Fig. 2. Systemic mosaic produced in broadbean plants which survived necrosis and wilt due to BWV infection.

A number of plant species from different families were tested for their reactions to BWV in the greenhouse. Freshly-extracted sap from infected broadbean was used as inoculum. Recovery inoculations were made from all inoculated plants to broadbean. Table 1 lists the type of reaction observed in the inoculated plants. Wilt was induced in pea (*Pisum sativum* L.) and globe amaranth (*Gomphrena globosa* L.) in addition to broadbean. Bean (*Phaseolus vulgaris* L.), mungbean (*Phaseolus aureus* Roxb.), cowpea (*Vigna unguiculata* L.), *Cassia occidentalis* L. and *Chenopodium* spp. produced local lesions (Figs. 3–5). The virus became systemic in *Chenopodium quinoa* L. and caused severe twisting of terminal leaves (Fig. 6). Mosaic symptoms were also induced in petunia, eggplant (*Solanum melongena* L.), tomato (*Lycopersicum* esculentum Mill.), pea (*Physalis floridana* Rydb.) and *Amaranthus caudatus* L. (Fig. 7). *Nicotiana tabacum* L. cv. Turkish, *N. glutinosa* L. and snapdragon (*Antirrhinum majus* L.) did not show symptoms, but

Table 1. Reaction of various plants to broadbean wilt virus BWV

Plants	Symptoms
Amaranthaceae:	
Amaranthus caudatus L. (amaranth)	Systemic mosaic
Gomphrena globosa L. (globe amaranth)	Necrotic local lesions, wilting, death
Chenopodiaceae:	
Beta vulgaris L. (sugar beet)	No reaction
Chenopodium album L. (goose foot)	Small chlorotic local lesions
C. amaranticolor Coste & Reyn.	Chlorotic local lesions
C. quinoa Willd.	Chlorotic local lesions with white center, twisting of terminal leaves
Spinacea oleracea L. (spinach)	No reaction
Compositae:	
Zinnia elegans Jacq. (zinnia)	No reaction
Cucurbitaceae:	
Cucumis melo L. var. *reticulatus* Naud. (cantaloupe)	No reaction
Cucumis sativus L. (cucumber)	No reaction
Cucurbita pepo L. (squash)	No reaction
Leguminosae:	
Cassia occidentalis L. (coffee senna)	Necrotic local lesions, vein necrosis*
Lathyrus odoratus L. (sweet pea)	No reaction
Phaseolus aureus Roxb. (mungbean)	Necrotic spots, necrotic rings
Phaseolus vulgaris L. (bean cv. Bountiful)	Necrotic local lesions with white centre
Pisum sativum L. (garden pea)	Systemic mosaic, wilting, death
Vicia faba L. (broadbean)	Tip necrosis, wilting, death, mottling in surviving plants
V. sativa L. (vetch)	No reaction
Vigna unguiculata L. (cowpea)	Necrotic local lesions
Scrophulariaceae:	
Antirrhinum majus L. (snapdragon)	Symptomless infection
Solanaceae:	
Capsicum annuum L. (pepper)	No reaction
Datura stramonium L. (Jimson-weed)	Chlorotic rings, yellowing*
Lycopersicum esculentum Mill. (tomato)	Systemic mosaic, leaf malformation
Nicotiana glutinosa L.	Symptomless infection
N. tabacum L. (tobacco cv. Turkish)	Symptomless infection
N. rustica L. (peasants tobacco)	Chlorotic zones*
Petunia hybrida Hort. (garden petunia)	Systemic mosaic, leaf malformation
Physalis floridana Rydb.	Mild systemic mosaic, recovery
Solanum carolinense L. (Carolina horse-nettle)	Chlorotic rings, yellowing*
S. melongena L. (eggplant)	Systemic mottling and crinkling
Tropaeoliaceae:	
Tropaeolum majus L. (nasturtium)	No reaction
Umbelliferae	
Petroselinum crispum (Mill.) Nym. (parsley)	No reaction

*Back inoculation on broadbean was negative.

recovery inoculations from these plants to broadbean showed typical tip necrosis. *C. occidentalis, Solanum carolinens* L. and *Datura stramonium* L. showed localized symptoms but attempts to recover the virus from these plants by recovery inoculation to broadbean were unsuccessful. Vetch (*Vicia sativa* L.), sweet pea (*Lathyrus odoratus* L.), pepper (*Capsicum annuum* L.), beet (*Beta vulgaris* L.), spinach (*Spinacea oleracea* L.), squash (*Cucurbita pepo* L.), cantaloupe (*Cucumis melo* L. var. *reticulatus* Naud.),

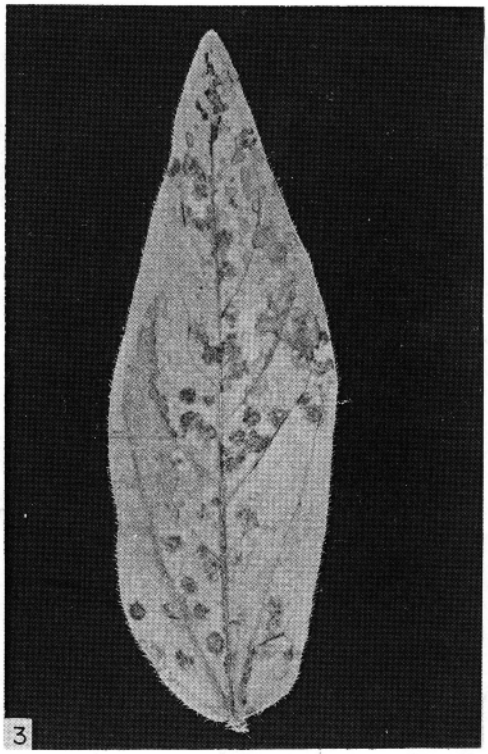

Fig. 3. Necrotic local lesions produced on primary leaves of mungbean 6 days after inoculation with BWV.

cucumber (*Cucumis sativus* L.), zinnia (*Zinnia elegans* Jacq.), nasturtium (*Tropaeolum majus* L.) and parsley (*Petróselinum cryspum* (Mill.) Nym.) did not show symptoms and recovery attempts failed.

Because of the discreteness of local lesions formed in mungbean and relative ease in growing this plant, its usefulness as an assay host was explored. Ten-fold dilutions of infected broadbean sap were made in 0.05 M phosphate buffer. Ten to twelve primary leaves of mungbean were inoculated with each dilution. Five days later the lesions were counted. The dilution curve was constructed by plotting the average number of lesions per leaf against the log of dilution (Fig. 8). Mungbean appeared to be a suitable assay host at 10^{-1} to 10^{-3} dilutions of infected broadbean sap. However, the use of this plant as an assay host for partially purified virus preparations produced inconsistent results probably due to the low concentration of the active virus.

Insect transmission

Green peach aphid (*Myzus persicae* Sulz.), and black broadbean aphid (presumably *Aphis fabae* Scopoli.), were tested for their transmission ability. Apterous insects, reared on healthy sugarbeet plants, were allowed to feed on infected broadbean plants for 1–2

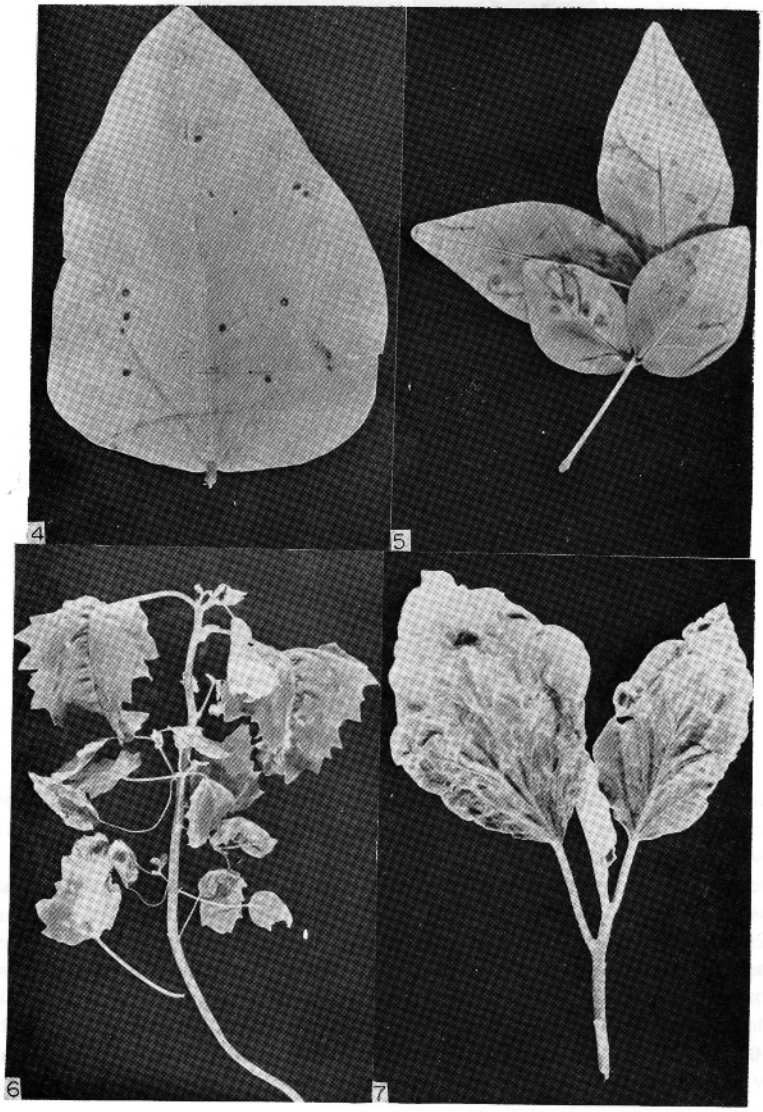

Fig. 4. Necrotic local lesions in primary leaves of cowpea inoculated with BWV.
Fig. 5. Leaves of *Cassia occidentalis* showing necrotic local lesions after inoculation with BWV.
Fig. 6. Systemic symptoms induced by BWV in *Chenopodium quinoa*.
Fig. 7. Leaves of BWV-infected eggplant showing systemic mosaic symptoms.

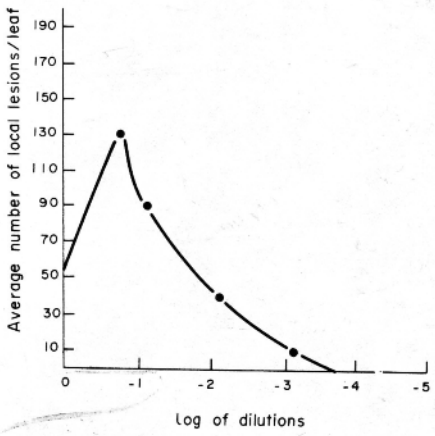

Fig. 8. Relationship between the number of local lesions produced on primary leaves of mungbean and dilution of BWV-infected broadbean sap.

min and transferred to young healthy broadbean plants. Five aphids were used per plant. Aphids fed on healthy broadbean plants were used as control. Plants were kept under insect proof plastic cages in the greenhouse. The insects were killed by insecticide application after 24 hr. Seventy per cent of plants inoculated with green peach aphids and 20% of those inoculated with black broadbean aphids showed typical tip necrosis after one week. Control plants showed no symptoms.

Properties of partially purified virus

The aqueous phase obtained after the initial clarification of the extract was dark brown. Clarifications with *n*-butanol or ether or addition of sodium sulfite, 2-mercaptoethanol, or EDTA to the homogenate did not remove the dark pigment. Some of this pigment remained with the preparations throughout the purification procedure.

When centrifuged density-gradient columns were examined in a dark room with a beam of light, only two bands were visible: an upper opalescent band and a lower brownish band. An additional u.v. absorbing band was detected between the upper and lower bands when the columns were analyzed with the density-gradient fractionator. The ultraviolet absorption spectrum of the upper band was typical for proteins (maximum absorption at 280 nm) whereas the spectra for middle and lower bands were typical of nucleoproteins (maximum absorption at 260 nm). The relative infectivity of different bands or their combinations were not determined but the bottom band was infectious when inoculated to broadbean plants. When this component was dialyzed, concentrated by ultracentrifugation and examined in the electron microscope, numerous isometric particles of about 30 nm were observed (Fig. 9).

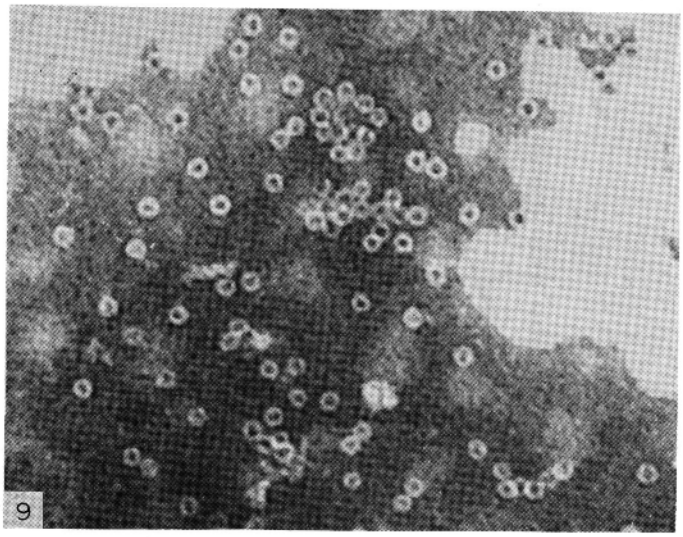

Fig. 9. Electron micrograph of partially purified BWV negatively stained in 2% potassium phosphotungstate, PH 7 (X 120,000).

Serology

In microprecipitin tests, the antiserum prepared against the virus gave a titer of 1:256. It also produced a strong precipitin band in gel-diffusion tests. A similar band was produced with antiserum against type II virus [21] (Fig. 10). A precipitin line was also

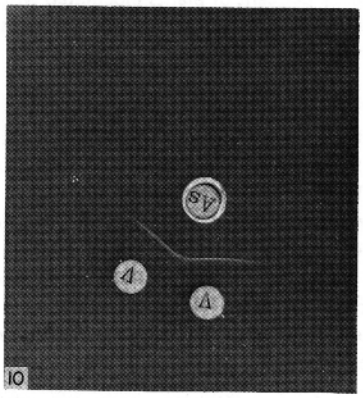

Fig. 10. Agar-gel-diffusion test with antiserum against type II BWV (AS) and partially-purified BWV (V). The antiserum was obtained from Dr. R.M. Gilmer of Cornell University.

produced when healthy broadbean sap was reacted against the locally-produced antiserum. This indicated presence of plant proteins in the partially-purified virus preparations used for injection and testing. The virus did not react with antisera against cucumber mosaic virus, alfalfa mosaic virus, broadbean true mosaic virus and cowpea mosaic virus.

Distribution

Suspected specimens from broadbean fields were collected from 1975 to 1977. Each specimen was used to inoculate young broadbean plants and formation of tip necrosis and wilt in inoculated plants was considered as evidence for the presence of BWV.

Limited search for the virus in broadbean fields in the vicinity of Shiraz, in the winter of 1975–1976 failed. A more extensive survey was made in the following growing season. Over 200 specimens which showed wilting or various types of necrosis were collected from various locations in the Fars province and indexed. Only two specimens from the vicinity of Shiraz and two specimens from the Mamassani area induced typical BWV symptoms in broadbeans. These results suggest that BWV has a limited occurrence in broadbean. There is no information about the presence of this virus in other parts of Iran.

The BWV isolate in the present study appears to be a new strain similar to the Australian strain [17] with respect to the type of symptoms produced in broadbean, pea, petunia, tomato, snapdragon, and *Chenopodium* spp. and failure to infect nasturtium and parsley. Our isolate, however, differed from the Australian strain in its failure to infect sugarbeet, vetch, pepper and spinach. Based on the reported host range in the other studies, it is even less similar to other BWV strains. Nasturtium ringspot virus [2, 12], for example, produces systemic mottling and ringspot in nasturtium. Parsley virus 3 [5, 6] produces a clear green mottle in broadbean, systemic chlorotic ringspot in petunia, oakleaf pattern in *N. tabacum* cv. Xanthi, chlorosis in *Chenopodium* spp. and severe stunting with leaf necrosis in parsley. In this study mungbean is reported as a host for BWV for the first time.

Broadbean did not appear to be a suitable virus source for purification under our conditions. However, Taylor *et al.* [19] have reported a yield of 1 mg per 100 g tissue for the Australian isolate. Our isolate was not purified satisfactorily by the modification of the method of Taylor *et al.* [19], since the final preparations contained a considerable amount of host proteins as indicated by the serological tests. Preliminary observations indicate that eggplant produces higher virus concentration and may be a more suitable source of virus for purification.

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