Bean Yellow Mosaic Virus on Broad Bean Plants in Libya

I. Identification of the causal agent

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INTRODUCTION

Several fields of broad bean (*Vicia fabae* L.) plants around Tripoli were inspected in the fall of 1970–1971 for the presence of virus diseases. In many of the fields surveyed, virus-like symptoms were noted on a large number of broad bean plants. These symptoms included varying degrees of mosaic intensity, leaf puckering and frequent development of green patches on chlorotic leaflet laminae.

Several reports of similar symptoms on broad beans in Egypt, the Sudan and Palestine have been made (1,3). In these countries three viruses; alfalfa mosaic, pea mosaic and bean yellow mosaic were identified from broad beans. Symptoms observed on these plants did not give an indication as to the identity of the virus or viruses involved. In the U.S.S.R., it was reported (7) that survey tests carried out in 1961 on broad bean fields revealed the presence of 18 viruses. Among those that were most serious and widespread were broad bean mottle, pea virus 'J', and pea leaf roll virus. Von Der Pahlen (7) identified bean yellow mosaic virus in crops of broad beans, pea and French beans in Argentina, but being severe only on broad beans.

The present work was undertaken to identify and study the virus or viruses involved in naturally infected broad bean plants showing the above described symptoms.

MATERIALS AND METHODS

The original source of infected plant material was obtained from a broad bean field at the Ministry of Agriculture Experimental Farm at Sidi El-Mesri near Tripoli. Mechanically inoculated broad bean seedlings were used as the source of the virus throughout the study. The virus isolate was also passed through susceptible bean varieties, then inoculated back to the broad beans.

Mechanical inoculations were done by grinding fresh and young infected leaves in a mortar together with a few drops of phosphate buffer or distilled water. The extracted sap was immediately rubbed on the surfaces of carborundum-dusted test plants. The inoculated surfaces were then rinsed with tap water. All experimental plants were grown in sterilized soil under greenhouse conditions.

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In all host range studies, back inoculation to broad beans from the tested plants were undertaken. Experiments dealing with the physical properties of the virus were conducted according to established procedures as described by Ross (4).

The aphid species employed in the insect transmission experiments were *Aphis gossypii* Glover and *Macrosiphum pisi* (Harris). The identity of these aphid species was confirmed by Dr. Shakir Hammad of the Faculty of Agriculture, University of Tripoli. All aphids used for transmissions were starved for 3–4 hours before commencing the acquisition feeding probe. Nonviruliferous aphids were allowed to feed on virus source plants for 10–15 minutes. The virus source plants were either young broad bean or bean leaves showing typical symptoms and known to have been inoculated with the virus under study. The aphids, after acquiring the virus, were moved to healthy broad bean seedlings, on which they were allowed to feed for 15 hours. The inoculation feeding period was terminated by spraying the aphids with malathion.

RESULTS

I. Host Range and Symptomology

Broad beans Broad bean seedlings, inoculated at 4–6 leaf stage, reacted systemically by first developing vein clearing on new growth about 6–8 days after inoculation. Not uncommonly this vein clearing progressed to some sort of net chlorosis. Other systemic reactions included mild to severe chlorosis and frequent development of green patches on the chlorotic back ground of the leaflets (Fig. 1). Several broad bean seedlings when inoculated with the virus directly obtained from naturally infected broad bean plants developed black necrotic lesions along the petioles, extending sometimes to a part of the stem. This type of necrotic reaction did not appear in later inoculation experiments after the virus had been passed through several susceptible bean and broad bean plants. This is

Fig. 1. Systemically infected broad bean leaflets with the virus isolate under study. General chlorosis and the appearance of green patches on the leaflet laminae were the usual responses.

an indication that the original virus source was probably a mixture of viruses or strains of one virus which were later screened to one virus or one strain of a virus as a result of several transfers through other hosts.

Beans Twelve varieties of beans *Phaseolus vulgaris* L. were tested as part of a host range study. Table 1 describes the type of reaction of each of the varieties tested to the virus inoculations. None of these varieties exhibited any form of necrotic reaction. A very common type of symptom produced by several of these varieties was the development of moderately numerous, well-defined, small chlorotic lesions on new growth leaves (Fig. 2) 6 days after inoculation. The varieties Harvester, Wade, Tender Green and Corneli produced severe to mild mosaic reactions. Back inoculations from all these infected plants to broad bean seedlings produced the usualy typical symptoms of the virus under study. The variety Romes Blanche de Guillet showed no noticeable reaction to the virus. However, upon back inoculation to broad bean seedlings, the latter developed the usual symptoms of the virus.

The varieties Bobis Mercato Mangiantutte, Extender, Tender Crop, Victory, and Jacson Wonder were not hosts of the present virus isolate.

Pea (*Pisum sativum* L) Local varieties of pea were found to be susceptible to the virus. Inoculated seedlings developed necrosis on some leaves and eventually there was a general collapse of the whole plant.

Chenopodium amaranticolor Coste and Reyn. In one experiment, young seedlings of *C. amaranticolor* inoculated with the purified virus preparation (5) reacted by forming faint chlorotic local lesions on the inoculated areas of the leaves 6 days after inoculation. In other experiments, using the usual infected crude sap instead of purified virus preparations, the tested plants failed to exhibit any type of reaction.

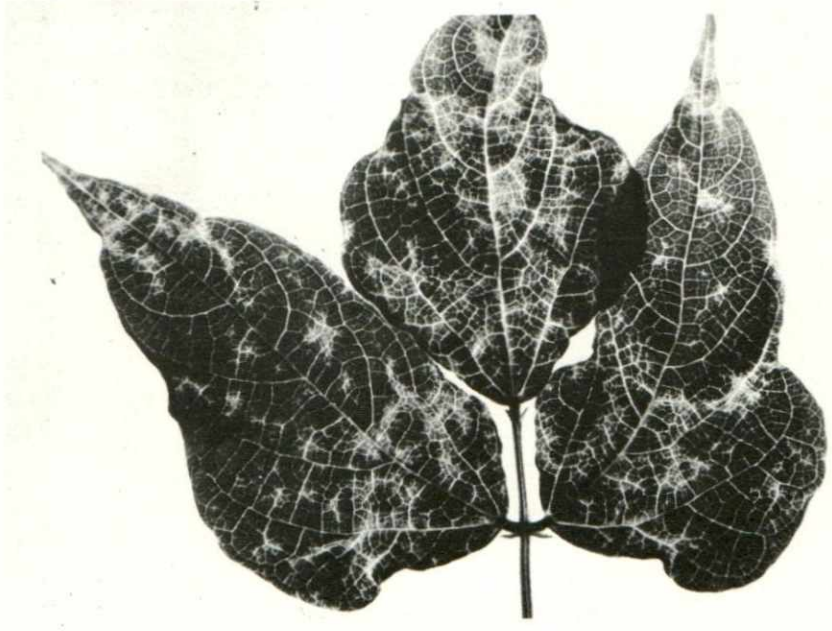

Fig. 2. Tender green bean leaf infected with the virus isolate. Small chlorotic lesions appear on the leaflets as the first noticeable reaction.

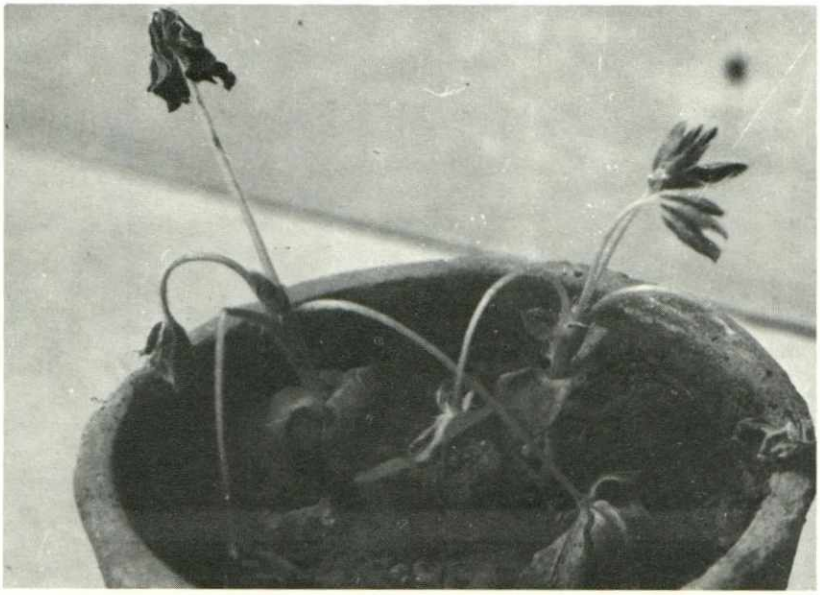

Fig. 3. Lupine seedlings inoculated with the virus under study. Symptoms include black necrotic local lesions on the cotyledons, followed by a general collapse of the seedlings.

Lupine (*Lupinus Termis* Forsk) Lupine seedlings reacted to the virus inoculation by forming black local necrotic lesions on the inoculated cotyledons and later by a general collapse and death of the whole plant (Fig. 3).

Inoculations and back inoculation tests showed that the following plants were not hosts of the present virus isolate: *Cucumis sativus* L., *Capsicum furtescens* L., *Nicotiana rustica* L., *N. tabaccum* L., *N. glutinosa* L., *Vigna sinessis* Savi; *Zinia elegans* Jacq., *Lycopersicon esculentum* L., Mill. *Gomphrena globosa* L., *Cucurbita pepo* L., *Brassica oleracea* L., and *Raphanus sativus* L.

II. Physical Properties

The thermal inactivation end point, the dilution end point, and the aging in vitro of the present virus isolate were determined by using fresh crude sap of infected broadbean leaves, and utilizing young broad bean seedlings as test plants. Experiments were repeated twice for the determination of each property. The thermal inactivation point of the virus isolate was found to be between 60 and 62°C. The virus withstood a dilution of 1:100 but not 1:1000. It lost its infectivity when stored in a crude juice at room temperature (about 25°C) for 24 hours, but not for 16 hours.

III. Insect Transmission

After acquiring the virus from infected bean or broad bean leaves *Aphis gossypii* Glover and *Macrosiphum pisi* (Harris) were found to be able to transmit the present isolate of BYMV when fed on healthy broad bean seedlings for 15 hours. Symptoms appeared on the test plants 12 days after inoculation when using insects that acquired the virus from infected broad bean leaves, and 18 days when using insects which acquired the virus from infected bean leaves.

Table 1 Reaction of twelve varieties of beans to the virus isolate

Tested bean varieties	Type of host's reactions	Back inoculations of broad beans
Stringless blue lake 228	Scattered chlorotic areas malformed leaves	Positive
Harvester	Green islands on chlorotic malformed leaves	Positive
Tender crop	No reaction	Negative
Bobis mercato		
Mangiantutte	No reaction	Negative
Wade	General mosaic reaction	Positive
Corneli	Mild mosaic	Positive
Seminole	Small to moderately large number of chlorotic lesions	Positive
Romes Blanch de Guillet	No noticeable reaction	Positive
Tender green	Large number of chlorotic lesions, progressing later to mosaic form of symptons	Positive
Extender	No reaction	Negative
Victory	No reaction	Negative
Jacson Wonder	No reaction	Negative

DISCUSSION

The studies of host range, symptomology, insect transmission, and physical properties indicated that the virus isolate dealt with in this study is a strain of bean yellow mosaic virus (BYMV). Systemic spread of the virus in many of the bean varieties tested and the complete absence of necrotic reaction on these varieties, the appearance of small chlorotic local lesions on leaves of *C. amaranticolor* Coste and Reyn, the types of reactions manifested by peas, broadbeans and lupins, and the failure of other tested plant species to be infected all constitute a strong indication of the presence of BYMV.

The insect transmission properties, the dilution end point, the thermal inactivation end point, and the aging in vitro of the present virus isolate, are all in agreement with those reported for BYMV (2). The electron microscopy study of purified preparations from infected broad bean leaves, that will be described in a subsequent report, further confirms the identity of the isolate as being a strain of BYMV. However, the Libyan isolate, based on the present study, appears to be distinct from the BYMV strains previously reported. It differs from the severe yellow mosaic strain described by Thomas et al (6) in failing to produce vein necrosis in inoculated bean leaves and chlorotic lesions on *N. tabaccum* and *N. rustica*. These species were found to be resistant to the Libyan strain. Furthermore, the Libyan strain, in contrast to the Zaumeyer's failed to produce local necrotic lesions on bean pods. El-Attar et al (1) reported from Egypt a BYMV strain that produced chlorotic spots on inoculated leaves of *Vigna sinensis*, followed by a mild systemic mottle. In the present study *V. sinensis* was found to be resistant to the present isolate. This isolate also differs from the type strain in not being infectious to *Soja max* Piper.

SUMMARY

Broad bean plants infected with bean yellow mosaic virus (BYMV) were used for the electron microscopy of the casual agent. The dip method revealed the presence of a few elongated-rod-shaped particles. Preparations from infected tissues using organic solvent mixture of ethylene glycol monethyl ether and ethylene glycol monobutyl ether and using borate buffer resulted in producing fairly clean preparations containing flexuous elongated virus-like particles with a length of 729 to 750 mu. This purified preparation was found to be infectious. However, the purified preparations from infected tissues following the phosphate buffer and differential centrifugation method yielded no virus-like particles when examined with the electron microscope.

LITERATURE CITED

1. El-Attar, S., F. Nour-Eldin, and S. A. Ghabrial, 1971. A strain of bean yellow mosaic virus naturally occuring on broad bean in the United Arab Republic. Agr. Res. Rev., Egypt, 49(3) : 285–290.
2. Evans, I. R., and F. W. Zettler, 1970. Aphid and mechanical transmission properties of bean yellow mosaic virus isolates. Phytopathology 60 : 1170–1174.
3. Nour, M. A., and J. Nour, 1962. Broad bean mosaic caused by pea mosaic virus in the Sudan. Phytopathology 52(5):398–403.
4. Ross, A. F., 1964. Identification of plant viruses in Plant Virology. Corbett, M. K. and H. D. Sisler, (eds). University of Florida Press, Gainesville.
5. Shagrun, M. A., 1972. Purification and electron microscopy study of virus-like particles from *Vicia fabae*. (In preparation).
6. Thomas, H. R., and W. J. Zaumeyer, 1953. A strain of yellow bean mosaic virus producing local lesions on tobacco. Phytopathology 43:11–15.
7. Von Der Pahlen, A. 1963. Bean yellow mosaic (Phaseobus virus 2 (Pierce) Smith) in crops of broad bean, pea, and french bean in the environs of Buenos Aires. Rev. Invest. agr., B. Aires, 16(1) : 87–92.
8. Zaumeyer, W. J., and H. H. Fisher, 1953. A new necrotic-lesion producing strain of yellow bean mosaic. Phytopathology 43 : 45–49.