Bean Yellow Mosaic Virus on Broad Bean Plants in Libya

II. Purification and electron microscopy

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INTRODUCTION

Virus-like symptoms on broad bean plants have been noticed to be prevalent in several fields around Tripoli (6). The suspected virus was found to be mechanically transmitted under greenhouse conditions to several host plants among which were broad beans, peas, lupins, and beans. Based on host range, symptomology and physical properties, the virus isolate in question was identified as being a strain of bean yellow mosaic virus (6).

In this study, the purification and the characterization of the virus isolate with the electron microscope will be reported. Bean yellow mosaic virus (BYMV) is believed to be one of the potato virus Y group (1). This group of viruses is delineated morphologically from others by having flexuous rod-shaped particles with a normal length ranging from 730 to 790 mu (1). Furthermore, the potato virus Y group is known to be difficult to experiment with in vitro conditions due probably to low concentration in host cells, and the aggregation of the virus particles during extraction and purification (3).

MATERIALS AND METHODS

A stock culture of the virus was maintained in young broad bean plants which were also used as source of the virus for purification experiments. Plants growing in six-inch pots were inoculated in the 4–6 leaf stage. Inoculations were carried out by rubbing the upper surface of carborundum-dusted healthy leaves. The inoculum was prepared by grinding the infected plant tissues together with a few drops of phosphate buffer at pH 7.2. The inoculated surfaces were then immediately rinsed with tap water, and incubated under greenhouse conditions.

For the extraction and purification work, infected plant materials were harvested 12 days after inoculations. Seventeen grams of infected leaf tissues were homogenized in a Waring blender in a 0.1 M phosphate buffer pH7, at 0°C to which 1 % 2 mercapto ethanol was added. The homogenized extract was strained through cheese cloth. To one volume of this strained homogenate 0.8 volume of organic solvent mixture of one part of ethylene glycol monoethyl ether and two parts of ethylene glycol monobutyl ether were added. The resulting mixture was clarified by low speed centrifugation in a Sorvall refrigerated centrifuge for about 10 minutes at 1,000 rpm. The clarified material was then dissolved in a 0.02 M borate buffer solution at pH 8.2, and reclarified once more at 10,000 rpm for 10 minutes. After being resuspended in the same borate buffer, the fluid was centrifugated for two hours at 39,000 rpm in Spinco preparative refrigerated ultracentrifuge. The pellet

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was dissolved in the borate buffer and the sample was ready for viewing with the electron microscope and for infectivity assays.

Another extraction and purification procedure was carried out using 0.1 M phosphate buffer at pH 7 to which 2 mercapto ethanol was added and without the use of organic solvents, following the same operations of differential centrifugations. The pellets obtained after the final high speed centrifugation in this method were dissolved in the same phosphate buffer for electron microscopy and infectivity tests. Control tests were carried out simultaneously with both methods using non-inoculated broad bean tissues.

The dip method for extraction of virus particles from freshly-cut edges of an infected leaf was also attempted for quick and preliminary search of virus-like particles. Infectivity tests of the final purified preparations were done by inoculating seedlings of *Chenopodium amaranticolor* to varify the presence of infectious particles of bean yellow mosaic virus in these preparations.

ELECTRON MICROSCOPY

Purified preparations from infected plant tissues as well as from healthy tissues were sprayed on collodion-coated grids without shadow-casting with heavy metals. The sprayed grids were examined with an RCA electron microscope model EMV-3.

RESULTS AND DISCUSSION

Using the dip method for the preliminary search for virus-like particles in infected leaf tissues, a few elongated rod-shaped particles were noticed with the electron microscope (Fig. 1). The purified preparations from infected tissues following the phosphate buffer and the differential centrifugation method yielded no virus-like particles when examined with the electron microscope. However, preparations from infected tissues following the organic solvent method and the use of the borate buffer were successful in producing fairly clean preparations containing flexuous elongated virus-like particles (Figures 2, 3). Preparations from healthy plant material identically treated showed no such particles. Seedlings of *Chenopodium amaranticolor* Coste & Reyn, inoculated with purified preparations containing the elongated virus-like particles reacted positively by developing chlorotic local lesions on leaf surfaces 6 days following inoculation.

The particles shown in the electron micrographs (Fig. 2, 3) measured 12–13 mu in diameter. The longest particles ranged from 1,600 to 1,800 mu in length. However, the common length of particles in all the electron micrographs examined, was between 720 and 750 mu. Though occasional presence of shorter particles was not uncommon. This variability in particle length could be due to the fragmentation of the complete virus particles in the course of the preparative treatments to which the extract was subjected. There is also the possibility that the shorter particles are sub-units of the complete virus that were normally present in the infected tissues at the time of extraction. Separating these particles by methods such as density gradient centrifugation, then assaying the serological and infectious properties of the different bands in the density gradient tubes, would be very valuable in providing information about the nature of the shorter particles and their relation to the normal length of the complete virus particles.

SUMMARY

A wide spread mosaic virus disease affecting broad bean plants around Tripoli was identified as being caused by bean mosaic virus infection. This was based upon the

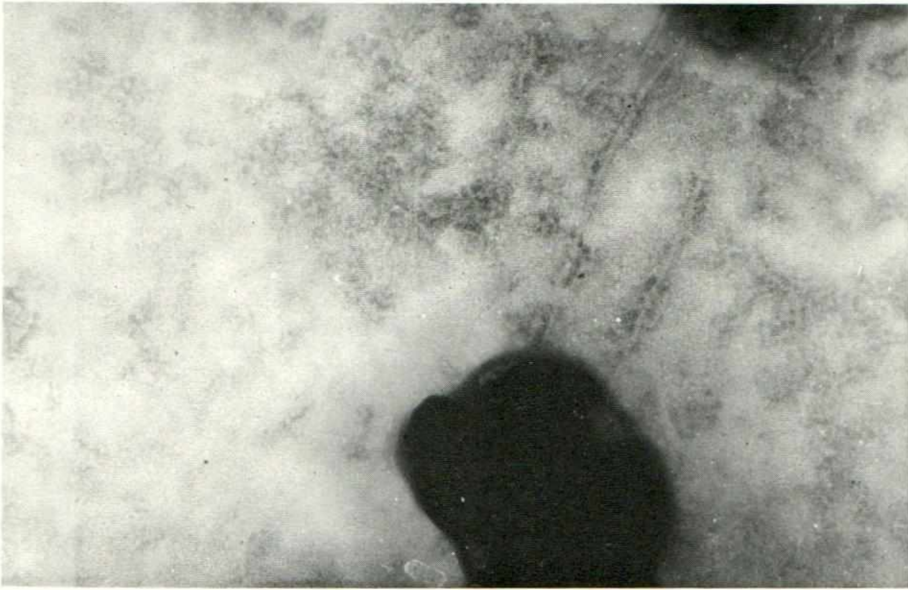

Fig. 1. An electron micrograph showing a rodshaped virus-like particle extracted by the dip method. Magnification is about 12,000 × .

results obtained from the study of the host range, physical properties and transmission of the virus. Broad bean, bean, lupins, and *C. Amaranticolor* Coste and Reyn were found to be hosts of this virus. The thermal inactivation point of the virus isolate was found to be between 60–62°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 for 24 hours but not for 16 hours. It was readily transmitted mechanically and by *Aphis gossypii* Glover and *Macrosiphum pisi* (Harris).

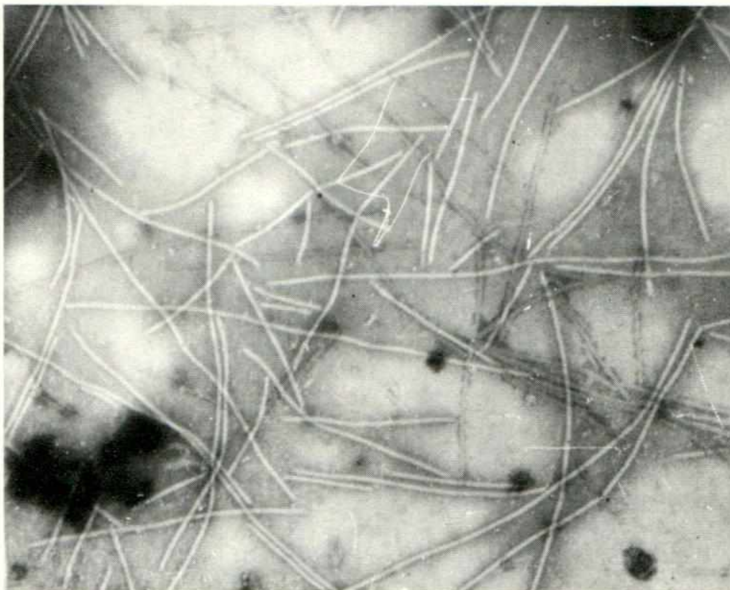

Fig. 2. Electron micrographs of the investigated virus showing virus particles of different lengths. Magnification is approximately 12,000 × .

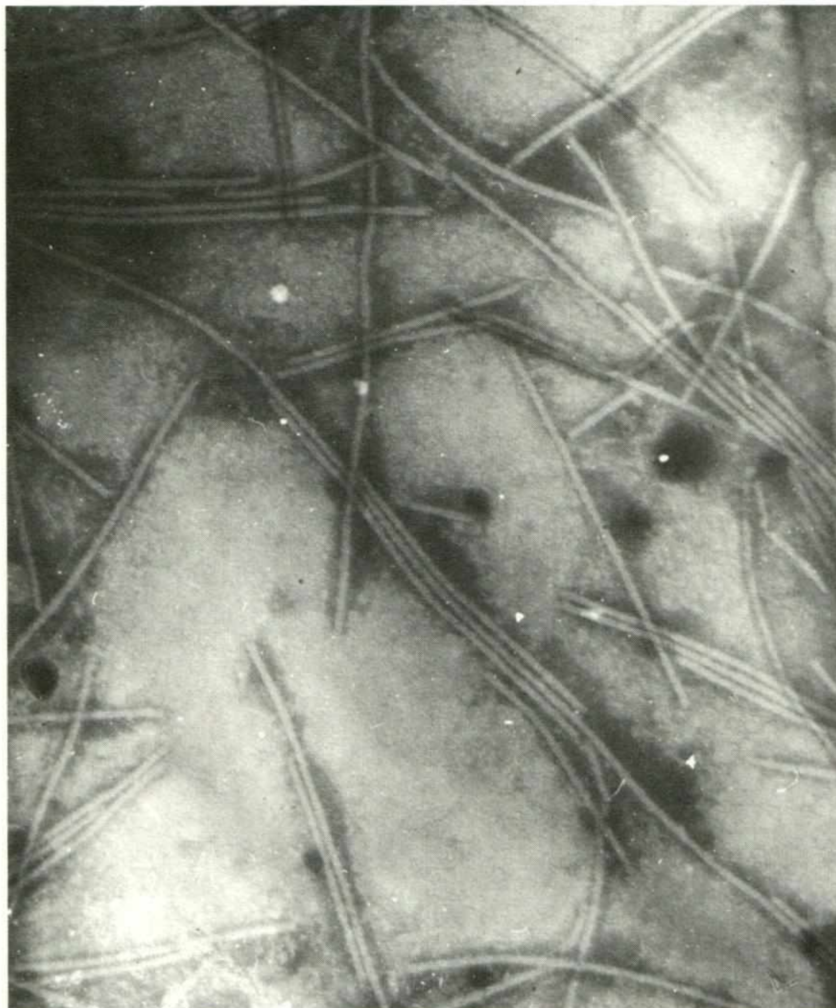

Fig. 3. Cleaner preparation of the bean yellow mosaic virus. The virus particle diameter ranges from 12 to 13 mu. Magnification is 126,000 × .

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