Isolation of Bean Yellow Mosaic Virus From Broad Bean Plants in Libya

H. YOUNES[1], M. SHAGRUN[1] AND J. KHALIL[1]

ABSTRACT

Mosaic symptoms on broad bean plants have been observed in several fields around Tripoli. The virus is transmitted by mechanical inoculation and by the aphids *Myzus persicae* and *Aphis crassivora*. It infects only plants in the Leguminosae and Chenopodiaceae. The TIP of the virus is between 62-64°C, the DEP is between $10^{-4} - 10^{-5}$ and the LIV is 8 days. The virus is antigenic and the antiserum titer is 1: 128. It has a serological relationship with bean yellow mosaic virus (BYMV) and pea seed-borne mosaic virus (PSBMV). On the basis of these results and those reported, it is concluded that the virus isolate in this study is most likely BYMV.

INTRODUCTION

Broad bean (*Vicia faba* L.) is one of the common field crops used as food for human and animal feed in Libya. Broad bean plants are infected by more than 30 viruses (17).

Shagrun (1973) reported that bean yellow mosaic virus caused mosaic symptoms on broad bean plants in Libya (15). A field survey of fava bean for viruses in six Arab countries showed the presence of nine viruses (8).

Mosaic symptoms on broad bean plants have been observed in several fields around Tripoli. The objective of this study is to identify and characterize the suspected virus or viruses that induce mosaic symptoms in naturally infected broad bean plants.

MATERIALS AND METHODS

Mechanical transmission:

Inoculations were made by rubbing carborundum dusted leaves of test plants with the sap from fresh and young infected broad bean leaves using the forefinger.

Host range:

Forty six plant species representing nine families were used to investigate host reactions to the virus.

[1] *Plant Protection Department, Faculty of Agriculture, University of Al-Fateh, Tripoli, Libya.*

Properties in the crude sap:

Standard procedures as described by Ross (13) were followed. Fresh sap was extracted from infected *V. faba* leaves 10-15 days after inoculation by homogenization of tissue in distilled water using a mortar and pestel. *V. faba* and *Chenopodium amaranticolor* plants as systemic and local lesion hosts, respectively, were inoculated for bioassay. Thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) experiments were performed.

Insect transmission:

Aphids of *Aphis craccivora* and *Myzus persicae* (Sulzer) were removed from rearing plants with a camel hair brush and placed in a petri-dish for 1-2 hrs starvation period, then were allowed to feed for varying periods on infected *V. faba* leaves. After feeding, 10 aphids were transferred to each of eight healthy *V. faba* seedlings. Ten fasting aphids were transferred to healthy seedlings without feeding on infected leaves served as control. Aphids were allowed to feed for 24 hrs on test plants after which all plants were sprayed with the aphicide *Pirimicarb* 50 WP.

Purification:

Systemically infected leaves of *V. faba* or *Pisum sativum* showing clear mosaic symptoms were harvested two weeks after inoculation. Virus was purified using the methods described by Granett and Provvidenti (5) and Huttinga (7).

Serology:

a) Antiserum preparation:

The virus suspension was mixed with a 0.85% saline solution (NaCl). A rabbit was given two successive intravenous injections at four days interval, followed by weekly two intramuscular injections composed of an emulsion of one ml of virus suspension and one ml of Freund's incomplete adjuvant. Bleeding was begun 2-4 weeks after the final injection.

b) Serological tests:

The microprecipitin and ring interface precipitin tests were employed as described by Ball (1). The two tests were made between the virus isolate and its antiserum and between the virus isolate and antisera of bean yellow mosaic virus (BYMV) and pea seed-borne mosaic virus (PSBMV).

RESULTS

Symptomatology and host range:

Of the nine families tested, species of only Chenopodiaceae and Leguminosae were susceptible to the virus infection as shown in Table 1.

Properties in the crude sap:

The virus was inactivated between 62°C and 64°C, withstood a dilution of 10^{-4}, and was still infectious after 8 days.

Table 1 — Host range studies and symptoms induced on test plants after mechanical inoculation with the virus isolate obtained from mosaic-affected *V. faba* plants.

Test Plant	Susceptibility and Symptoms
Chenopodium amaranticolor	Chlorotic local lesions
C. quinoa	Chlorotic local lesions
Cicer arietinum	Necrosis of the tips of the plants and severe yellowing of the foliage, then complete necrosis of the whole plant.
Dolichus lablab	Not susceptible
Glycine max.	Not susceptible
Lathyrus odoratus	Mild mosaic
Lens esculenta	Mottling and severe curling of the new leaves and necrosis of the old growth.
Lupinus termis	Yellowing of the new leaf tips, necrosis of the leaves, distortion and reduction of the leaflets.
Medicago sativa	Not susceptible
Phaseolus vulgaris cvs. 'Blue Lake 274 Bush', 'Bountiful Stringless', 'Contender', 'Extender', 'Harvester', 'Romano', 'Scarlet Runner', 'Tender Green'	Not susceptible
'Kentucky Wonder'	Mild mosaic
Pisum sativum cvs. 'Alaska', 'Laxton Progress No. 9', 'Local', 'Dark Green Perfection'	Mild mosaic
'Deep Freeze', 'Oregon', 'Melting Sugar Edible Pod'	Not susceptible
Trifolium alexandrium	Mild mosaic
Trigonella foenum-graecum	Mosaic
Vicia faba	Vein clearing, mottling and mosaic
V. sativa	Mild mosaic
Vigna sinensis	Not susceptible

Insect transmission:

A. craccivora was able to transmit the virus with an acquisition feeding period of up to 25 min. *M. persicae* had the ability to transmit the virus up to 50 min. (maximum time tested) of feeding on diseased leaves.

Purification:

The ultraviolet spectrum of the purified virus showed maximum absorption (A) at 260 nm and the A260/280 was 1.54. The purified virus was infectious when *V. faba* plants were inoculated.

Serology:

The virus isolate proved to be antigenic and produced antiserum with a titer of

1: 128 with the microprecipitin test. There was a serological reaction between the broad bean virus isolate and antisera of BYMV and PSBMV when the microprecipitin test was used but not with the ring interface test.

DISCUSSION

The virus isolate produced local lesions on *C. amaranticolor* and this was similar to the symptoms reported by Nour and Nour (12) for PMV on *C. amaranticolor*. Symptoms appearing on *V. faba, L. odoratus* and *P. sativum* were similar to those described for PMV by other workers (3, 10, 11, 12). No symptoms were produced on *M. sativa, V. sinensis, G. max* and *D. lablab*. These results are in agreement with those of Murphy and Pierce (10), Wiess (19), Chaudhuri (2) and Nour-Eldin *et. al.* (11) who reported that these plants were not susceptible to PMV.

Several investigators differentiated between BYMV and PMV by the failure of PMV to infect *P. vulgaris* (4, 6, 10, 14, 16, 18, 19). In this study, among the nine cultivars of *P. vulgaris* tested, only 'Kentucky Wonder' showed mild mosaic. The virus isolate infected *P. sativum* cvs. 'Alaska' and 'Laxton Progress No. 9'. This was reported by Doolittle and Jones (3) and Murphy and Pierce (10) for PMV.

The virus isolate was transmitted by the aphids *A. craccivora* and *M. persicae* in a non-persistent manner. The rate of transmission with *M. persicae* was not reduced up to 50 min. acquisition feeding period (maximum time tested). Chaudhuri (2) studied the transmission of PMV by aphids and found that *M. persicae* caused most infection when allowed to feed on diseased plants for 2 min. He also found that the rate of transmission of BYMV was greatly reduced after 30 minutes.

The virus isolate was antigenic and had a serological relation with BYMV and PSBMV.

Comparing the results of this study with those reported for BYMV and PMV, we conclude that our virus isolate is closely similar to these two viruses. According to the latest classification and nomenclature of viruses, BYMV and PMV are considered synonyms (9). The virus causing broad bean mosaic disease is, therefore, most likely BYMV.

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LITERATURE CITED

1. Ball, E.M. 1974. Serological tests for the identification of plant viruses. *Amer. Phytopathol. Soc. Inc.*, St. Paul, Minn., USA, 31pp.
2. Chaudhuri, R.P. 1950. Studies on two aphid-transmitted viruses of leguminous crops. *Ann. Appl. Biol.* 37: 342-354.
3. Doolittle, S.P. and F.R. Jones. 1925. The mosaic disease in the garden pea and other legumes. *Phytopathology* 15: 763-773.
4. Goodchild, D.J. 1956. Relationships of legume viruses in Australia. I. Strains of bean yellow mosaic virus and pea mosaic virus. Aust. *J. Biol. Sci.* 9: 213-230.

5. Granett, A.L. and R. Provvidenti. 1975. Partial purification and serological relationship of three strains of bean yellow mosaic virus. *Ann. Appl. Biol.* 81: 413-415.
6. Hagedorn, D.J. and J.C. Walker. 1950. The relation of bean virus 2 to pea mosaic in Wisconsin. *Phytopathology* 40: 684-698.
7. Huttinga, H. 1973. Properties of the potyvirus group. I. A simple method to purify bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus and potato virus Y^N. Neth. *J. Plant Pathol.* 79: 125-129.
8. Makkouk, K.M., L. Bos, O.I. Azzam, S. Koumari and A. Rizkallah. 1988. Survey of viruses affecting faba bean in six Arab Countries. *Arab J. Plant Protec.* 6: 53-61.
9. Matthews, R.E.F. 1982. *Classification and nomenclature of viruses. Intervirology* 17 (1-3): 1-200.
10. Murphy, D.M. and W.H. Pierce. 1937. *Common mosaic of the garden pea, Pisum sativum. Phytopathology* 27: 710-721.
11. Nour-Eldin, F., M.T. El-Banna, S. El-Attar and S.A. Eid. 1966. Pea and broad bean mosaic diseases caused by pea mosaic virus. *UAR J. Res.* 46: 132-137 (in Arabic).
12. Nour, M.A. and J.J. Nour. 1962. Broad bean mosaic caused by pea mosaic virus in Sudan. *Phytopathology* 52: 398-403.
13. Ross, A.F. 1964. *Identification of plant viruses: Properties in crude juice.* Pages 77-80 in: *Plant Virology* (Corbett, K.M. and H.D. Sisler, eds.). University of Florida Press, Gainesville, 527pp.
14. Schroeder, W.T. and R. Provvidenti 1966. Further evidence that common pea mosaic (PV2) is a strain of bean yellow mosaic virus (BV 2). *Plant Dis. Reptr.* 50: 337-340.
15. Shagrun, M. 1973. Bean yellow mosaic virus on broad bean in Libya. I. Identification of the causal agent. *Libyan J. Agr.* 2: 33-38.
16. Stubbs, M.W. 1937. Certain viruses of the garden pea. *Phytopathology* 27: 242-266.
17. Thornberry, H.H. 1966. Index of plant virus diseases. *Agr. Handbook No. 307, USA Dept. of Agri.* 446P.
18. Varma, A. and A.J. Gibbs. 1967. Preliminary studies on sap-transmissible viruses of red clover, *Trifolium pratense* L. in England and Wales. *Ann. Appl. Biol.* 59: 23-30.
19. Weiss, F. 1939. A key to the typical viruses of leguminous crops. Plant Dis. Reptr. 23: 352-361.

عزل فيروس موزيك الفاصولياء الأصفر من نباتات الفول في ليبيا

حسنى يونس، محمد شقرون، وجبر خليل

المستخلص

لوحظت أعراض الموزيك على نباتات الفول بعدة مزارع بمنطقة طرابلس. وتبين أن مسبب المرض ينتقل بسهولة بالعدوى الميكانيكية وبواسطة حشرتي مَنّ الخوخ الأخضر *Myzus persicae* ومَنّ الكرنب *Aphis craccivora* بالطريقة غير الباقية. ويصيب الفيروس نباتات من الفصيلتين البقولية والرمرامية فقط. تقع درجة الحرارة المميتة للفيروس بين 62-64° م، ودرجة التخفيف النهائية بين $10^{-4} - 10^{-5}$ ومدة استمرارية نشاطه بالعصارة الخام هي 8 أيام. ثبت أن الفيروس مناعي، ودرجة تخفيف المصل المضاد هي 1 :128، كما وجدت علاقة سيرولوجية بينه وبين فيروس موزيك الفاصولياء الأصفر وفيروس موزيك البسلة المنقول بواسطة البذور. وعلى أساس هذه النتائج وما هو مسجل عن الفيروسات التي تصيب الفول، فإن الفيروس المسبب لموزيك نباتات الفول هو في الغالب فيروس موزيك الفاصولياء الأصفر.