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# Broad Bean Mosaic Caused by Pea Mosaic Virus

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#### ABSTRACT

Various types of mosaic diseases affecting broad bean are wide spread in Tripoli. Results on symptomatology, host range, and physical properties showed that viral isolates under study were probably strains of pea mosaic virus. The virus was readily transmitted mechanically to *Vicia faba*, *Pisum sativum*, and *Lathyrus odoratus*. Thermal inactivation occured between 52–60°C. The longevity in vitro at room temperature  $(20-24^{\circ}C)$  was about 120 hr. The dilution end point was between  $10^{-3}$  and  $10^{-5}$ . This is the first report to indicate that broad bean mosaic in The Socialist People's Libyan Arab Jamahiriya (S.P.L.A.J.) could be caused by pea mosaic virus.

### INTRODUCTION

Broad bean, *Vicia faba* L., is one of the most common field crops in the Libyan Jamahiriya for animal and human feed. It is also used in soil reclamation.

Various types of mosaic symptoms on broad bean plants have been observed in several fields. In general these symptoms consisted primarily of variations in degree of stunting and foliage distortion. Newly formed leaves on affected plants usually showed different kinds of mottling and mosaic patterns. Several reports of viral diseases with similar symptoms have been made in different countries. Allam and El-Kady (1), Krayev (5, 6), Nour-Eldin *et al.* (8), Nour and Nour (9), and Stubbs (13) reported that broad bean mosaic diseases could be caused by different strains of pea mosaic virus. Bean yellow mosaic virus was also reported (4, 10, 11) to cause a mosaic disease on broad bean plants in Egypt and Libyan Jamahiriya.

The investigation reported herein was conducted to identify the virus or viruses that induce mosaic symptoms in naturally infected broad bean plants.

## MATERIALS AND METHODS

Source of virus isolates. Naturally infected broad bean plants, showing different degrees of mosaic symptoms, were collected from the College of Agriculture Farm in Tripoli. These plants served as the source of virus isolates for preliminary transmissions. Thereafter, mechanically inoculated broad bean seedlings were used as the source of the virus throughout the present study. All extractions were made 10–15 days

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after inoculation. Test plants were cultivated in autoclaved soil and kept in an insectproof, air-conditioned greenhouse.

Mechanical transmission. Inoculum was prepared by triturating fresh and young infected leaves in distilled water with a mortar and pestle, and the juice was squeezed from the pulp through two layers of cheesecloth. Inoculations were made by rubbing Carborundum-dusted (300-mesh) leaves of test plants with the expressed sap using the forefinger or inoculum-saturated cheesecloth pads.

Host range. Nineteen species of test plants were used to check the host range of obtained virus isolates. At least five seedlings of each species were sap-inoculated. The species tested included: Vicia faba L., Glycine max (L.) Merr., Pisum sativum L., Lathyrus odoratus L., Phaseolus vulgaris L., Vigna sinensis (Torner) Savi, Lupinus termis Forsk, Lycopersicon esculentum Mill, Nicotiana tabacum L., N. rustica L., N. glutinosa L., Vinca rosea L., Datura stramonium L., Gomphrena globosa L., Physallis floridana Rybd., Petunia hybrida Vilm, Spinacea oleracea L., Capsicum frutescens L., and Cucurbita pepo L. Similar number of test plants rubbed with distilled water served as controls. Reproduction of mosaic symptoms after back-inoculations to broad bean seedlings was the criterion used to determine if a plant was infected. Inoculum for back-inoculations was obtained from individual plants. Back-inoculations were made 30 days after the test plants had been inoculated.

*Physical properties. Thermal inactivation.* Fresh sap extracted from infected broad bean leaves was used for the thermal inactivation experiments. Sap was drawn into thin-walled glass tubes. Tubes of extract were heated in a constant-temperature waterbath for 10 min at the indicated temperature, then quickly immersed in ice water. Broad bean seedlings were inoculated for bioassays.

Longevity in vitro. Fresh sap extracted from infected broad bean leaves was immediately inoculated on the leaves of broad bean seedlings. The remaining extract was

Species inoculated	Virus isolate		
	Α	В	С
Vicia faba L.	+	+	+
Glycine max (L.) Merr.	-		-
Pisum sativum L.	+	+	+
Lathyrus odoratus L.	+	+	+
Phaseolus vulgaris L.	-	-	-
Vigna sinensis (Torner) Savi	_	-	-
Lupinus termis Forsk	-	-	-
Lycopersicon esculentum Mill	-	-	-
Nicotiana tabacum L.	-	-	-
N. rustica L.	-	-	-
N. glutinosa L.	-		-
Vinca rosea L.	-	-	-
Datura stramonium L.	_	-	-
Gomphrena globosa L.	-	-	-
Physallis floridana Rybd.	-	-	-
Petunia hybrida Vilm		-	-
Spinacea oleracea L.	-	-	-
Capsicum frutescens L.	_	-	-
Cucurbita pepo L.	-	-	-

Table 1 Results of host range studies with virus isolates obtained from mosaic-affected broad bean plants.

(+ = negative, - = negative)

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stored in stoppered flask at room temperature (20–24°C). Aliquots were used to inoculate broad bean plants at 5, 24, 48, and 120 hr after extraction.

Dilution tolerance. Sap extracts from broad bean leaves were diluted 0,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  with distilled water, and rubbed on the leaves of broad bean seedlings.

### **RESULTS AND DISCUSSION**

Of the 19 species tested, only Vicia faba, Pisum sativum, and Lathyrus odoratus, were susceptible to virus infection (Table 1). The first symptom to appear, on the newly formed foliage, was a very slight vein clearing about 7–10 days after inoculation of seedling plants. No local necrotic lesions were observed. Mottling of the new foliage





Fig. 1. A) Leaflets from broad bean plants affected with mosaic disease showing different degrees of mottling. B) Healthy leaflets.



Fig. 2. Symptoms produced by virus infection in pea. A) Leaflets with mild mosaic. B) Healthy leaflets.

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Treatment	Virus isolate		
	А	В	С
I. Thermal inactivation (°C):			
50	+	+	+
52	+	+	+
54	+	+	+
56	+	+	+
58	+	1.45	-
60	+	-	
II. Aging in vitro (hr):			
0	+	+	+
5	+	+	+
24	+	+	+
48	+	+	+
120	-	+	+
III. Dilution tolerance:			
0	+	+	+
10 <sup>-1</sup>	+	+	+
10 <sup>-2</sup>	+	+	+
10 <sup>-3</sup>	+	+	+
10-4	+		-
10 <sup>-5</sup>	+	-	_

Table 2 Physical properties of virus isolates obtained from mosaicaffected broad bean plants.

was the only disease symptom produced. Systemic infection with mottling or mosaic was observed on all of the hosts infected. Growth of plants infected with the virus was not retarded, and no distortion of the foliage occured. Foliage produced by further growth borne the marble type of mottle pattern, which consisted of rather large chlorotic areas bounded by light green or normal green tissue. In *Vicia faba*, symptoms were quite severe with a very pronounced mottling (Fig. 1); however, in *Pisum sativum* and *Lathyrus odoratus* symptoms were light with a mild mosaic on leaflets (Fig. 2).

Thermal inactivation of the viral isolates occurred between 52–60°C, and infectivity was lost in sap heated over 60°C. The longevity in vitro at room temperature (20–24°C) was about 120 hr. The dilution end point was between  $10^{-4}$  and  $10^{-5}$ ; however, most infectivity was lost in dilutions greater than  $10^{-3}$  (Table 2).

The obtained results showed that the virus isolates failed to infect *Phaseolus vulgaris*. This information ruled out the possible presence of bean yellow mosaic virus, since *P. vulgaris* is susceptible to this virus (4, 10, 11, 12). Studies on physical properties appeared to be in close agreement with that reported for pea mosaic virus (PMV), besides that PMV is not transmissible to *P. vulgaris* (2, 3, 7, 8, 9, 12, 13).

The results on symptomatology, host range, and physical properties indicated that viral isolates under study are probably strains of PMV. Therefore, it can be concluded that mosaic diseases affecting broad bean in S.P.L.A.J. could be caused by pea mosaic virus.

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مرض تبرقش أوراق الفول المتسبب عن الإصابة بفيرس تبرقش أوراق البازلاء عبد الشافي فضل الله حسني يونس المستخلص

لوحظ انتشار أنواع مختلفة من الأمراض الفيروسية التي تسبب تبرقش أوراق الفول . وبدراسة الأعراض المرضية ، والعوائل النباتية ، وكذا الخواص الطبيعية للمسبب وجد أنها تتفق مع خواص فيروس تبرقش أوراق البازلاء . وقد ثبت أن هذا الفيروس ينتقل بواسطة التلقيح بالعصير والعوائل التي يصيبها هي الفول والبازلاء وبازلاء الزهور ، كما لوحظ أن الفيروس يحتفظ بنشاطه عند تعرض العصير لدرجات حرارة بين ٥٢ – ٢٠٠ م ، ولكن هذا النشاط يفقد عند استعمال درجات حرارة أعلى من ٢٠٠ م ، وأن العصير المستخرج من نباتات مصابة يمكنه أن يحتفظ بقدرته لمدة ١٢٠ ساعة عند درجة حرارة ٢٠ – ٢٤٠ م ، كما أن القدرة على إحداث الإصابة تفقد باستعال تخفيفات أعلى من ١ : ١٠٠٠ .