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دراسات فيروس الموزايك الأصفر للفاصوليا على نبات الفول في سرت.

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الملخص

لوحظ إنتشار أعراض الموزايك بشكل واسع علي أوراق نبات الفول *Vicia faba L.* بحقل في منطقة سرت. جمعت عشر عينات من الحقل المصاب واختبرت باستخدام اختبار اليزا غير المباشر (Indirect ELISA)، وذلك باستخدام ثلاثة أمصال مضادة لفيروسات تعرف بإصابتها لهذا المحصول، وهي فيروس موزايك الفول (BBMV) وفيروس موزايك الخيار (CMV) و فيروس موزايك الأصفر للفاصوليا (BYMV) تم الحصول عليها من المركز العالمي للبحوث الزراعية في الأراضي الجافة (ICARDA)، لوحظ التفاعل الموجب مع مصل فيروس الموزايك الأصفر للفاصوليا، حفظت عزلات الفيروس في المعمل والعينة رقم 6 استخدمت كمصدر للفيروس لأجل الدراسات أخرى والتي شملت تأكيد نتائج اختبار إليزا، النقل الميكانيكى، الخواص الفيزيائية للفيروس في العصارة الخام والمدى العوائلى والأعراض. بناء علي اختبار إليزا غير المباشر تم تأكيد تعريف عزلة الفيروس أنها سلالة من BYMV. وتبين نجاح عزلة الفيروس في الإنتقال الميكانيكي. وكانت درجة الحرارة المميتة للفيروس مابين 55 - 60°م وقد فقد الفيروس قدرته علي الإصابة عند درجة التخفيف 10^{-3} وعند بقاءه بالعصارة الخام في المعمل لمدة 72 ساعة عند درجة حرارة 22-25°م. دراسة المدى العوائلي والأعراض تضمنت أكثر من 20 نوع نباتي ممثلة لثمانية عائلات نباتية Chenopodiaceae; Cruciferae; Cucurbitaceae ; Fabaceae; Solanaceae; Umbelliferae; Amarantaceae; Compositae. أظهرت أن المدى العوائلي للفيروس (BYMV) لم يكن محصورا بإصابة نوع نباتي معين فقط بل علي العكس تماما، وبالنسبة للأعراض فتبين أنها اختلفت علي حسب النوع النباتي.

Abelmoschus esculentus reacted with chlorotic local lesions with systemic mosaic with BYMV inoculation. Contradictory with other studies that reported no symptoms were appeared on *Abelmoschus esculentus* with BYMV inoculation (7&10) .

The following hosts, *Gomphrena globosa*; *Lycopersicon esculentum*; *Nicotiana glutinosa*, were not infected by the BYMV. These results agreed completely with those previously reported (2,5,6).

Other hosts, *Cucurbita pepo*; *Cucumis melo*; *Lens esculentam*, were also not infected by the BYMV. These results agreed completely with those were previously reported (6,7,10) . In contrast, *Lens esculentam* reacted with mild mosaic when inoculated by BYMV (15) and mottling symptoms with severe curling of the new leaves of *Lens esculenta* when inoculated with BYMV isolate (14).

The BYMV isolate failed to infect *Datura stramonium.* Similar results were recorded by (2&6)

Raphanus sativas; Brassica oleraceae; Eruca sativa; Brassica compestris; Lactuca sativa, Were not infected by the BYMV. These results are similar to those obtained by (10).

According to the present investigation, BYMV has a wide host range and not limited to fabaceae. These results differ from other report that indicated the host range was limited to leguminous plants (2).

The variation of symptoms occurred on same hosts that inoculated with BYMV may be due to different strains of the virus (BYMV).

while contradictory results were obtained by Several workers, who observed local lesions on leaves of *Chenopodium amaranticolor* (6,7,10,12,14,15).

Capsicum annum reacted with mild mosaic, upper leaves malformation, Chlorotic local lesions and chlorosis on old leaves with BYMV inoculation. These results are similar to those described by (7&10), while, not agree with other that found *Capsicum annum* was not infectable by BYMV (5).

The mild systemic mosaic symptoms obtained from the inoculation of *Solanium melongona* with BYMV, contradicts with those obtained in previous study (10), when this virus failed to produce symptoms on the same plant.

Chlorotic local lesions with ringed spots induced on *Datura metel.* as a result of inoculation with the BYMV. In contrast, with other study found that *Datura metel.* was not infectable by BYMV (7) .

Table (). Reaction of hosts to inoculation with BYMV.

Host name	Symptoms
Vicia faba	Sm
Lupinus termis	Sm, LLy, ULmal, ULy
Cicer arientium	Sym, Y, PD
Phaseolus vulgaris Tender green.	Sm, CLL
Pisum sativam	MSm
Vigna sinensis	CLs, ULmal, MSm, CLL
Arachis hypogea	ULmal, MSm, ULy
Glycine max	MSm, LLy
Capsicum annum	CLL, ULmal
Solanium melongona	MSm
Datura metel	CLL, RS
Abelmoshus esculentus	CLL, Sm

CLL : Chlorotic local lesions. CLs : Chlorotic spots. ULy : Upper leaves yellowing.
LLy : Lower leaves yellowing. MSm : Mild Systemic mosaic. PD : Plant Death.
RS : Ringed Spots. Sm : Systemic mosaic. Sym : Systemic yellow mosaic.
ULmal : Upper leaves malformation. Y : Yellowing.

Physical properties:

Physical properties of the tested virus are important parameter to indicate it's stability outside the host (in vitro), these properties might differ according to the virus strain (Ross, 1964 cited after 11). In the present study, the thermal inactivation point of the virus was between 55 - 60 ^{0}C. . The Dilution end point was 10^{-2} and The virus remained infectious when stored in crude sap for 48 hours but not till 72 hours at 22 - 25 ^{0}C. These results were not in complete harmony with those reported by (15&10), their works indicated that the BYMV isolate has lost its infectivity at sap dilution 10^{-3} - 10^{-4}. While quite contradictory results were obtained by (3,5,7&14) who revealed that the thermal inactivation point of BYMV isolate was 65 ^{0}C., the dilution end point ranged between 10^{-3}- 10^{-5} and the longevity in vitro ranged from 2 to 7 days (3), it was Found that the thermal inactivation point of the BYMV isolate was between 60 - 62 ^{0}C. for 10 minutes, it lost its infectivity when diluted to 1:1000 or stored at room temperature for 24 hours (5), an investigation in Libya, showed that the thermal inactivation point of BYMV isolate was 62 - 64^{0}C., with dilution end point ranged between 10^{-4} - 10^{-5} and longevity in vitro was 8 days (14), and the other isolate of BYMV from Libya was inactivated at 60 - 65 ^{0}C. and when diluted from 10^{-3} - 10^{-4}, or stored for 3 days at 20 - 25 ^{0}C. (7).

Host range and symptomology:

Inoculation of BYMV isolate produced systemic symptoms and local symptoms on different plant species which belong to different families (Table 2). The present study, revealed that the systemic and local symptoms produced in Leguminous plants as a result of inoculation with the BYMV isolate, were similar to those previously described (7&10), except that symptoms which appeared on *Vigna sinensis* and *Glycin max*, were not the same. The present work showed chlorotic spots, mild systemic mosaic on *Vigna sinensis* and mild systemic mosaic on *Glycin max.* These results agreed with those previously reported (5) while in other studies the virus isolates induced chlorosis of growth tip on Vigna sinensis, and produced no symptoms on *Glycin max* (7&10). Whereas, no symptoms were observed on both *Vigna sinensis* and *Glycin max* (14).

The present work showed that *Chenopodium amaranticolor* was not infectable by the BYMV isolate. This result agreed completely with (5).

RESULTS AND DISCUSSION

Serological diagnosis of the virus isolates:

By using indirect ELIAS test all the virus isolates produced positive reaction with Bean yellow mosaic potyvirus (BYMV) as shown in the table (1) . this result is the first report on the occurrence of BYMV on faba bean in Sirt region. BYMV had been identified before in other parts of Libya on faba bean : in Tripoli (12 &14) in the regions near to Tripoli and Tripoli itself (7), in Al-Jabal El-Akder (10) and in Sebha (9) and on Pea in West of Libya (15&16).

Table (1) . Detection of some viruses infect faba bean by indirect ELISA test .

No. of tested samples	Antisera of viruses infect faba bean		
	BBMV	CMV	BYMV
1	-	-	+
2	-	-	+
3	-	-	+
4	-	-	+
5	-	-	+
6	-	-	+
7	-	-	+
8	-	-	+
9	-	-	+
10	-	-	+

\+ Presence of the virus .

\- No presence of the virus .

BBMV (Broad Bean Mottle Virus) .

CMV (Cucumber Mosaic Virus) .

BYMV (Bean Yellow Mosaic Virus) .

Characterization of isolate no. 6

Isolate no. 6 was used to study physical properties, host range and symptomology of this virus.

obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA). Ten virus isolates were tested against these anti sera through indirect ELISA test. The ELISA value were expressed as adsorbance at 405 nm measured by Bio-Rad ELISA reader. In each set of test, healthy sample prepared in coating buffer were included as control. The development of a yellow colour with an adsorbance values of at least double that of the healthy control were considered positive.

4- Identification of isolate No. 6

Isolate no. 6 that gave positive reaction with BYMV was used as a source of the virus in the present work and the identification of the virus was based on studying its physical properties, host range and symptomology. Experiments of the mentioned studies, were conducted in agricultural experimental station in Benghazi, except the serological diagnosis was conducted in plant virus laboratory.

4.1- Physical properties:

Thermal inactivation point, Dilution end point and longevity in vitro, were studied in the infectious sap of *Vicia faba* leaves. Each treated sap, was used to inoculate 8 out of 10 faba bean plants and the results were based on the number of infected plants out of inoculated ones. The tested plants were kept in an insect-proof greenhouse. Daily observation was conducted for two months.

4.2- Host range and symptomology:

Faba bean leaves showing symptoms , were ground in 0.1 M potassium phosphate buffer (PH 7.0), with mortar and pestle. Extracted sap was squeezed through a double layer of cheesecloth and used in mechanical inoculate test. Test seedlings included 25 plant species, belonging to 8 different families. 20 out of 25 Seedling of each species, were mechanically inoculated by the isolated virus. The inoculated seedlings were kept in an insect-proof greenhouse. Daily observation were conducted for two months. Symptomless plants were checked for virus infection by back inoculation to *Vicia faba* plants.

area under faba bean cultivation in Libya was around 10.000 ha. during 2006 cropping season (8). Productivity of the crop is affected by a number of factors including viruses. More than 40 viruses were recorded to affect faba bean (4&13).

Virus-like symptoms on broad bean plants were noticed to be prevalent in field near Sirt. Preliminary examination by indirect-ELISA test was carried out on samples collected from that broad bean field, indicating that bean yellow mosaic virus (BYMV) is the causal agent of mosaic symptoms in the field that under the present study. So this research aimed to identify the virus based on: Serological diagnosis, physical properties and host range and symptomology.

MATERIALS AND METHODS

1- Isolation.

Field observations have been done in Sirt region during the beginning of winter season of 2009- 2010 to collect leaf samples showed mosaic symptoms of faba bean plants, which suspected to be due to virus infection. Ten samples from the infected field were collected then an experiment was carried out in the green house inside the plant viruses laboratory of plant protection department of Omar Al-Mukhtar University, in order to isolate and identify the causal agent. Each isolate was inoculated to ten plants, then the tested plants kept under observation in the green house and periodically sprayed with insecticide (Mospy), to prevent the contamination through insect transmission.

2- Samples preservation in the laboratory:

The infected leaf samples showing mosaic symptoms were collected in plastic bags and then were desiccated in the lab, each sample was kept after that in labeled plastic containers having calcium chloride separated from the sample by tissues. The closed containers labeled with the sample number and kept in the refrigerator until time of examination.

3 - Serological diagnosis of the virus isolates:

Three Antisera of different viruses usually infect broad bean plants, included Broad bean mottle bromovirus (BBMV), Cucumber mosaic cucumovirus (CMV) and Bean yellow mosaic potyvirus (BYMV), were

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Studies on Bean Yellow Mosaic Virus (BYMV) On Broad Bean In Sirt

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Abstract

Mosaic symptoms on leaves of broad bean plants were observed in field of Sirt region. Ten samples from the infected field were collected and tested for viruses present by indirect ELISA, using antisera of three viruses (BBMV, CMV, BYMV), which are common to infect broad bean plants and were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA). Positive reactions were obtained with the antiserum of BYMV. The isolates of the virus were kept in the laboratory and the isolate no. 6 was used as a source of the virus for further studies, included confirming the results of indirect ELISA test, mechanical transmission, physical properties in crude sap, host range and symptoms studies. On the basis of serology, the virus was confirmed to be a strain of BYMV. This isolate was successfully transmitted through mechanical transmission, had thermal inactivation point, between 55 -60 ^{0}C., it lost its infectivity at sap dilution 10^{-3} and when stored in crude sap in vitro for 72 h at 22 - 25^{0}C. Study of host range and symptoms, included more than 20 plant species representing eight plant families, Chenopodiaceae; Cruciferae; Cucurbitaceae; Fabaceae; Solanaceae; Umbelliferae; Amarantaceae; Compositae. Revealed that the host range of the BYMV was not restricted to specific plant family but quite the opposite, and the symptoms studies showed that the reactions of tested plants differed according to plant species.

INTRODUCTION

Faba bean (*Vicia faba L.*) is an important legume crop belongs to family fabiaceae and cultivated in different parts of Libya as an important food crop for human consumption. It is considered as the main protein source for a large part of population in many Arab countries. it is used for animal feed and as a green manure to improve the organic matter of soil (1). The total