Distribution of Some Viruses Affecting Faba Bean In El-Beheira Governorate and Screening of Some Faba Bean Cultivars for Resistance to The Most Prevalent One

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ABSTRACT

Field survey studies carried out during the growing seasons 2000/2001 and 2001/2002 in El-Beheira governorate revealed the presence of *Bean yellow mosaic virus* (BYMV), *Broad bean mottle virus* (BBMV), *Broad bean true mosaic virus* (BBTMV), *Broad bean stain virus* (BBSV) and Pea seed borne mosaic virus (PsbMV). BYMV was found to be the most prevalent virus, while BBMV was the least one. BYMV was detected in 99 and 98 samples out of 114 and 155 tested samples in the growing seasons 2000/2001 and 2001/2002, respectively. The percentage of mosaic and mottle diseases incidence ranged from 27 to 77% during the two seasons survey. A total of fourteen faba bean cultivars were screened for resistance to infection with BYMV; the most prevalent one using infection percentage, disease index and concentration as a parameters for evaluation under greenhouse conditions. Results showed that all the tested cultivars were highly susceptible to BYMV. Giza 2 cultivar was found to be the highest susceptible and Nobaria 1 cultivar was the least one.

INTRODUCTION

Surveys of faba bean plantations for viruses were conducted in many Arab countries (Fortass and Bos, 1991; Makkouk *et al*., 1987; Makkouk *et al*., 1988; Mouhanna *et al*., 1994; and Najar *et al*., 2001).

Bean yellow mosaic virus (BYMV) is a wide spread potyvirus on faba bean that has been reported in Egypt (Rizkallah, 1977; Allam *et al.,* 1979 ; Makkouk *et al*., 1988; Makkouk *et al*., 1994 ; El-Hammady *et al*., 2002 and Fegla *et al*., 2003)

The objective of this work is to determine the distribution and prevalence of viruses infecting faba bean in different locations at El-Beheira governorate using indirect ELISA. Screening of faba bean cultivars for resistance to the most prevalent virus was also conducted.

MATERIALS AND METHODS

1. Distribution and prevalence of some viruses infecting faba bean

Survey studies were conducted in some locations of El-Beheira governorate during the growing seasons 2000/2001 and 2001/2002. The most common symptoms detected in the fields were different types of mosaic, mottle, leaf curling, reduction of the leaf blade and stunting of cvs. Giza 461, Giza 3, Giza 843 and Nobaria 1 plants. Mosaic and mottle diseases incidence was estimated by counting the number of plants showing mosaic and mottle symptoms out of 100 consecutive plants in each of four patches selected, at random, from each inspected field.

114 and 155 samples, during the growing seasons 2000/2001 and 2001/2002, respectively with different mosaic and mottle symptoms were separately collected, in plastic bags, at random from diseased faba bean plants of 3 different regions in El-Mahmodia, El-Rahmania and Hosh Isa locations of El-Beheira governorate.

Samples were indexed by indirect ELISA described by Younes (1995) and Fegla *et al*., (1997) for infection with *Broad bean mottle virus* (BBMV), *Broad bean true mosaic virus* (BBTMV), *Broad bean stain virus* (BBSV), *Pea seed-borne mosaic virus* (PsbMV) and *Bean yellow mosaic virus* (BYMV). The used antisera for such viruses were kindly supplied by Dr. Khalid Makkouk, ICARDA, Aleppo , Syria .

2. Screening of faba bean cultivars for resistance to BYMV

This experiment was carried out to evaluate faba bean cultivars for resistance to BYMV under greenhouse conditions.

Fourteen cultivars used in this study , namely : Sakha 1 , Sakha 2 , Giza 3 , Giza 461 , Giza 643 , Giza 716 , Giza 717 , Giza 843 , Masr 1 , Giza 2 , Giza 40 , Giza 429 , Giza 674 and Nobaria 1 were obtained from Agricultural Research Center . Cultivars were sown during the growing season of 2003 / 2004 in 25 cm pots (10 seeds/pot) containing a sterilized soil – peat moss – sand mixture. Three replicates were used for each cultivar and each replicate consisted of one pot. Seedlings of faba bean cultivars were mechanically inoculated with BYMV after 2 weeks of planting. Inocula were prepared by grinding infected faba bean leaf tissues with 0.1M phosphate buffer, pH 7.0, containing 0.5% 2-mercaptoethanol using an extraction ratio of 1:5 (W/V).

Inoculated plants were observed daily for symptoms development. Percentage of infection, disease index and virus concentration were calculated four weeks after inoculation.

2.1 Disease index:

Disease incidence was recorded as number of infected plants and rated using the following scale:

10 = 100% of leaves showing lethal systemic wilt.
8 =< 100% of leaves showing lethal systemic wilt .
6 = Severe systemic mosaic and curling symptoms.
4 = Moderate systemic mosaic symptoms.
2 = Mild systemic mosaic symptoms.
0 = no symptoms .

Disease index (DI) was calculated from the disease rating by the following formula as reported by Raupach *et al.,*(1996) .

$$\text{Disease index} = \frac{[\Sigma(\text{rating no. X no. plants in rating}) \times 100]}{(\text{Total no. plants} \times \text{highest rating})}$$

؛ index categories applied by Makkouk and Kumari (1995) were used to evaluate faba bean cultivars for resistance:-

DI = 0.0	highly resistance
DI = 0.1 – 20.0 %	resistant
DI = 20.1 – 50 %	susceptible
DI > 50 %	highly susceptible

2.2 Virus concentration:

Leaf samples of fourteen tested cultivars were collected from randomly selected healthy and infected faba bean plants with BYMV. Each virus sample was taken from the third top leaf. Virus free as well as virus infected samples of each cultivar were ground in coating buffer (0.05M carbonate , pH 9.6) using an extraction ratio 1:10 (W/V) and assayed by indirect ELISA as described before .

RESULTS

1. Distribution and prevalence of some viruses infecting faba bean

Results of surveys revealed that, the incidence of mosaic and mottle diseases ranged from 41 to 77% and from 27 to 68% during the first and the second seasons, respectively (Tables 1 and 2).

Obtained data of the first growing season 2000/2001, presented in Tables (1 and 3) showed that BYMV was the most prevalent virus. Out of 114 tested samples, BYMV was detected in 99 samples followed by PsbMV in 85 samples and then BBTMV in 64 samples, which in turn was followed by BBSV in 26 samples and finally by BBMV in 14 samples representing 86.8%, 74.6%, 56.14%, 22.8% and 12.3%, respectively from the whole previously inspected samples.

Regarding the single infections, it was found that 7 out of 99 samples were infected with BYMV, 7 out of 85 samples were infected with PsbMV, 2 out of 26 samples were infected with BBSV and 3 out of 14 samples were infected with BBMV.

As for double infection, the combination between PsbMV + BYMV was the most common (25 samples), followed by BBTMV + PsbMV (14 samples).Triple infection BBTMV + PsbMV + BYMV was the most prevalent occurred in 29 samples , followed by BBSV + PsbMV + BYMV (3 samples) . Tetra infection was only found among BBTMV + BBSV + PsbMV + BYMV (10 samples). 11 samples were found to be infected by the five tested viruses. Three samples showed negative results with the tested virus antisera (Table 3).

At the second growing season 2001/2002, 155 infected samples were collected. Data presented in Tables (2 and 4) showed that BYMV had the highest frequency being present in 98 samples, (63.2%) as a single infection.

Mixed infections were not detected. Fifty seven samples showed negative results with the five tested virus antisera.

4-Screening of faba bean cultivars for resistance to BYMV

Infection percentage, disease index and virus concentration were used as a parameters for screening faba bean cultivars for resistance to BYMV.

4.1-Disease index

Data concerning infection percentage and disease index presented in Table (5) were found to be varied according to the tested cultivar. However, obtained values indicated that the tested cultivars were highly susceptible to BYMV. Using disease index as a main parameter for resistance , cv. Giza 2 was found to be the highest susceptible (100 %) , followed by cv. Giza 40 (87.5 %) and cv. Masr 1 (85.8 %) , while cv. Nobaria 1 was the least one in this respect (58.3 %) , followed by Giza 717 (69.2 %) and cv. Giza 843 (70.0 %) .

4.2-Virus concentration

Relative concentrations of BYMV determined by indirect ELISA as absorbance values at 405 nm are presented in Table (6). Results reveal that BYMV concentration was varied according to the tested cultivars.

However, virus concentration coincides in part with the disease index. Higher concentration was detected in cvs. Giza 429 , Giza 40 and Giza 843 infected with BYMV. On the other hand , lower concentration was observed in cvs. Giza 461 and Giza 717.

Table 1. Occurrence and relative prevalence of some viruses infecting faba bean in some faba bean producing areas in El-Beheira governorate during the growing season 2000/2001.

Location	Mosaic and mottle diseases incidence%	No. of tested samples	No. of samples found infected with					
			BYMV	BBMV	BBTMV	BBSV	PsbMV	Unknown
El-Zohour location	41	13	9	0	0	2	11	–
Dirout location	55	10	5	0	0	0	10	–
Ariamoon location	46	13	13	0	3	3	13	–
Houd El- Beer location	50	18	15	0	10	0	6	3
Houd El-Bahragan location	74	12	12	0	12	0	8	–
El-Gezera location	56	20	20	0	18	0	12	–
Ezbet El-Modeer location	77	13	10	5	10	10	10	–
Ezbet Emara Location	60	9	9	7	9	9	9	–
El-Kardoud location	58	6	6	2	2	2	6	–
Total		114	99	14	64	26	85	3
%			86.8	12.3	56.14	22.8	74.6	2.6

Table 2. Occurrence and relative prevalence of some viruses infecting faba bean in some faba bean producing areas in El-Beheira governorate during the growing season 2001/2002 .

Location	Mosaic and mottle diseases incidence%	No. of tested samples	No. of samples found infected with					
			BYMV	BBMV	BBTMV	BBSV	PsbMV	Unknown
El-Zohour location	27	27	20	0	0	0	0	7
Dirout location	34	9	4	0	0	0	0	5
Ariamoon location	43	17	13	0	0	0	0	4
Houd El- Beer location	56	15	7	0	0	0	0	8
Houd El-Bahragan location	51	19	11	0	0	0	0	8
El-Gezera location	42	12	5	0	0	0	0	7
Ezbet El-Modeer location	68	17	17	0	0	0	0	–
Ezbet Emara Location	33	20	8	0	0	0	0	12
El-Kardoud location	54	19	13	0	0	0	0	6
Total		155	98	0	0	0	0	57
%			63.2	0.0	0.0	0.0	0.0	36.8

Table 3. Viruses causing single and mixed infections in samples collected from naturally infected faba bean plants in fields distributed in some locations of El-Beheira governorate during the growing season 2000/2001 .

Viruses \ Location	El-Mahmodia		El-Rahmania		Hosh Isa	
	No. of samples	%	No. of samples	%	No. of samples	%
BBMV	0	0.0	0	0.0	3	10.7
BBTMV	0	0.0	0	0.0	0	0.0
BBSV	2	5.6	0	0.0	0	0.0
PsbMV	0	0.0	7	14	0	0.0
BYMV	7	19.5	0	0.0	0	0.0
BBTMV+PsbMV	0	0.0	14	28	0	0.0
PsbMV+BYMV	21	58.3	0	0.0	4	14.3
BBTMV+PsbMV+BYMV	3	8.3	26	52	0	0.0
BBSV+PsbMV+BYMV	3	8.3	0	0.0	0	0.0
BBTMV+BBSV+PsbMV+BYMV	0	0.0	0	0.0	10	35.7
BBMV+BBTMV+BBSV+PsbMV+BYMV	0	0.0	0	0.0	11	39.3
Unknown	0	0.0	3	6.0	0	0.0
Total	36		50		28	

Table 4. Viruses causing single and mixed infections in samples collected from naturally infected faba bean plants in fields distributed in some locations of El-Beheira governorate during the growing season 2001/2002 .

Location / Viruses	El-Mahmodia		El-Rahmania		Hosh Isa	
	No. of samples	%	No. of samples	%	No. of samples	%
BBMV	0	0.0	0	0.0	0	0.0
BBTMV	0	0.0	0	0.0	0	0.0
BBSV	0	0.0	0	0.0	0	0.0
PsbMV	0	0.0	0	0.0	0	0.0
BYMV	37	69.8	23	50	38	67.9
Unknown	16	30.2	23	50	18	32.1
Total	53		46		56	

Table 5. Disease index of BYMV infection on faba bean cultivars.

Cultivars	BYMV		
	Rate*	Infection%	Disease index%
Sakha 1	29/30	96.7	73.3
Sakha 2	29/30	96.7	78.3
Giza 3	30/30	100	80.0
Giza 461	30/30	100	72.5
Giza 643	30/30	100	85.0
Giza 716	28/30	93.3	75.8
Giza 717	29/30	96.7	69.2
Giza 843	29/30	96.7	70.0
Masr 1	29/30	96.7	85.8
Giza 2	30/30	100	100
Giza 40	30/30	100	87.5
Giza 429	30/30	100	78.3
Giza 674	29/30	96.7	75.0
Nobaria1	28/30	93.3	58.3

* No. of infected plants / No. of tested plants .

Table 6. Concentration of BYMV in faba bean cultivars determined by indirect ELISA as absorbance values at 405 nm.

Cultivars	Indirect ELISA absorbance values (E 405 nm) for BYMV		
	Healthy	Infected	I / H
Sakha 1	0.56	1.61	2.87
Sakha 2	0.64	2.47	3.85
Giza 3	0.34	1.04	3.05
Giza 461	0.55	1.14	2.07
Giza 643	0.51	1.21	2.37
Giza 716	0.49	1.64	3.34
Giza 717	0.28	0.62	2.21
Giza 843	0.41	1.72	4.19
Masr 1	0.30	0.89	2.96
Giza 2	0.41	1.48	3.60
Giza 40	0.36	1.55	4.30
Giza 429	0.40	1.90	4.75
Giza 674	0.43	1.73	4.02
Nobaria1	0.49	1.77	3.61

- ELISA absorbance values are averaged of two replicates.

- The absorbance values of at least double that of the healthy control were considered positive.

I = Infected, H = Healthy

DISCUSSION

Field surveys were conducted during two growing seasons to study the prevalence and distribution of viruses infecting faba bean in some locations along El-Beherira governorate. Five viruses, namely: BBMV, BBTMV, BBSV, PsbMV and BYMV were detected using indirect ELISA in different locations but at different rates. These viruses were detected in other countries by some authors (Mouhanna *et al*., 1994 ; Abraham *et al*., 2000 ; El-Muahdidi *et al*., 2001 ; Najar *et al*., 2001) and in Egypt (Mazyad *et al*., 1975 ; Rizkallah, 1977 ; Allam *et al.,* 1979 ; Omar *et* al., 1990 ; Makkouk *et al.,* 1994 ; El-Afifi and El-Dougdoug, 1997 ; Sallam, 2000 ; El-Hammady *et al*., 2002 and Fegla *et al*., 2003). However, such viruses were detected for the first time in El-Beheira governorate.

BYMV was the most prevalent, while BBMV was found to be the least one. Such results are in line with those recorded by Fegla *et al*. (2003). Contradictory results were obtained by El-Hammady *et al*. (2002) who found that BBMV was the most prevalent virus, followed by BBTMV.

The incidence of mosaic and mottle diseases symptoms ranged from 27 – 77% during the two seasons survey. The high and the low incidence were found in El-Zohour (27%) and Ezbet El-Modeer (77%) locations, respectively.

Control through the use of resistant cultivars is probably the cheapest and most effective way of combating virus diseases. Fourteen faba bean cultivars were screened for resistance to BYMV. Evaluation was based on infection percentage, disease index and virus concentration. Unfortunately results showed that all tested cultivars were highly susceptible. Similar conclusion has been reached by Makkouk and Kumari (1995).

According to the available literature, no work has bean done on prevalence and distribution of viruses infecting faba bean in El-Beheira governorate, so the present study could be considered the first report of the occurrence of such viruses in El-Beheira governorate.

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الملخص العربى

انتشار بعض الفيروسات التى تصيب الفول فى محافظة البحيرة واختبار بعض أصناف الفول لمقاومة أكثرهم انتشارا

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أوضحت نتائج الحصر التى أجريت فى محافظة البحيرة وجود فيروس الموزاييك الأصفر للفاصوليا (BYMV) ، فيروس تبرقش الفول (BBMV) ، فيروس الموزاييك الحقيقى للفول (BBTMV) ، فيروس تلون بذور الفول (BBSV) وفيروس موزايك البسلة المنقول بالبذرة (PsbMV) وكان فيروس BYMV هو الأكثر انتشاراً بينما فيروس BBMV الأقل انتشاراً وقد وجد BYMV فى 99 و 98 عينة من 114 و 155 عينة مختبرة فى موسمى الزراعة 2001/2000 و 2002/2001، على التوالى. وكانت النسبة المئوية للنباتات المصابة بالموزاييك والتبرقش تتراوح ما بين 27 و 77٪ وذلك خلال موسمى الحصر . وتحت ظروف الصوبة تم اختبار مدى قابلية 14 صنف مختلف من الفول للإصابة بفيروس BYMV باستخدام النسبة المئوية للاصابة، دليل المرض وتركيز الفيروس كوسائل للتقييم. وقد أوضحت النتائج أن كل الأصناف المختبرة كانت شديدة القابلية للإصابة وكان صنف جيزة 2 هو أشدها قابلية للإصابة وصنف نوبارية 1 هو الأقل فى شدة الإصابة .