BARLEY YELLOW DWARF VIRUS IN EGYPT: NATURAL INCIDENCE, TRANSMISSION, *AND WILD* HOSTS

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Abstract

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Symptoms suggestive of barley yellow dwarf virus (BYDV) infection were observed on some wheat and barley plants in experimental fields at Giza during the growing season of 1987. Later on, symptoms were also observed in some farmer fields in Upper and Lower Egypt. The virus was identified on the basis of symptomatology, virus transmission, and serological reactions (ELISA) using BYDV-PAV antiserum. Natural infection of BYDV was detected in fields of wheat, barley, oat, maize and sorghum located in Giza, Beni Suweif, Qualubia, Sharkia, Monofia, Gharbia, and Kafr El-Shaikh provinces. The virus was transmitted by the aphids *Rhopalosiphum padi* and *Schizaphis graminum* from barley cv. G1 R2 V7 to wheat cv. Giza 163. After inoculation, the incubation period of the virus in wheat plants before symptom development was 45-49 days at 30-32 C. Five species of graminaceous weeds (*Avena fatua, Diplachne malabrica, Eleusine indica, Paspalum distichum* and *Polypogon monspeliensis*) out of twelve tested with ELISA, were found to be sources of BYDV infection. Upper leaves of wheat and oat plants contained higher virus concentrations than the lower leaves. The virus was found to be translocated to all tillers of a plant within 30 days. The effect of different buffers and storage conditions of diseased samples on virus detection by ELISA was evaluated.

Introduction

In the Mediterranean region, barley yellow dwarf virus (BYDV) attracted attention over the last decade as an economically important disease of cereals (5,6). BYDV was recorded in 1980 in Egypt (3). Fcur cereal aphids *Rhopalosiphum maidis* (Fitch.), *R. padi* (L.), *Schizaphis graminum* (Rond.), and *Sitobion avenae* (Fabr.) collected from three wild plants (*Bromus catharticus, Hordeum murinum* and *Panicum* sp.) were found earlier to carry BYDV (3).

BYDV infection has been observed in wheat and barley fields in Giza since 1985 with mild symptoms and low infection rates. BYDV infected plants often are widely dispersed but incidence may increase later in the season if the aphid population is high.

Over the last two years, BYDV incidence has increased in Lower and Middle Egypt. Even though BYDV is now considered one of the most important problems of Egyptian cereal crops, only limited studies has been conducted (3).

A study on the natural occurrence of BYDV, virus transmission and graminaceous weeds as source of infection was conducted in Egypt during 1990-1991 and is reported in this paper.

Materials and Methods

1. Virus isolation and identification

Barley variety G1 R2 V7 was the source of virus infection. Seedlings of wheat cv. Giza 163 at the one-leaf stage were used as the assay host.

Virus-free aphid colonies of two species (*R. padi* and *S. graminum*) were supplied by the aphid screening laboratory, ARC. Nymphs and wingless adults of aphids were allowed to feed on detached BYDV-infected leaves in Petri dishes for 24 hours. Aphids (5 insects/plant) were then allowed to feed on healthy assay seedlings for 3 days. Insects were then manually removed and test seedlings were kept for 60 days in the greenhouse with periodical insecticide spray. Plants showing BYDV-like symptoms were tested by ELISA using the PAV serotype.

2. Field observations and plant sample collection

Wheat, barley, oat, maize and sorghum fields were examined in the 1990 and 1991 growing seasons at Giza, Beni Suweif, Sharkia, Qualubia, Monofia, Gharbia and Kafr El-Shaikh. Visual observation by counting plants with suspected BYDV symptoms was used to assess virus occurence. Different types and severity of BYDV-diseased plant samples were collected in plastic bags and brought to the laboratory and stored at room temperature (27° C) before being tested with ELISA. Graminaceous weeds showing BYDV-like symptoms also were collected from cereal fields and tested in the same manner. Apparently healthy cereal and weed plants were collected and used as a negative control for ELISA-tests.

3. Virus detection with ELISA

The double antibody sandwich method (2) was used throughout the investigation to detect virus presence. Wheat, barley, oats, sorghum and wild grasses were tested against the PAV serotype. Maize samples were tested at Purdue University (Dr.R.Lister) for PAV and RMV serotypes.

Plates were coated with 1 g/ml gamma-globulin. The conjugate dilution used was 1/1,000. There was no reaction with crude extracts from healthy leaves. Reactions were read at 405 nm using a Multiskan spectrophotometer (Titertek).

Samples were homogenized in a phosphate buffer saline (PBS) extraction buffer, pH 7.4, containing 0.05 % Tween 20,2 % polyvinyl pyrrolidone (PVP) and 0.2 % ovalbumin. For sample extraction, tris-buffer at various pH levels and different additives were evaluated in comparison with the standard PBS.

Results

1. Natural disease incidence

Barley yellow dwarf virus was present in all locations sampled (Table 1). Barley, oats, wheat maize, and sorghum were infected (Table 1). The PAV isolate of BYDV was present in the majority of the cereal samples collected. However, only the RMV isolate was detected by serology in maize plants showing symptoms (Table 1).

Table 1. Incidence of barley yellow dwarf virus (BYDV) infection in cereal crops, collected from fields surveyed in different locations, during 1990 and 1991 growing seasons. Results are based on ELISA tests using the PAV serotype of BYDV.

Location	Crop	Field observation of infection (%) during		ELISA tests % BYDV positive
		1990	1991	1991
	Wheat	57	26	24
	Barley	-	74	75
	Oat	30	61	82
	Maize. **	58	45	84
	Sorghum	-	10	16
	Rice	0	-	-
Beni Suweif				
	Wheat	47	61	54
Qualubia				
	Wheat	45	49	-
	Maize. **	35	-	20
Sharkia				
	Wheat	28	35	50
	Maize. **	8		100
Gharbia				
	Wheat	-	23	0
	Maize. **	7	-	33
Monofia				
	Wheat	-	23	-
	Maize.**	2	-	0
Kafr El-Shaikh				
	Wheat	-	8	-

* = Samples were considered positive when their ELISA values were more than three times those of healthy controls.
** = Samples reacted positively with RMV and MAV serotype.

2. Virus Identification

Barley yellow dwarf virus was identified in this investigation by the following:

2.1. Symptomology: Natural BYDV infection on barley (*Hordeum vulgare*) produced diffused bright yellow discoloration starting from the leaf tip and associated with stunted growth. Oats (*Avena sativa*) showed purple yellow leaf tip which extended to the leaf base in an elongated oval shape and plants were also stunted. Symptoms on wheat (*Triticum aestivum*) and triticale included yellowing and dwarfing but were less pronounced than on barley. Symptoms on maize (*Zea mays*) included faint chlorosis and slight stunting and, occasionally, reddening of leaves.

BYDV symptoms observed were similar to those reported by Conti (3).

2.2. Serological reactions: A double antibody sandwich ELISA was used to detect BYDV in samples of different cereals using the PAV type antiserum. Results are summarized in Table 1.

2.3. Insect transmission: Virus-free aphid colonies of *R. padi* and *S. graminum* were able to transmit the virus from barley to wheat seedlings at the one-leaf stage (Table 2). Successful transmission was determined by symptomology and a positive ELISA reaction.

3. Weed as possible sources for virus infection

Symptoms observed on weeds are recorded in Table 2 and were sometimes similar to those of diseased cereal crops. Five species of wild graminaceous plants (*Avena fatua, Diplachne malabrica, Polypogon monospeliensis,*

Table 2. Transmission of barley yellow dwarf virus (BYDV) from barley to wheat seedlings by two aphid species (*Rhopalosiphum padi* and *Schizaphis graminum)*. BYDV was dectected by ELISA using PAV serotype.

Aphid species	Symptoms observed	transmission
R. padi	Yellow leaf tip,stunting	4/15
S. graminum	No symptoms	0/15
S. graminum	Yellow leaf tip, stunting	3/14

* Samples were considered positive when their ELISA values were three times those of healthy controls.

** Numerator is number of plants infected, denominator is number of plants inoculated.

Table 3. Graminaceous wild plants as BYDV sources of infection. Weed samples collected from BYDV-diseased fields of wheat, barley, and oat in Giza during the growing season of 1991. Samples were tested by ELISA using PAV serotype.

Weed species	Symptoms	BYDV infection[a]
Cyperaceae		
1. *Cyperus rotundus*	Symptomless	0/2
Fam. Gramineae		
2. *Avena fatua*	Yellows, stunting leaf roll dry leaf tips	1/1
3. *Brachiaria cruciformis*	Yellow, leaf tips, stunting	0/1
	symptomless	0/1
4. *Cynodon dactylon*	dry leaf tips, stunt	0/1
5. *Diplachne malabrica*	yellows, stunting, leaf roll	1/1
6. *Echinocloa colonum*	symptomless	0/2
7. * *Eleusine indica*	reddening of leaves, stunting	1/2
8*. *Imperata cylindrica*	reddening of leaves, stunting	0/3
9. *Lolium multiflorum*	symptomless	0/1
10. *Paspalum distichum*	yellows, stunting, dry leaf tips	1/2
11. *Phalaris minor*	mosaic-like	0 /1
12. *Polypogon monspeliensis*	elongated leaf blotches, stunting small leaves	6/8
	symptomless	0/1

a = Numerator is number of plants infected, denominator is number of plant tested (ELISA). Samples were considered infected when ELISA values were three times those of healthy controls.

* = Samples reacted positively with RMV serotype.

Paspalum distichum, and *Eleusine indica*) out of twelve tested by ELISA, had a positive reaction with the PAV serotype. *E. indica* reacted positively with the RMV serotype (Table 3).

4. Intra and interhost movement of BYDV

Interhost movement of BYDV was detected in both oat and wheat by ELISA tests using the PAV serotype. Intrahost movement of the virus was detected in oats (Table 4). Oat flowers and upper leaves had higher virus concentrations than middle and lower leaves (Table 4). The virus was detected in all tillers/plant within 30 days after single diseased plants had appeared.

Table 4. Intrahost concentration and interhost dissemination of barley yellow dwarf virus (BYDV) on wheat and oat host plants. Naturally infected plants were tested by ELISA using PAV serotype.

Date of collection & growth stage	crop	Symptoms observed	Tested parts	BYDV infection[a]
Interhost dissemination				
April 4	Oat	Yellows, stunt	Leaf	1/1
early		dry leaf tip		
Heading		Symptomlless	Leaf	0/1
May 4	Oat	Yellows, stunt	Leaf	9/9
Flowering		dry leaf tip		
		Symptomess	Leaf	2/2
May 11	Oat	Yellows, stunt	Leaf	2/2
		dry leaf tip	Flower	1/1
		Symptomless	Leaf	1 /1
			Flower	1/1
Intrahost concentration				
May 4	Oat	Yellows, stunt,	Upper leaves	2/2 (0.250).[b]
Flowering	Leaf	dry leaf tip	Middle leaves	2/3 (0.142)
			Lower leaves	2/2 (0.101)
April 4	Wheat	-	Upper leaves	2/2 (0.102)
Flowering	Leaf		Lower leaves	1/2 (0.065)

[a] Numerator is number of plants infected, denominator is number of plant tested (ELISA). Samples were considered positive when their ELISA values were ten times those of healthy controls.
[b] Values between brackets are ELISA readings at 405 mm.

5. Effect of storage on testing samples by ELISA

To determine the best storage conditions, BYDV-infected oat samples were stored at room temperature, in the refrigerator, and freezer. Some samples were kept on silica gel. ELISA analysis was conducted about three weeks after storage. Samples stored with silica gel in the refrigerator or in the freezer produced higher ELISA values than other treatments (Table 5).

No significant differences were observed in virus extraction when the Tris buffer had various pH levels and different additives were compared with PBS buffer.

Discussion

This is the first report from Egypt where BYDV was identified by symptomatology, insect transmission, and serological reactions by ELISA.

In our tests, some symptomless samples gave a positive reaction in serological tests, suggesting that disease symptoms alone are not a reliable indication for the presence of the virus. Since 4-8 weeks are required after infection before symptoms are produced, symptomless samples which gave a positive ELISA reaction may represent recent field infection.

Some plants with BYDV-like symptoms gave a negative ELISA reaction. Such plants may have been infected with a BYDV serotype other than PAV, or there may be other factors contributing to the development of BYDV-like symptoms.

Results obtained in this study showed that five of the 12 wild graminaceous species can play the role of BYDV reservoir in the Egyptian environment.

We demonstrated that at least two aphid species were vectors of BYDV in Egypt: *R. padi* and *S. graminum*. They also may be vectors for BYDV in oats. Elnagar et al. (4) reported that four aphid species collected from three wild plants carried BYDV.

Table 5. Effect of storage on testing of oat leaves by ELISA. Naturally infected samples were tested using PAV serotype. Period of storage was about three weeks.

Storage treatment	Number of Samples tested	ELISA value (A405)
Room temperature	3	0.222
Room temperature with silica gel	3	0.140
Refrigerator	3	0.777
Refrigerator with silica gel	3	1.254
Freezer	3	0.839
Freezer with silica gel	3	0.579
Negative Reaction		
Blank		0.070
Healthy		0.107

The presence in Egypt of many BYDV-susceptible crops and wild hosts in addition to the aphid vectors may lead to BYDV epidemics when climatic conditions are favorable. Moreover, recent surveys in maize and sorghum fields during the growing season of 1991 indicated that BYDV infection on such crops was prevalent in Fayum and Delta locations (1). The possible role of maize and sorghum in the BYDV epidemic in wheat and barley in Egypt requires further studies.

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الملخص

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لوحظت أعراض مشابهة لتلك التي تتسبب عن فيروس تقزُّم واصفرار الشعير على بعض نباتات القمح والشعير في حقول تجارب محطة البحوث بالجيزة في الموسم 1987. ولوحظت الإصابة الفيروسية نفسها فيما بعد في بعض حقول المزارعين في مصر العليا والدلتا. وتم تعريف الفيروس على أساس الأعراض، والتفاعلات السيرولوجية (ELISA) باستعمال مصل مضاد خاص بالسلالة PAV. وجدت الإصابة الطبيعية بالفيروس على كل من القمح، الشعير، الشوفان، الذرة والذرة الرفيعة في مناطق: الجيزة، بني سويف، القليوبية، الشرقية، المنوفية، الغربية وكفر الشيخ. أمكن نقل الفيروس بواسطة نوعين من حشرات المن هما *Rhopalosiphum padi* و *Shizaphis graminum* من نباتات شعير صنف GIR2V7 إلى القمح صنف جيزة 163. كانت فترة الحضانة للفيروس داخل النباتات 45-49 يوماً تحت درجة حرارة تتراوح بين 30-32° م. وجدت خمسة أنواع من الاعشاب النجيلية (*Avena fatua*، *Diplachne malabrica*، *Polypogon monspeliensis*، *Paspalum distichum*، *Eleusine indica*) بأنها حاملة للفيروس من أصل 12 نوعاً جمعت من حقول القمح والشعير والشوفان المصابة. كما وجد باستخدام الإختبار نفسه بأن تركيز الفيروس في الأوراق العليا لنباتات القمح والشوفان كان أعلى بقليل من تركيزه في الأوراق السفلى. كما وجد أن الفيروس ينتقل إلى كل إشطاءات النبات المصاب في خلال 30 يوماً. كما درس أيضاً تأثير اختلاف بعض المحاليل المنظمة وظروف التخزين على الكشف عن الفيروس باستخدام طريقة اليزا.

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