Identification and Importance of Barley Yellow Dwarf Virus in Morocco

M. EL YAMANI, National Institute for Agronomic Research, Settat, B.P. 290, Morocco, and J. H. HILL, Department of Plant Pathology, Iowa State University, Ames 50011-1020

ABSTRACT

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Rhopalosiphum padi-specific, *R. maidis*-specific, and nonspecific strains of barley yellow dwarf virus (BYDV) were identified in Morocco during 1980–1982. Five species of aphids were identified as vectors of the virus. Inoculation of 12 grass species endemic to the area showed that two were immune and 10 were susceptible, of which three were symptomless. Early inoculation (stages 1 and 2 on Feekes scale) of bread wheat cultivar Nesma 149 with BYDV induced 43% yield loss, whereas later inoculation (stages 5 and 6) caused 29% loss.

The barley yellow dwarf disease (BYD) was first described by Oswald and Houston (8) in 1951 in California. The disease was shown to be induced by a virus (BYDV) that can infect members of the Gramineae family. Since that date, BYD has been reported from different parts of the world (2,13). The virus is transmitted by aphids in a persistent manner. Rochow (12) and Rochow and Muller (15) distinguished five isolates of the virus on the basis of aphid-transmission specificity. Under epiphytotic conditions, BYD can cause great damage on wheat, oats, or barley, and losses induced by the virus have been documented worldwide (1,2,4,17).

J. Burleigh (1980, *personal communication*) estimated a 75% incidence of BYD in the bread wheat cultivar Nesma 149 and a 90% incidence in the durum wheat cultivar Kyperounda in the Tessaout area of Morocco; no other data have been available on BYD in North Africa. Because cereals constitute the staple food and occupy about 5 million hectares in Morocco (7), BYD could be a significant economic problem in the country, a problem compounded by the regular use of old and low-yielding cereal cultivars. The objective of this study was to describe BYDV strains, vectors, and host range in Morocco and to assess potential crop loss caused by BYDV.

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MATERIALS AND METHODS

Identification and transmission of BYDV strains in Morocco. Plants belonging to the genera *Hordeum, Triticum, Avena*, and *Phalaris* and showing BYDV-like symptoms were collected in the Rabat, Casablanca, and Marrakech areas of Morocco during 1980–1982 and used as source plants for aphid acquisition and transmission bioassays. The aphid species used were *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion (Macrosiphum) avenae* (Fab.), *Schizaphis graminium (Rond.), and Sipha (Rungsia) maydis* (Passerini). The first four species were routinely used in transmission; the last was used only to test its ability as a virus vector. The aphids were collected

in the field and identified by A. Sekkat (Ecole National d'Agriculture, Meknes, Morocco). Virus-free cultures of each of the four aphid species used for strain identification were started from two to four newly emerged nymphs. Each species was cultured in separate cages on barley plants (*Hordeum vulgare* L. 'Arig 8') that were renewed every 4–5 wk. Periodically, aphids from each species in the virus-free cultures were transferred directly to healthy oats (*Avena byzantina* Koch 'Coast Black') to ensure that the colonies remained virus-free. *S. maydis* collected on oat plants showing characteristic BYDV symptoms in the field were used directly to infest seedlings of Coast Black oats.

To test for virus transmission, all aphid species were given a 2-day acquisition access period on detached leaves of BYDV-infected hosts at 15 C, followed by a 3-day inoculation period (12). During inoculation, five aphids were caged on healthy seedlings of Coast Black oats. After inoculation, plants were sprayed with nicotine sulfate and transferred to the greenhouse. Four weeks later, Coast Black plants showing symptoms of BYDV after primary inoculation with one of the aphid species were used for strain identification. The plants were used as new sources for virus acquisition by all four aphid species through one or more cycles of transmission to the same test plant (12). Plants were observed for symptom development at least 4 wk after inoculation.

Host range. Seedlings of 12 grass species in 10 different genera were grown from seed and tested for susceptibility to BYDV (nonspecific PAV isolate Rabat 2, Table 1) by inoculation with *R. padi*. Two weeks after inoculation, the seedlings were used as source plants for reinoculation tests to the Coast Black oats with *R. padi*. In control experiments, seedlings from the same species were infested with *R. padi* from virus-free cultures.

Measurement of yield loss. Nesma 149, a cultivar of wheat used widely by growers in Morocco, was planted during the 1981–1982 season (late November) in field plots eight rows wide and 6 m long surrounded by a border 2 m wide of the same cultivar. Guard plots 2 m wide and 6 m long separated inoculated plots. The guard plots were kept clean of any vegetation by regular plowing. Treatments applied to separate plots included inoculation with a PAV-like isolate of BYDV (Rabat 2, Table 1) before tillering (Feekes growth stages 1 and 2) (5), inoculation with the same isolate at early elongation (stages 5 and 6), plots sprayed biweekly with the insecticide dimethoate (Roxion) until heading, and no inoculation or spray. Treatments were arranged in a randomized complete-block design with four replications. For inoculation, viruliferous aphids (*R. padi* maintained on Coast Black oats infected with BYDV) on diseased leaf pieces (100 aphids per leaf piece) were evenly distributed in the plots (50 pieces per plot). Yield measurements included the plot grain yield, head weight, and 1,000-kernel weight. The number of tillers per linear meter, the number of diseased plants per square meter at growth stages 8 and 9, and the number of tillers with blasted florets per square meter at growth stage 11 were also measured. Data were analyzed by ANOVA. The experimental procedures were similar to those described by Smith and Sward (17).

Table 1. Aphid transmission of barley yellow dwarf virus from cereal and grass samples collected in Morocco during 1980–1982

Species infected Virus isolate	Number of Coast Black oats plants infected after inoculation with aphids[y]				
	Rhopalosiphum padi	*R. maidis*	*Sitobion avenae*	*Schizaphis graminium*	*Sipha maydis*
Barley					
Khemisset 1	0	7	0	...[z]	...
Tiflet 1	9	5	0	0	...
Rabat 4	11	8	11	10	...
Casa 2	8	0	0	1	...
Eljadida 2	10	11	9	7	...
Marrakech 1	11	6	4	0	...
Marrakech 2	10	7	0	4	...
Oats					
Khemisset 2	4	8	5	...	...
Rabat 2	12	1	8	...	...
Rabat 3	11	5	6		12
Agadir 1	6	...	...	...	...
Phalaris sp.					
Rabat 1	5	4	11	...	...
Wheat					
Rabat 5	7	6	8	6	...
Casa 1	5	...	5	...	...
Casa 3	12	6	7	5	...
Eljadida 1	7	7	8	...	...

[y]Data represent the number of symptomatic plants of 12 inoculated in each test.
[z]Not included.

Table 2. Barley yellow dwarf virus (BYDV) strains in Morocco as indicated by recurrent aphid transmission patterns from plants previously inoculated with four aphid species

BYDV isolates used for recurrent transmission experiments	No.	Number of plants infected after inoculation with aphids[y]			
		Rhopalosiphum padi	*R. maidis*	*Sitobion avenae*	*Schizaphis graminium*
PAV-like[z]					
Rhopalosiphum padi	11	10	6	10	7
R. maidis	8	8	6	10	8
Sitobion avenae	11	12	9	10	9
Schizaphis graminium	10	8	0	7	0
RVP-like					
R. padi	9	3	0	0	2
R. maidis	5	12	5	0	4
RMV-like					
R. maidis	7	3	10	0	3

[y]Data represent the number of symptomatic plants of 12 inoculated in each test. Five aphids per plant were used; acquisition period was 2 days and inoculation period, 3 days.
[z]PAV-, RPV-, and RMV-like groups had 12, 3, and 1 isolates, respectively. Transmission data are for representative members of the groups only.

RESULTS

Identification of BYDV strains and aphid vectors. Transmission tests performed on grass samples collected during 1980–1982 revealed that each aphid species tested could be a potential vector (Table 1). *R. padi, R. maidis, Sitobion avenae*, and *Schizaphis graminium* recovered the virus from 94, 94, 73, and 75% of the samples tested, respectively, and *Sipha maydis* recovered the virus from the sample it colonized.

Because the proportion of Coast Black seedlings that became infected after each inoculation depended largely on the aphid species and the virus isolate, the results suggested that more than one strain of BYDV infected those samples, and further transmission experiments were necessary. Plants showing symptoms after initial inoculation by each of the four aphid species (Table 1) served as source plants for additional transmission attempts by the same four aphid species. Results of these experiments are shown in Table 2. *R. padi* and *Sitobion avenae* consistently transmitted a group of 12 isolates, whereas transmission by *R. maidis* and *Schizaphis graminium* was more inconsistent. Thus, these isolates are consistent with the description of the

PAV strain of BYDV. An additional three isolates were transmitted regularly by *R. padi* but less efficiently by the other three aphid species. This aphid transmission pattern is similar to that reported for the RPV strain of the virus. Finally, one isolate was efficiently transmitted by *R. maidis* and inefficiently by *R. padi* and *Schizaphis graminium*, which suggested similarity to the RMV strain of BYDV (12).

Host range. Ten of the 12 plant species inoculated with the isolate Rabat 2 (Table 1), which is similar to the PAV strain of BYDV, became infected (Table 3). Symptoms varied from none to severe chlorosis and stunting. *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., and *Lolium italicum* A. Br. showed no symptoms, but BYDV could be recovered from these plants when they were used as source plants for transmission to Coast Black oats (Table 3). BYDV could not be recovered from *Panicum crus-galli* (L.) Beauv. or *Poa pratensis* L. The reaction of plants of *L. perenne* L. was diverse, with some plants showing stunting and others remaining symptomless. Control plants of the respective species showed no symptoms after mock inoculation with aviruliferous aphids, and BYDV could not be recovered from them.

Measurement of yield loss. Inoculation of the plants with the PAV-like isolate of BYDV at growth stages 1 and 2 resulted in more diseased plants per square meter than any other treatment (Table 4). The number of tillers per meter of row did not vary significantly from one treatment to another. However, compared with the sprayed treatment, early inoculation increased the number of blasted heads per square meter by 100% and decreased head weight by 37%, 1,000-kernel weight by 31%, and grain yield by 43%. Late inoculation had no effect on blasted heads per square meter but reduced head weight by 21%, 1,000-kernel weight by 15%, and grain yield by 29%. No statistically significant difference ($P = 0.05$) was detected between the sprayed and the unsprayed uninoculated checks for any parameter measured. Regression analysis revealed a weak negative correlation between the number of diseased plants per square meter and yield loss ($r = -0.36$).

This investigation documents the occurrence of different strains of BYDV in Morocco. In these preliminary studies, strain identification was based on data from aphid transmission experiments. More recent experiments, utilizing aphid transmission and serological techniques, have also documented the presence of the PAV, RPV, and MAV strains of BYDV in Morocco (*unpublished*). In addition, crop loss assessment data have demonstrated the potential importance of this disease in Morocco. The diversity of grass species susceptible to the virus, as well as numerous aphid vectors, provides the potential for disease epiphytotics that could induce a significant reduction in cereal production in this country. Additional studies are in progress to clarify the ecology of this disease and evaluate germ plasm for resistance to BYDV in Morocco.

DISCUSSION

We have reported the presence of PAV-, RPV-, and RMV-like strains of BYDV in the semiarid area of Morocco. The diversity of BYDV strains found is in agreement with that reported from other regions of the world (11,12). PAV-like isolates were collected most frequently and also appear to be the most prevalent in Syria and Tunisia (6). These isolates may represent the most prevalent strain occurring in this region and possibly the entire lower Mediterranean basin. PAV-like isolates were transmitted efficiently by *R. padi* and *Sitobion avenae* and less efficiently by *R. maidis* and *Schizaphis graminium*. The transmission of RPV- and RMV-like isolates by presumed nonvectors was erratic and inefficient, suggesting that these isolates may not have been pure RPV and RMV strains of BYDV or that transcapsidation could have occurred in doubly infected plants. Alternatively, this transmission pattern has also been demonstrated previously by Rochow (14) for strains RPV, RMV, and SGV of BYDV.

Transmission of BYDV by *R. padi, R. maidis, Sitobion avenae*, and *Schizaphis graminium* is in agreement with previous reports (12). These species tend to be the most common in the area, but this does not exclude the presence of other aphid species and their possible role in the virus spread. For example, transmission by *Sipha maydis* is not in agreement with the work of Slykhuis et al (16), who were unsuccessful in transmitting the virus with *Rungsia* sp. and *Sipha* sp. found on diseased barley, although both *S. agropyrella* and *S. kurdjimori* have been reported to transmit BYDV (19). This, therefore, is the first report of transmission of BYDV by *S. maydis*.

Several previous studies have examined the host range of BYDV (2,3, 10,18), with sometimes conflicting results. Our data are in good agreement with those previously reported from

Table 3. Reactions of some grass species inoculated by a Moroccan PAV-like isolate of barley yellow dwarf virus vectored by *Rhopalosiphum padi*

Grass species[w]	Reactions of inoculated plants[x]	Number of infected Coast Black oats of 12 seedlings retroinoculated[y]
Bouteloua curtipendula (Mich.) Torr.	Leaf yellowing	5
Bromus inermis Leyss.	Leaf yellowing	7
B. mollis L.	Leaf yellowing and stunting	6
Cynodon dactylon (L.) Pers.	No visible symptoms	8
Dactylis glomerata L.	No visible symptoms	6
Festuca arundinacea Schreb.	Leaf yellowing	8
Lolium italicum A. Br.	No visible symptoms	7
L. perenne L.	Stunting	6
Panicum crus-galli (L.) Beauv.	No visible symptoms	0
Phleum pratense L.	Leaf yellowing	7
Poa pratensis L.	No visible symptoms	0
Zea mays L., a land race	Leaf yellowing and stunting	...[z]

[w]Seed of *Z. mays* was provided by S. Belaid, INRA, Morocco; all other seed was from the collection of C. Boulet, IAV, Morocco.
[x]Reactions were read at least 4 wk after inoculation.
[y]Inoculated grass species were used as source plants and Coast Black oats were used as test plants in experiments for recovery.
[z]Not done.

Table 4. Effect of barley yellow dwarf virus inoculation on yield and yield components of bread wheat cultivar Nesma 149 at Sidi Elaîdi, Chaouia, Morocco

Treatment	Diseased plants/m² at stage 9[y]	No. of tillers/m of row	No. of heads with blasted florets/m²	Head weight (g)	1,000 Kernel weight (g)	Total yield/ha (t)
Early inoculation (stages 1 and 2[y])	17 a[z]	166 a	20 a	0.79 a	33.13 a	1.07 a
Late inoculation (stages 5 and 6[y])	5 b	161 a	10 b	0.99 ac	40.50 b	1.33 ac
Insecticide sprayed	1 b	163 a	10 b	1.25 b	47.81 c	1.89 b
Unsprayed, uninoculated	2 b	148 a	12 b	1.15 bc	45.07 bc	1.70 bc
$LSD_{5\%}$	4.21	...	7.92	0.24	6.59	0.54

[y]Stages of plant development on Feekes scale (5).
[z]Means followed by the same letters are not significantly different at $P < 0.05$.

California and Greece, which have similar meteorologic conditions. The susceptibility of *L. perenne, C. dactylon,* and *D. glomerata* may be significant because these species can maintain the virus and vectors indefinitely in Morocco. The susceptible species *L. italicum, Festuca arundinacea* Schreb., and *Zea mays* L. are grown almost all year for forage. In addition, *Z. mays* is grown in the spring for grain. These species may all be significant as oversummering hosts for BYDV in Morocco.

Results demonstrating significant effects of inoculation of the bread wheat cultivar Nesma 149 with BYDV at two growth stages are important because the experiment was conducted at Chaouia, a major cereal-growing area in Morocco. The data demonstrate adverse effects of early inoculation on grain yield and various yield components except for the number of tillers per meter of row. Similar results, except for the tiller number, have been reported previously (4,9,17). Inoculation at growth stages 5 and 6 induced significant reductions in head, 1,000-kernel, and total grain weight. These data agree with those of Gill (4) but not with those of Smith and Sward (17), who found no adverse effect of late inoculation (stages 5 and 6) with BYDV on wheat yield or yield components. No significant differences were found in the parameters measured between untreated uninoculated plots and the sprayed plots. Similar results were obtained by Smith and Sward (17). Little natural infection occurred during the year this study was conducted (*unpublished*).

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LITERATURE CITED

1. Bayon, F., and Ayrault, J. P. 1977. Importance de la jaunisse nanisante de l'orge (JNO ou BYDV) en Poitou-Charentes: Incidence économique, nuisibilités et approche des moyens pratique de lutte. Ann. Phytopathol. 9(3):235-239.
2. Bruehl, G. W. 1961. Barley yellow dwarf. Monogr. 1. American Phytopathological Society, St. Paul, MN. 52 pp.
3. Fargette, D., Lister, R. M., and Hood, E. L. 1982. Grasses as a reservoir of barley yellow dwarf virus in Indiana. Plant Dis. 66:1041-1045.
4. Gill, C. C. 1980. Assessment of losses on spring wheat naturally infected with barley yellow dwarf virus. Plant Dis. 64:197-203.
5. Large, E. C. 1954. Growth stages in cereals: Illustration of Feekes scale. Plant Pathol. 3:128-129.
6. Makkouk, K. M., Azzam, O. I., Skaf, J. S., El Yamani, M., Cherif, C., and Zouba, A. 1987. Situation review of barley yellow dwarf virus in West Asia and North Africa. Page A.8 in: Abstracts of CIMMYT Workshop on Barley Yellow Dwarf, Udine, Italy.
7. MARA. 1978. Principales productions végétales-statisiques agricoles série PV 4 Ministere de l'Agriculture et de la réforme Agraire, Rabat, Morocco. 145 pp.
8. Oswald, J. W., and Houston, B. R. 1951. A new virus disease of cereals, transmissible by aphids. Plant Dis. Rep. 35:471-475.
9. Oswald, J. W., and Houston, B. R. 1953. The yellow dwarf virus disease of cereal crops. Phytopathology 43:128-136.
10. Panayotou, P. C. 1982. Some aspects on barley yellow dwarf virus host range. Plant Dis. Prot. 89:595-603.
11. Plumb, R. T. 1974. Properties and isolates of barley yellow dwarf virus. Ann. Appl. Biol. 77:89-91.
12. Rochow, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
13. Rochow, W. F. 1970. Barley yellow dwarf virus. No. 32 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
14. Rochow, W. F. 1982. Identification of barley yellow dwarf viruses: Comparison of biological and serological methods. Plant Dis. 66:381-384.
15. Rochow, W. F., and Muller, I. 1971. A fifth variant of barley yellow dwarf virus in New York. Plant Dis. Rep. 55:874-877.
16. Slykhuis, J. T., Zallinsky, F. J., Hannah, A. E., and Richards, W. R. 1959. Barley yellow dwarf virus on cereals in Ontario. Plant Dis. Rep. 43:849-854.
17. Smith, P. R., and Sward, R. J. 1982. Crop loss assessement studies on the effects of barley yellow dwarf virus in wheat in Victoria. Aust. J. Agric. Res. 33:179-185.
18. Stoner, W. N. 1976. Reaction of some grasses to barley yellow dwarf virus. Plant Dis. Rep. 60:593-596.
19. Sylvester, E. S. 1980. Circulative and propagative transmission by aphids. Annu. Rev. Entomol. 25:257-286.