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Serodetection of viruses associated to barley yellow dwarf (BYD) on cereals in Algeria

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Résumé de l'article

La recherche des virus associés à la jaunisse nanisante de l'orge et de leurs vecteurs a été effectuée dans différentes zones céréalieres en Algérie (Guelma, Constantine, Alger, Sidi-bélabès, Adrar) en 1997 et 1998. Le *Rhopalosiphum padi* est présent dans toutes les zones de culture, alors que *R. maidis*, *Sitobion avenae*, *S. fragariae* et *Schizaphis graminum* ne montrent qu'une distribution locale. Dans la plupart des parcelles, on observe un nanisme et jaunissement de l'orge (*Hordeum vulgare*), un rougissement et un nanisme de l'avoine (*Avena sativa*) et du blé (*Triticum aestivum*). Des tests sérologiques ont été effectués sur ces cultures en DAS-ELISA (RMV et SGV) ou en TAS-ELISA révélés par un anticorps monoclonal du CYDV-RPV et par différents anticorps monoclonaux du BYDV-PAV (CpA et CpB) et du BYDV-MAV. Le BYDV-PAV est mis en évidence dans toutes les zones étudiées. Peu de plantes se sont révélées porteuses des autres virus (RMV, SGV, BYDV-MAV, CYDV-RPV). Les fréquences relatives des sérotypes BYDV-PAV CpA et CpB sont variables suivant les cultures et les années. Les symptômes induits par les isolats des sérotypes BYDV-PAV CpA et BYDV-PAV CpB d'Algérie sur l'orge sont légers à graves. Le comportement de 21 isolats de BYDV-MAV a été analysé vis-à-vis d'anticorps monoclonaux distinguant deux sérotypes de ce virus. Uniquement un sérotype, le plus répandu en Europe, a été mis en évidence.

Serodetection of viruses associated to barley yellow dwarf (BYD) on cereals in Algeria

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Surveys on viruses associated with Barley Yellow Dwarf (BYD) and their vectors were carried out in Algerian cereal areas (Guelma, Constantine, Algiers, Sidi-bélabès, Adrar) in 1997 and 1998. *Rhopalosiphum padi* was present in all zones of culture, whereas *R. maidis, Sitobion avenae, S. fragariae* and *Schizaphis graminum* had only local distributions. In most areas BYD-like symptoms, i.e. dwarfing and yellowing of barley *(Hordeum vulgare)*, dwarfing and reddening of oat *(Avena sativa)* and wheat *(Triticum aestivum)*, were observed. Serological tests were done on these crops using DAS-ELISA (RMV and SGV) or TAS-ELISA using monoclonal antibodies specific to CYDV-RPV or using different variant specific BYDV-PAV (CpA and CpB) and BYDV-MAV monoclonal antibodies. BYDV-PAV was prevalent and few plant samples carrying RMV, SGV, BYDV-MAV or CYDV-RPV were detected. The relative frequencies of BYDV-PAV CpA and CpB serotypes were variable depending on the area and the crop season. The range of symptoms induced on barley by both Algerian BYDV-PAV CpB and BYDV-PAV CpA serotypes was mild to severe. Twenty-one BYDV-MAV isolates were compared using monoclonal antibodies, which distinguish two serotypes of this virus. Only one serotype was detected. This same serotype is also the most prevalent in Europe.

[Sérodétection des virus associés à la jaunisse nanisante de l'orge sur les céréales en Algérie]

La recherche des virus associés à la jaunisse nanisante de l'orge et de leurs vecteurs a été effectuée dans differentes zones céréalières en Algérie (Guelma, Constantine, Alger, Sidi-bélabès, Adrar) en 1997 et 1998. Le *Rhopalosiphum padi* est présent dans toutes les zones de culture, alors que les *R. maidis, Sitobion avenae, S. fragariae* et *Schizaphis graminum* ne montrent qu'une distribution locale. Dans la plupart des parcelles, on observe un nanisme et jaunissement de l'orge *(Hordeum vulgare)*, un rougissement et un nanisme de l'avoine *(Avena sativa)* et du blé *(Triticum aestivum)*. Des tests sérologiques ont été effectués sur ces cultures en DAS-ELISA (RMV et SGV) ou en TAS-ELISA révélés par un anticorps monoclonal du CYDV-RPV et par différents anticorps monoclonaux du BYDV-PAV (CpA et CpB) et du BYDV-MAV. Le BYDV-PAV est mis en évidence dans toutes les zones étudiées. Peu de plantes se sont révélées porteuses des autres

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virus (RMV, SGV, BYDV-MAV, CYDV-RPV). Les fréquences relatives des sérotypes BYDV-PAV CpA et CpB sont variables suivant les cultures et les années. Les symptômes induits par les isolats des sérotypes BYDV-PAV CpA et BYDV-PAV CpB d'Algérie sur l'orge sont légers à graves. Le comportement de 21 isolats de BYDV-MAV a été analysé vis-à-vis d'anticorps monoclonaux distinguant deux sérotypes de ce virus. Uniquement un sérotype, le plus répandu en Europe, a été mis en évidence.

INTRODUCTION

The viruses associated with Barley yellow dwarf (BYD) have a worldwide distribution and cause damages on straw cereals (D'Arcy and Burnett 1995). Six distinct viruses associated with BYD have been identified (Cheng *et al.* 1996; Murphy *et al.* 1995). Variants of these viruses have been characterized by their biological and/or their serological properties and/or their specific aphid vectors. Serological variants of BYDV-MAV (Lister and Sward 1988) have been associated with distinct symptoms on oat (*Avena sativa* L.) (Quiroz *et al.* 1991). Two groups of BYDV-PAV have been determined from their capsid amino-acid sequences (Chay *et al.* 1996; Mastari *et al.* 1998;) and they differ by one (Chay *et al.* 1996) or several epitopes (Mastari and Lapierre 1999). In the different countries of the Mediterranean basin, immuno-enzymatic tests revealed the general presence of BYDV-PAV and sometimes also of BYDV-MAV, CYDV-RPV, RMV or SGV whose frequencies vary according to the zones of culture (Benbelkacem 1991; El-Yamani and Hill 1990; Makkouk *et al.* 1990; Moriones and Garcia-Arenal 1991). The importance of phloem necrosis induced by these viruses (limitation of sap flow) explains the dramatic effect of drought on BYD-infected plants (Ibriz 1992) frequently found in this basin.

In Algeria the presence of BYDV-PAV, BYDV-MAV and CYDV-RPV has been recorded (El-Yamani and Bencharki 1996). During the 1997 and 1998 seasons high aphid populations and severe expression of BYD symptoms were observed in the different cereal crop areas. The objectives of this work were to study the geographical distribution of Algerian aphid populations of the potential vectors *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (F.), *S. fragariae* (Walk) and *Schizaphis graminum* (Rondani) and to characterize the viruses associated to BYD and serotypes BYDV-PAV and BYDV-MAV infecting these cereal crops.

MATERIALS AND METHODS

Survey sites

Surveys were carried out from the end of March to the end of May in the four experimental stations of the Institut Technique des Grandes Cultures, 3 sites at Algiers 2°-4° east (El-Harrach, Oued-smar and Blida), 1 site at Constantine 6°-8° east, Guelma 6°-8° east and Sidi-bélabès 0.5°-1° west) devoted to wide cereal crops and in a south Algerian station (Adrar 2°-4° center) of the Institut National de la Recherche Agronomique.

Symptoms and aphid populations of viruses associated with BYD

The percentage of plants expressing symptoms or inhabitated by aphids was directly estimated from 100 to 200 plants randomly collected in different parts of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) plots. In Algiers area the number of infested plant was measured only at Blida site. Aphid populations were estimated only once and at the near flowering stage. A plant was considered infested when at least one nymph or an adult aphid was found. Determination of aphid species was done in the laboratory.

Plant sampling

Fifty or fifty-five plants showing symptoms and eventually infested by aphids were collected in each of 29 fields in

1997 (9 durum wheat, 9 bread wheat, 6 barley and 5 oat) and 10 in 1998 (6 durum wheat and 4 bread wheat). The number of samples tested in 1997 and in 1998 was 580 and 200, respectively.

Aphid transmission

Apterous aphids of *R. padi* and *R. maidis* collected in the fields were caged on one-leaf stage barley plants cv. Plaisant for 2 d and then killed. The isolates were regularly transferred to fresh plants using a French clone of each aphid species.

Severity of the isolates

Virus-free *R. padi* and *R. maidis* were allowed to feed for 48 h on barley infected with PAV and RMV isolates, respectively. Apterous aphids (*R. padi* on barley cv. Plaisant, and *R. maidis* on oat cv. Coast-Black) were then maintained for 2 d on 10 healthy seedlings (one-leaf stage). After 4 wk at 17°C and a 14–h photoperiod in a growth chamber, fresh shoot biomass was measured.

Storage of leaf samples

Field samples (last extended leaves) were dried at room temperature and stored at 4°C for 2 to 4 mo before testing.

Serological tests

Dried samples were pulverized in liquid nitrogen and blended in 10 volumes of phosphate buffered saline containing polyvinylpyrrolidone (1%) and bovine serum albumin (0.1%). Virus-free barley plants were used as controls. Control samples were ground in a roller in the presence of five volumes of the same buffer. The polyclonal (PCAbs) [BYDV-PAV, BYDV-MAV] and then the following monoclonal antibodies (MAbs) prepared in mouse were obtained from INRA, Versailles : PM63 (for PAV / MAV detection), P2 (BYDV-PAV CpB specific), P14 (BYDV-PAV CpA specific) equivalent to 1C2 from Chay *et al.* (1996), M2 (BYDV-MAV specific), M4 (detects only BYDV-MAV A, a serotype distinct from BYDV-MAV B). MAC 92 [CYDV-RPV specific] and MAFF2 (considered as BYDV-MAV specific) MAbs were prepared in rat and provided by Adgen (Scotland, Great Britain). DAS-ELISA kits for the detection of RMV and SGV serotypes were obtained from Sanofi (France). In DAS-ELISA and TAS-ELISA, microplates were first coated with polyclonal antibodies (PCAbs), and incubated at 33°C for 4 h and 2 h, respectively. Then leaf extracts were incubated overnight at 4°C in duplicate wells (100 µL extract per well). In TAS-ELISA, microplates were incubated 2 h at 33°C with MAbs and then 2 h at 33°C with rabbit PCAbs (mouse or rat anti-IgG) conjugated to alkaline phosphatase (Biosys, France). After 2 h substrate incubation at room temperature, for alkaline phosphatase reaction, absorbance values of wells were measured at 405 nm with a Molecular Devices Emax microplate reader. Samples were considered as positive when O.D. (optical density) values were greater than three times the means of the results for uninfected control leaves.

RESULTS

Geographical distribution of viruses associated with BYD aphid vector populations

The main aphid vector species of viruses associated with BYD *(R. padi, S. avenae, S. fragariae, R. maidis,* and *S. graminum)* were found in Algerian cereal crops (Fig. 1) during spring and the beginning of summer in 1997 and 1998. *R. padi* was prevalent in barley crops (Fig. 2) and similarly in other cereal

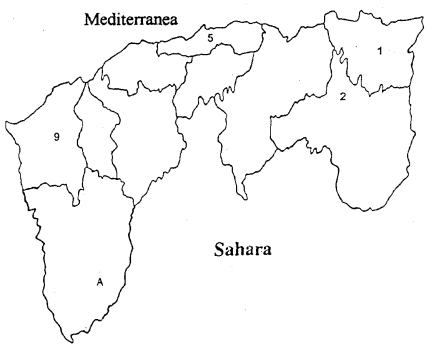

Figure 1. Algerian cereal areas surveyed in 1997 and in 1998 (1: Guelma; 2: Constantine; 5: Algiers; 9: Sidi-bélabès; A: Adrar).

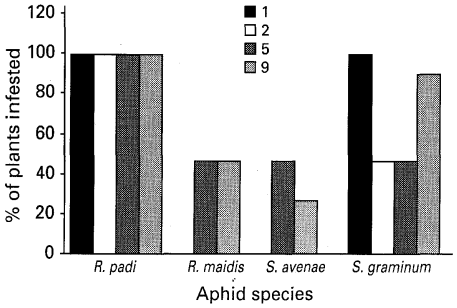

Figure 2. Frequencies of aphid-infested barley plants from plots of designated crops in Algeria in 1997 (1: Guelma; 2: Constantine; 5: Algiers; 9: Sidi-bélabès).

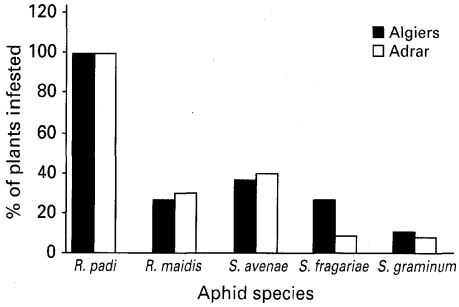

Figure 3. Frequencies of aphid-infested wheat plants from plots of designated crops in Algeria in 1998 (Algiers and Adrar).

crops surveyed. *S. graminum* was observed in the four cereal crop areas but not at Blida, one of the three sites of the Algiers area. *S. avenae* and *R. maidis* were less frequent and detected only in the Algiers and Sidi-bélabès areas. In 1998, only *R. padi* was prevalent in the Algiers area and in the Adrar region, compared to the other aphid species. However, high populations of *S. fragariae* were found on bread wheat at Blida in 1998 (Fig. 3).

Distribution and impact of BYD-like symptoms

BYD-like symptoms were observed in all cereal areas surveyed (Fig. 1). The most significant difference in disease severity was observed in the Algiers crop area between Oued-smar and El-Harrach. At Oued-smar, oat plants were mildly affected, whereas at El-Harrach, plants were very stunted. At Blida, barley plants were also very stunted and oat plants expressed only mild symptoms. At Sidi-bélabès and at Constantine moderate symptoms were observed in all cereal crops. Severe symptoms were observed in oat at Guelma and in bread and durum wheat at Adrar (Table 1).The symptoms seen may not have been due to BYD.

Identification and distribution of viruses associated to BYD

Only 24.5 % (191/780) of the plants tested were positive with one or several of the specific antisera tested (Table 2). In a few cases, two viruses were detected simultaneously by in the plants (Table

Table 1. Relative virus symptom expression in cereals in Algeria

Areas and sites	No. of plants examined	No. of plants showing symptoms	Symptom type
Guelma	250[a]	166 (O)	severe
Constantine	304[a]	280 (O,B,W)	moderate
Algiers			
Blida	150[a], 225[b]	290 (O,B,W)	mild, severe
Oued-smar	202[a]	150 (O)	mild, severe
El-Harrach	217[a]	180 (O)	severe
Sidi-bélabès	269[a]	240 (O,B,W)	moderate
Adrar	341[b]	300 (W)	severe

[a] in 1997; [b] in 1998.
O: oat; B: barley; W: wheat.

Table 2. Number of isolates belonging to the different BYDV serotypes on cereals in Algeria

Year	M Abs or PC Abs	TAS-ELISA						DAS-ELISA		No. of samples tested
		PM63	P2	P14	M2	MAFF2	MAC92	RMV	SGV	
1997	*Areas and sites*									
	Guelma	16	14	5	2	0	1	0	0	80
	Constantine	26	23	9	3	0	1	0	0	100
	Algiers									
	Blida	13	9	8	2	0	0	0	0	100
	Oued-smar	10	8	4	0	0	0	0	0	100
	El-Harrach	14	9	4	4	0	0	0	0	100
	Sidi-bélabès	21	16	12	5	0	0	0	0	100
1998	Algiers									
	Blida	27	21	8	5	0	1	4	0	100
	Adrar	9	9	4	0	0	0	0	0	100
Total		136	109	54	21	0	3	4	0	780

Specificity of monoclonal (M) and polyclonal (P) antisera used: PM63 (M): BYDV-PAV, BYDV-MAV; P2 (M): BYDV-PAV CpB; P14 (M): BYDV-PAV CpA; M2 (M): BYDV-MAV B; MAFF2 (M): BYDV-MAVvic; MAC92 (M): CYDV-RPV; RMV (P): RMV; SGV (P): SGV.

3). BYDV-PAV was the prevalent serotype and the frequency of CpA (54 isolates) and CpB (109 isolates) was dependent on the area and on the year of sampling (Table 2). One hundred and thirty-six isolates were not detected by the specific MAbs, but were detected by PM63, which recognizes both BYDV-PAV and BYDV-MAV. These isolates cannot be grouped within BYDV-PAV or BYDV-MAV serotype. The 21 MAV isolates detected by MAb M2 were not clearly detected by MAFF2. CYDV-RPV, SGV and RMV isolates were rare in all areas except at Blida in 1998 where 4% of the positive ELISA reactions were of the RMV serotype (Table 2).

Severity of BYDV-PAV and RMV isolates

Oat plants infected by RMV isolates expressed reddening of the oldest leaves and only slight decreases in biomass (0-8%). In contrast, barley plants infected either by BYDV-PAV CpA or BYDV-PAV CpB isolates showed a wide range in the extent of dwarfing. The shoot biomasses of barley infected with some isolates were reduced to less than 90% of the control (Table 4).

DISCUSSION

Surveys conducted in the main cereal areas of Algeria during 1997 and 1998 seasons during the near flowering stage of wheat showed the general presence of high aphid populations. In all these areas, *R. padi* was prevalent and was frequently associated with yellowing and/or reddening of most leaves on all plants. The survey was limited to one to three sites per area and therefore gives only preliminary data on the distribution of the different aphid species. Despite the high percentage of plants showing BYD-like symptoms, ELISA tests to detect viruses associated to BYD were negative for 3/4 of the samples. Other authors (Makkouk *et al.* 1989) have obtained similar results. The hot weather during the surveys may have reduced both virus concentrations in the plants and in dried leaf samples. It cannot be excluded that undetected viruses associated to BYD or other pathogens inducing similar symptoms were present in these areas. The presence of pathogen complexes or strain variations may explain differences in severities between the same crop in the

Table 3. O.D.$_{405\ nm}$ values in ELISA of some isolates collected in Algeria

	TAS–ELISA							DAS–ELISA		Serotypes detected
M Abs or PC Abs	PM63	P2	P14	M2	M4	MAFF2	MAC92	PC	PC	
Virus detected	BYDV-PAV/MAV	BYDV-PAV CpB	BYDV-PAV CpA	BYDV-MAV B	BYDV-MAV A	BYDV-MAVvic	CYDV-RPV	RMV	SGV	
Areas and sites										
Guelma	**0.804**	**0.948**	0.004	0.006	0.002	0.098	**3.000**	0.000	0.000	**BYDV-PAV CpB, CYDV-RPV**
Constantine	**0.848**	**0.264**	**0.632**	0.004	0.008	0.076	0.000	0.024	0.002	**BYDV-PAV CpA**
	0.000	0.000	0.000	0.000	0.000	0.000	**2.944**	0.028	0.018	**CYDV-RPV**
Algiers										
Blida	**1.776**	0.028	0.048	0.036	0.000	0.150	0.028	0.004	0.008	(1)[a]
	0.000	0.000	0.000	0.000	0.060	0.000	0.000	**0.744**	0.018	**RMV**
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.950**	**SGV**
Oued-smar	**1.498**	0.008	0.034	0.028	0.000	0.086	0.014	0.026	0.044	(1)[a]
El-Harrach	**1.738**	0.058	0.036	**0.452**	0.002	0.136	0.016	0.018	0.016	**BYDV-MAV B**
Sidi-Bélabès	**1.304**	**1.100**	0.042	0.024	0.004	0.132	0.012	0.038	0.018	
Infected control										
PAV A	**0.530**	**0.238**	**0.728**	0.000	0.000	0.000	nt[b]	nt	nt	
PAV B	**0.486**	**0.632**	0.070	0.000	0.000	0.000	nt	nt	nt	
PAV Am	**0.328**	**0.224**	**0.632**	0.000	0.000	**0.458**	nt	nt	nt	
MAV A	**0.618**	0.000	0.000	**0.200**	**0.798**	0.000	nt	nt	nt	
MAV B	**0.596**	0.000	0.000	**0.698**	0.000	0.000	nt	nt	nt	
RPV	0.000	0.000	0.000	0.000	0.000	0.000	**1.760**	nt	nt	
RMV	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.508**	nt	
SGV	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.404**	
Healthy control	0.006	0.006	0.006	0.016	0.018	0.152	0.090	0.038	0.028	

[a] Probably new BYDV serotype.
[b] nt : not tested.

Table 4. Severity of disease symptoms induced by BYDV-PAV and RMV isolates found in Algeria[1]

Disease symptom	Barley		Oat
	BYDV-PAV A	BYDV-PAV B	RMV
Mild	2	2	4
Moderate	4	6	0
Severe	11	3	0

[1] Number of isolates causing mild (0-10%), moderate (11-30%) and severe (> 30%) reduction in biomass, respectively.

different Algerian areas where the same cultivars are seeded. It is also possible that aphid populations landing in the crops in the different areas determined the severity of the disease (Comeau 1987).

BYDV-PAV was the most frequent strain detected, as is generally found in cereal crops around the world. The predominance of BYDV-PAV can be explained by its capacity to be transmitted by several aphid species, including *S. avenae* and *S. fragariae* which are sometimes locally abundant in Algeria. The wider host range of BYDV-PAV compared to CYDV-RPV (Beuve and Lapierre 1992, 1993; D'Arcy 1995) strengthens its prevalence in most countries. Even though only three CYDV-RPV isolates were found during this study, their detection corroborates the presence of this serotype already reported in the Maghreb (El-Yamani and Bencharki 1996). RMV and SGV serotypes were also not frequently detected in these areas despite the relatively common presence of their vectors. A better estimation of their frequency requires improvement of the ELISA tests for RMV and SGV which are less sensitive than those available for BYDV-PAV, BYDV-MAV and CYDV-RPV. Serological variability of BYDV-PAV and BYDV-MAV isolates from Algeria collected in the different crop areas was investigated. As found in other countries (Chay *et al.* 1996; Mastari *et al.* 1998) BYDV-PAV CpA and BYDV-PAV CpB were detected in all cereal areas, indicating a similar adaptation of the two serotypes whatever the climate where cereals are grown. In contrast to the situation in Europe and in the USA, in a few cases CpA frequency was higher than CpB, which may indicate incomplete identity of the reservoirs or distinct vector efficiency between the two serotypes. Two new serotypes (CpAm and CpBz) of BYDV-PAV have been recently discovered in Europe (Mastari *et al.* submitted paper). These two new serotypes would have been detected in the 163 Algerian BYDV-PAV isolates, suggesting that they are absent or very infrequent as in Europe.

In a few samples only MAb PM63 gave a clear positive reaction in ELISA. The relatively high O.D. values obtained with this MAb and absence of reaction of usual PAV and MAV MAbs indicates that probably new viruses associated to BYD or serotypes are present in Algeria. Aphid transmission assays will help to characterize these isolates. Two serotypes of BYDV-MAV have been detected in Europe. The A serotype is extremely rare and the B serotype has been found only in Belgium (Lapierre, unpublished results). Although a limited number of BYDV-MAV isolates have been tested, a similar situation may be encountered in Algeria where only BYDV-MAV B was found. Curiously, MAFF2 was not or very faintly reactive with these Algerian BYDV-MAV isolates. RMV or RMV like isolates are very variable (D'Arcy *et al.* 1989; Fan *et al.* 1994; Geske *et al.*1996) and variants showing severe symptoms in oat have been described in the USA (Yount and Carroll 1983). No such biological differences have been observed between the four Algerian RMV isolates collected in the Blida area, which expressed only mild

symptoms on oat. Comparison of RMV isolates from the different continents will be useful to more accurately determine the geographical influence on their properties.

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