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Comparison and differentiation of Wheat Yellow Mosaic Virus(WYMV), Wheat Spindle Streak Mosaic Virus (WSSMV) and Barley Yellow Mosaic Virus (BaYMV) isolates using WYMV monoclonal antibodies

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Abstract

Twelve monoclonal antibodies (MAbs) were obtained by immunizing mice with a French isolate (F1) of wheat yellow mosaic virus (WYMV). Three of these (3D12, 2C1, 6C3) belong to the IgM class and the nine others to the IgG class (3D8, 3H1, 2B8, 1F2, 3C10, 4F12, 3H9, 1G5, 54). In antigen-coated plate (ACP) ELISA and indirect double antibody sandwich (IDAS) ELISA, all MAbs recognize the WYMV (F1) both in the form of purified particles and in wheat leaf extract. The analysis of numerous French isolates of WYMV shows a variable reactivity with MAbs 3D8, 3H1, 2B8, 3C10, 3H9 and 1G5 in IDAS – and ACP-ELISA. The Japanese isolate of WYMV and United States isolates of wheat spindle streak mosaic virus (WSSMV) were detected in IDAS- and ACP-ELISA by ten of the MAbs tested showing that the wheat bymoviruses originating from the three locations share a high epitopic homology. French isolates of barley yellow mosaic virus (BaYMV; pathotypes 1 and 2) were only detected in ACP-ELISA with MAbs 6C3, 3D8, 3H1 and 2B8 whereas the two Japanese strains (I-1, II-1) of MaYMV were recognized with these and also with that of 3C10. In IDAS-ELISA, the two Japanese strains were clearly detected by MAbs, 6C3, 3D8, 3H1, 1F2, 3C10 and 1G5 and the British and Belgian (pathotype 2) isolates only by that of 6C3. Only the Japanese strain of BaYMV, 1-1 could be detected with MAb 3H9 in this ELISA system.

Introduction

Wheat bymoviruses have been described, in Japan as wheat yellow mosaic virus (WYMV) [Inouye, 1969] and in Canada as wheat spindle streak mosaic virus (WSSMV) [Slykhuis and Polak, 1969]. The particles of these two viruses have common properties [Usugi and Saito, 1979; Usugi *et al.*, 1989] and produce similar modifications of the cells of infected plants [Ruan *et al.*, 1991]. However, slight differences in symptomatology and serological relationships led Usugi and Saito [1979] to propose that WSSMV is a strain of WYMV. In Europe, wheat bymoviruses have either been called WSSMV [Signoret *et al.*, 1979; Proeseler and Stanarius, 1983; Rubies-Autonell and Vallega, 1987] or WYMV [Lapierre *et al.*, 1985].

Using polyclonal antibodies, it has been demonstrated in Asia [Usugi *et al.*, 1989], in Europe [Chen and Adams, 1991; Huth, 1988] and in America [Carroll *et al.*, 1993; Carroll *et al.*, 1995] that wheat bymoviruses were strongly related to barley yellow mosaic virus (BaYMV), the type member of the bymovirus genus although these viruses have different hosts.

To study the antigenic variability of WYMV, we prepared MAbs against a French WYMV isolate from the 'Centre' region of France. In this paper, we describe the reactivity of these MAbs with isolates of WYMV, WSSMV and BaYMV from different origins.

Materials and methods

Purification of the French WYMV isolate (F1)
Virus was purified using wheat leaves (cv Pemel) collected at Saunay (Loir et Cher) at the early stages of mosaic virus expression. Batches of 200 g were ground in 01. M citrate buffer, pH 7.2, containing 0.5 M urea, 0.005 M EDTA and 0.5% 2-mercaptoethanol, filtered through cotton wool, lightly clarified (5000 g, 15 min) then stirred in the presence of 1/3 volume of chloroform at 4 °C for 45 min. After centrifugation (5000 g, 15 min), the supernatant was ultracentrifuged through a sucrose cushion (20%, 5 ml) in 35 ml tubes at 180,000 g for 100 min. The pellet was resuspended in 0.1 M citrate buffer, pH 7.2, and kept at 0 °C overnight. The suspension was then placed over a solution of caesium chloride of an initial density of 1.32 in 4.5 ml tubes. The tubes were ultracentrifuged in a VTI 55 rotor at 50,000 rpm for 5 h at 4 °C. The virus-containing fractions were collected and centrifuged at 45,000 rpm for 5 h in a R70 rotor. The purified virus was resuspended in 1 ml of distilled water.

Production of hybridomas and ascites
Two 20-week old female BALB/c mice were immunised using the technique described by Mirza *et al.* [1987]. Twenty μg of solubilized virus in 0.1 M phosphate buffer, pH 7.2, was emulsified in the same volume of Freund's adjuvant and injected intramuscularly. On day 12, a booster injection containing the same quantity of virus and Freund's adjuvant was administrated. On day 15, samples of popliteal and inguinal lymph nodes were collected. The lymph cells were fused with Sp 2/O myeloma cells in the presence of polyethylene glycol (PEG) 4000. Hybrid cells were selected according to their rate of development on the selective medium (RPMI/1640) supplemented with 10% foetal calf serum (FCS) and HAT (hypoxanthine/aminopterine/thymidine) inactivated by heat.

Ten days after fusion, the capacity of the hybridoma culture supernatant to recognize the antigen was tested in IDAS- and ACP-ELISA and the hybridomas chosen were cloned and multiplied. The cells of each of the selected subclones were injected into two pristane-primed mice. The ascites were recovered ten days after injection following the method of Galfre and Milstein [1981].

Determination of isotypes and titration of MAbs
The isotypes of the MAbs were determined by the culture supernatant test using the method described by Thomas *et al.* [1986].

The ascite immunoglobulins were partially purified by precipitation with saturated ammonium sulphate (1v/9v). The titre of these antibodies was determined by ACP-ELISA with a leaf extract of WYMV (F1)(1w/10v)(Table 2).

Origin and purification of the IgG polyclonal sera
The anti-WYMV and anti-BaYMV polyclonal sera produced in rabbits were kindly provided by Drs W. Huth and M. J. Adams, respectively. The anti-WYMV serum was prepared using a French isolate from the 'Centre' region [Huth, 1988] and the anti-BaYMV from Wiltshire (GB) [Adams, 1991]. IgGs were purified following the protocol described by Clark and Adams [1977].

Virus isolates: sources and preparation (Table 1)
Samples were ground (1w/10v for fresh leaves and 1w/100v for desiccated leaves) in 0.1 M citrate buffer, pH 7.2, containing 0.5 M urea, then filtered through cotton wool and lightly clarified (5000 g, 15 min) before testing.

Immunoenzymatic detection of WYMV and BaYMV
Double antibody sandwich (DAS) ELISA. The technique used was essentially that described by Clark and Adams [1977]. Microtitre plates (Greiner 96 wells) were coated with polyclonal antibody, at 1 μg/ ml in coating buffer (2.93 g $NaHCO_3$, 1.59 g Na_2CO_3/litre) pH 9.6. After incubation for 2 h at 37 °C, the plates were blocked using 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS: 8 g NaCI, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4.12\ H_2O$, 0.2 g KCI, 0.2 g NaN_3/ litre) containing 0.05% Tween 20 (PBS-T) for 1 h at 37 °C. Leaf tissue was extracted in 0.1 M citrate buffer, 0.5M Urea pH 7.2 (1w/10v or 1w/100V), added to the wells and left overnight at 4 °C. Rabbit IgG anti-WYMV or anti-BaYMV coupled to alkaline phosphatase was diluted 1/1000 with PBS-T and added to the wells for 3 h at 37 °C. After addition of p-Nitrophenyl phosphate (p-NP) at 1 mg/ml in 10% diethanolamine, pH 9.8, the plates were left at room temperature, and absorbance values at 405 nm were measured several times. All reactants were used at 100

Table 1. Bymovirus isolates used in this study

Virus	Isolates	Plants	Cultivars	Origins	Suppliers
WYMV	**(F1)**	Wheat[a]	Pernel	Saunay (Loir et Cher) France	Mr Didelot
	(F2)	Wheat[a]	Soissons	Bourges (Cher) France	Mr Didelot
	(F3)	Wheat[a]	Scipion	La Chapelle du noyer (Eure et loir) France	Mr Didelot
	(F4)	Wheat[a]	Rossini	Pithiviers le Vieil (Indre) France	Mr Didelot
	(F5)	Wheat[a]	Soissons	Guilly (Indre) France	Mr Didelot
WYMV	**(J)**	Wheat[b]	Norion 61	Japan	Dr Kashiwazaki
WSSMV 1 (soil inoculation)	**(USA)**	Wheat[b]	-	(New York) USA	Dr Carroll
WSSMV 2 (mechanicaly inoculation)	**(USA)**	Wheat[b]	-	(New York) USA	Dr Carroll
BaYMV 1 (common strain)	**(F1)**	Barley[a]	Plaisant	Levet (Cher) France	Mr Delorme
	(F2)	Barley[a]	Plaisant	Reims (Marne) France	Mr Vermast
	(F3)	Barley[a]	Plaisant	Cuperly (Marne) France	Mme Verhaegen
BaYMV 2 (new pathotype)	**(F1)**	Barley[a]	Express	Sillery (Marne) France	Mr Vermast
	(F2)	Barley[a]	Rebelle	Reims (Marne) France	Mr Jouannic
	(G. B)	Barley[b]	Target	England	Dr Adams
	(B)	Barley[b]	-	Belgium	Dr Kummert
BaYMV I -1	**(J)**	Barley[b]	New Golden	Japan	Dr Kashiwazaki
BaYMV II - 1	**(J)**	Barley[b]	New Golden	Japan	Dr Kashiwazaki

BaYMV 1 and 2 - common and new pathotype, respectively, in Europe (Adams, 1989; Huth, 1989; Hariri *et al* 1990).
BaYMV I - 1 and II - 1- strains characterized in Japan (Kashiwazaki et al; 1989).
a - Fresh leaves.
b - Desiccated leaves.

Table 2. Reactivity of MAbs in IDAS- and ACP-ELISA with WYMV (F1)

	MAbs		IDAS-ELISA			ACP-ELISA		
Name	Class	Dilution of ascites	Purified virus[a]	Leaf extract[b]	Healthy control	Purified virus	Leaf extract	Healthy control
3D12	IgM	1.10^{-3}	**0.879** ± 0.078[c]	**0.789** ± 0.058	0.099 ± 0.005	**2.912** ± 0.042	**2.747** ± 0.159	0,240 ± 0.038
2C1	IgM	5.10^{-3}	**0.765** ± 0.052	**0.850** ± 0.112	0.041 ± 0.005	**2.412** ± 0.067	**1.930** ± 0.129	0,141 ± 0.032
6C3*	IgM	5.10^{-3}	**0.720** ± 0.067	**0.655** ± 0.043	0.235 ± 0.017	**2.589** ± 0.099	**1.957** ± 0.135	0,210 ± 0.067
3D8	IgG	5.10^{-3}	**0.890** ± 0.075	**0.901** ± 0.051	0.115 ± 0.045	**2.745** ± 0.099	**2.750** ± 0.153	0,219 ± 0.035
3H1	IgG	5.10^{-3}	**0.910** ± 0.082	**0.976** ± 0.088	0.148 ± 0.031	**2.911** ± 0.060	**2.781** ± 0.128	0,160 ± 0.042
2B8	IgG	5.10^{-3}	**1.190** ± 0.111	**0.921** ± 0.031	0.134 ± 0.020	**2.812** ± 0.075	**1.705** ± 0.305	0,148 ± 0.031
1F2	IgG	5.10^{-1}	**1.299** ± 0.099	**0.642** ± 0.089	0.112 ± 0.012	**2.789** ± 0.074	**2.010** ± 0.198	0,131 ± 0.019
3C10	IgG	5.10^{-3}	**0.965** ± 0.088	**0.890** ± 0.118	0.072 ± 0.009	**2.813** ± 0.069	**2.515** ± 0.188	0,222 ± 0.038
4F12	IgG	3.10^{-3}	**1.119** ± 0.075	**0.705** ± 0.075	0.051 ± 0.011	**2.147** ± 0.081	**1.715** ± 0.290	0,129 ± 0.024
3H9	IgG	1.10^{-3}	**0.270** ± 0.137	**0.310** ± 0.039	0.075 ± 0.012	**0.578** ± 0.118	**0.385** ± 0.128	0,210 ± 0.026
1G5	IgG	1.10^{-3}	**0.305** ± 0.059	**0.311** ± 0.045	0.095 ± 0.016	**0.495** ± 0.036	**0.255** ± 0.139	0,120 ± 0.054
5F4	IgG	1.10^{-3}	**0.379** ± 0.045	**0.351** ± 0.035	0.062 ± 0.027	**0.512** ± 0.030	**0.498** ± 0.137	0,142 ± 0.040

a - 1µg/ ml in citrate buffer.

b - 1w/ 10v of citrate buffer.

c - Absorbance at 405 nm values after 1 hour substrate - enzyme - incubated period for ACP-ELISA and 2 hours for IDAS-ELISA (standard deviation for 2 repetitions and 2 replications). Absorbance at 405 nm values equal or above two times the healthy control absorbance at 405 nm value is considered as positive.

μl per well. The plates were washed with PBS-T after each stage.

Antigen-coated plate (ACP) ELISA The technique used was similar to that described by Voller *et al.* [1979]. Microtitre plates was coated with the purified particles of WYMV (F1)(1 μg/ ml) or leaf extract diluted in coating buffer and maintained overnight at 4 °C. After rinsing, the wells were saturated with 1% BSA and incubated at 37 °C for 1 h. The plates were rinsed and coated with MAbs (1μg/ml of PBST buffer) and incubated at 37 °C for 1 h. After rinsing, the plates were coated with rabbit anti-mouse IgG coupled to alkaline phosphatase (1μg/ml), incubated at 37 °C for 3 h, rinsed and finally p-NP substrate was added as in DAS-ELISA.

Indirect double antibody sandwich (IDAS) ELISA. IDAS-ELISA was followed by D'Arcy *et al.* [1989] with some modifications. The microtitre plates were coated with polyclonal antibodies (1 μg/ml in the coating buffer). After incubation for 2 h at 37 °C, the plates were blocked with BSA for 1 h at 37 °C. The antigen, prepared as for DAS-ELISA, was added to the wells and incubated overnight at 4 °C. The second antibodies were anti-WYMV Mabs (1μg/ml of PBS-T buffer, 2 h at 37 °C), and the third antibodies were rabbit anti-mouse IgG (1μg/ml) coupled to alkaline phosphatase. After incubation for 3 h at 37 °C, p-NP was added as in DAS-ELISA.

Results

Production of hybridomas
Twenty hybridomas specifically detected WYMV (F1) in leaf extracts in ACP-ELISA. Among these, 12 hyrbidomas selected for the quality of their secreting cells were subcloned. Analysis of 160 subclones made it possible to select a secreting cell line for each of the 12 MAbs. Three of these MAbs were of the IgM type (3D12, 2C1, 6C3) and the others were of the IgG type. Only antibody purified by affinity chromatography from ascites fluid was used in subsequent assays (Table 2).

Reactivity of MAbs with WYMV (F1)
The 12 MAbs that detected WYMV (F1) in leaf extracts, also detected purified WYMV (F1) in IDAS- and ACP-ELISA. In both ELISA systems, MAbs, 3H9, 1G5 and 5F4 did not recognize the virus as readily as the other MAbs (Table 2).

Reactivity of MAbs with French WYMV isolates
WYMV from 20 origins were tested with 7 WYMV Mabs in ELISA. Only 4 representative isolates are shown (Table 3). In IDAS-ELISA, isolates 1 and 2 were detected with all Mabs, isolate 3 with Mabs 6C3, 3D8, 3H1, 2B8 and 3C10 and isolate 4 only with MAb 6C3. In ACP-ELISA isolate 1 was detected with all Mabs and isolates 2, 3 and 4 only by MAbs 6C3 and 2B8.

Reactivity of MAbs with American WSSMV and Japanese WYMV isolates
The ten MAbs (6C3, 3D8, 3H1, 2B8, 3C10, 4F12, 1F2, 3H9, 1G5, 5F4) detected a Japanese isolate of WYMV, and U. S. isolates of WSSMV inoculated mechanically or through the soil (Table 4), but some relative differences occurred. For example, MAbs 6C3 and 3D8 recognized WYMV (J) more readily than WYMV (F1), while WSSMV was poorly recognized by the polyclonal antibody and most of the MAbs whereas MAb 1G5 reacted more strongly with it than with either of the WYMV isolates. The distinct responses of these MAbs to these isolates indicated that virus concentrations alone could not explain this behaviour.

Reactivity of the MAbs with the different BaYMV isolates
None of the 12 MAbs reacted in IDAS-ELISA with the BaYMV 1(F1) whereas in ACP-ELISA the MAbs 3D12, 2C1, 6C3, 3D8, 3H1 and 2B8 readily detected this virus (Table 5). Nine of the Mabs were used to compare different isolates of BaYMV from Europe and Japan in IDAS-and ACP-ELISA. As a control these isolates were tested with WYMV and BaYMV polyclonals antibodies in DAS-and ACP-ELISA. All the isolates reacted strongly with the polyclonal antibodies (Table 6). In IDAS-ELISA, the British isolate and especially Belgian BaYMV 2 and the two Japanese isolates of this virus were recognized by the MAb 6C3. The MAbs 3D8, 3H1, 1F2, 3C10 and 1G5 also recognized the two Japanese strains whereas MAb 3H9 only recognized the strain BaYMV I-1. In ACP-ELISA, all the isolates of BaYMV were detected strongly by 6C3 and weakly by 3D8 and 3H1. The isolates of British and Belgian BaYMV 2 were not recognized by Mab 2B8.

Table 3. Detection of WYMV (F) isolates with MAbs and PAb in IDAS-DAS and ACP-ELISA

ELISA			Isolates[a] 1[a]	2	3	4	Healthy control
IDAS		6C3	**0.789** ± 0.065[b]	**1.047** ± 0.065	**0.824** ± 0.078	**0.568** ± 0.062	0.059 ± 0.045
		3D8	**1.023** ± 0.077	**0.789** ± 0.078	**0.412** ± 0.069	0.101 ± 0.045	0.047 ± 0.034
	MAbs	3H1	**1.112** ± 0.082	**0.802** ± 0.089	**0.437** ± 0.042	0.152 ± 0.074	0.069 ± 0.035
		2B8	**0.982** ± 0.186	**0.605** ± 0.075	**0.291** ± 0.025	0.076 ± 0.048	0.045 ± 0.015
		3C10	**1.145** ± 0.076	**0.895** ± 0.048	**0.301** ± 0.039	0.099 ± 0.065	0.122 ± 0.048
		3H9	**0.351** ± 0.071	**0.700** ± 0.073	0.071 ± 0.075	0.124 ± 0.078	0.073 ± 0.068
		1G5	**0.364** ± 0.038	**0.487** ± 0.040	0.154 ± 0.057	0.159 ± 0.090	0.097 ± 0.058
DAS	PAb	WYMV	**1.187** ± 0.051	**1.235** ± 0.048	**0,879** ± 0.081	**0,785** ± 0.075	0.198 ± 0.047
ACP		6C3	**2.147** ±0.078	**2.152** ± 0.095	**2.215** ± 0.092	**2.512** ± 0.085	0.075 ± 0.036
		3D8	**2.887** ±0.115	0.111 ± 0.052	0.141 ± 0.052	0.071 ± 0.009	0.111 ± 0.073
		3H1	**2.801** ±0.065	0.155 ± 0.058	0.112 ± 0.079	0.150 ± 0.038	0.175 ± 0.045
	MAbs	2B8	**2.114** ±0.125	**1.421** ± 0.046	**1.085** ± 0.079	**0.408** ± 0.047	0.087 ± 0.063
		3C10	**2.625** ±0.106	0.078 ± 0.049	0.078 ± 0.080	0.099 ± 0.079	0.122 ± 0.034
		3H9	**0.405** ±0.112	0.087 ± 0.062	0.105 ± 0.101	0.089 ± 0.111	0.112 ± 0.085
		1G5	**0.354** ±0.078	0.265 ± 0.047	0.180 ± 0.099	0.189 ± 0.089	0.180 ± 0.065
	PAb	WYMV	**2.124** ±0.049	**2.047** ± 0.089	**2.258** ± 0.058	**2.154** ± 0.068	0.153 ± 0.053

a - Refer to table 1 for a description of isolates. Leaf extract (1w/ 10v in citrate buffer).

b - Absorbance at 405 nm values after 1 hour substrate - enzyme - incubated period for ACP-ELISA and 2 hours for IDAS-and DAS-ELISA (standard deviation for 2 repetitions and 2 replications).

Absorbance at 405 nm values equal or above two times the healthy control absorbance at 405 nm value is considered as positive.

Discussion

Twelve MAbs directed against WYMV (F1) reacted with this isolate in IDAS- and ACP-ELISA. However, using different WYMV isolates the reactivity of these antibodies varied depending on the system of detection and the isolates of this virus. In IDAS-ELISA, MAb 6C3 was the antibody which recognized all the French isolates. In this system of detection, Mabs 3D8, 3H1 2B8, 3C10, 3H9 and 1G5 showed variable behaviour compared to MAb 6C3. In ACP-ELISA the MAbs 6C3 and 2B8 recognized all the isolates. These results show that if the same epitopic motifs existed in the different French isolates of WYMV, they were not as well exposed in each of them and/or they had different affinities with some of these antibodies.

Both American isolates of WSSMV and Japanese isolate of WYMV reacted with the 10 MAbs tested. However, different reactivities of these MAbs were observed with these isolates compared to the French isolate F1. These differences could also be explained either by the different environment of these epitopes or possibly distinct amino acid sequences of their capsid proteins. The similar susceptibility of some wheat cultivars in the USA to WSSMV [Lommet *et al.*, 1986] and in France to WYMV (Lapierre, unpublished results) and the strong serological relationship between them suggests that they are different strains of the same virus.

Two categories of monoclonal antibodies could be defined based on their reactivity to the French isolates of BaYMV. In ACP-ELISA the MAbs 6C3, 3D8 and 3H1 were the only ones to recognize all these iso-

Table 4. Detection of WYMV (J) and WSSMV (USA1, USA2) with MAbs in IDAS-and DAS-ELISA

ELISA			Isolates[a]				
			WYMV[a1] (F1)	WYMV[a2] (J)	WSSMV[a2](USA1)	WSSMV[a2](USA2)	Healthy control
IDAS		6C3	**0.744** ± 0.035[c]	**1.053** ± 0.058	**0.776** ± 0.030	**0.868** ± 0.067	0.087 ± 0.014
		3D8	**1.145** ± 0.047	**1.704** ± 0.093	**0.409** ± 0.024	**0.509** ± 0.052	0.069 ± 0.012
		3H1	**1.126** ± 0.020	**1.230** ± 0.077	**1.114** ± 0.058	**1.222** ± 0.057	0.089 ± 0.032
		2B8	**1.212** ± 0.123	**0.776** ± 0.088	**0.772** ± 0.075	**0.690** ± 0.030	0.074 ± 0.022
		3C10	**1.009** ± 0.082	**0.518** ± 0.065	**1.089** ± 0.056	**1.234** ± 0.080	0.078 ± 0.082
	MAbs	4F12	**1.350** ± 0.089	**0.750** ± 0.065	**0.521** ± 0.010	**0.218** ± 0.090	0.090 ± 0.018
		1F2	**1.125** ± 0.052	**0.782** ± 0.092	**0.952** ± 0.052	**0.795** ± 0.002	0,08 ± 0.052
		3H9	**0.286** ± 0.078	**0.320** ± 0.067	**0.204** ± 0.101	**0.299** ± 0.067	0.087 ± 0.007
		5F4	**0.197** ± 0.058	**0.275** ± 0.066	**0.204** ± 0.030	**0.212** ± 0.020	0.078 ± 0.010
		1G5	**0.323** ± 0.086	**0.319** ± 0.055	**1.150** ± 0.075	**0.749** ± 0.071	0.078 ± 0.041
DAS	PAb[b]		**1.245** ± 0.093	**0.696** ± 0.078	**0.301** ± 0.070	**0.255** ± 0.047	0.069 ± 0.032

a - Refer to table 1 for a description of isolates (a1= 1w/ 10v, a2= 1w/100v of citrate buffer).

b - Polyclonal antibodies of WYMV

c - Absorbance at 405 nm values after 1 hour (standard deviation for 2 repetitions and 2 replications).

Absorbance at 405 nm values equal or above two times the healthy control absorbance at 405 nm value is considered as positive.

lates. The fact that this recognition only occurred in ACP-ELISA with french BaYMV isolates although it also occured in IDAS-ELISA with WYMV (F1) indicates that the same epitopes are exposed differently on the particles of the two viruses. The positive response of the polyclonal antibodies to BaYMV in DAS-ELISA, despite the substantially reduced reactivity because of their coupling to alkaline phosphatase, could be explained by a cooperation among the monoclonal antibodies represented in the WYMV polyclonal antiserum.

The motifs recognized by 6C3 exposed in native particles of WYMV (IDAS-ELISA) were also exposed in native particles of British and Belgian isolates of pathotype 2 and the two Japanese strains of BaYMV. The two Japanese isolates of BaYMV were recognized moreover by Mabs 3D8, 3H1, 1F2, 3C10 and 1G5 whereas BaYMV I-1 was also recognized by MAb 3H9. The reactivity of MAbs 1F2 and 3C10 with the two Japanese isolates of BaYMV and of 3H9 with one of them was unexpected. These two isolates are also distinguished with a monoclonal antibody to BaYMV (Hariri unpublished results). These MAbs interacted with the epitopes situated on the native particles of these 2 Japanese isolates.

Although the French isolates of BaYMV made it possible to distinguish 2 groups of MAbs, the behaviour of the foreign isolates of this virus increased the number of antibody groups to 4. The analysis of a larger range of European and Asian isolates having undergone a purification cycle and treated in the form of intact particles and capsid protein subunits would make it possible to determine their potential in characterizing different serotypes of this virus.

The amino acid sequences of the capsid protein of WSSMV from the south of France [Sohn *et al.*, 1994] and 3 isolates of BaYMV [Kashiwazaki *et al.*, 1990; Peerenboom *et al.*, 1992; Benediek *et al.*, 1993] share a high homology. The variable zones between these 2 viruses are situated all along this protein but especially on the first (N-terminal) third. Furthermore, the capsid protein of two isolates of BaYMV [(G and JII] only have nine different amino acids situated in the first 70 of the N terminal zone. The relationship between the sequences data and the interactions described here among the MAbs of WYMV and the different isolates of BaYMV were not elucidated. Neither the position of the epitopes on the N terminal zone nor the amino acid sequences of most of the isolates of BaYMV studied, in particular BaYMV (J) I-1, are known. If all the variable sequences of the capsid protein of the BaYMV isolates are situated in the N terminal zone, a sequence of this zone is recognized by 3H9 in WYMV (F1) and in BaYMV I-1.

Table 5. Reactivity of MAbs in IDAS- and ACP-ELISA with Ba YMV (F1)

	IDAS-ELISA				ACP-ELISA			
	Isolates[a]				Isolates			
MAbs	WYMV (F1)	Healthy control	BaYMV 1 (F1)	Healthy control	WYMV (F1)	Healthy control	BaYMV 1 (F1)	Healthy control
3D12	**0.803** ± 0.078[b]	0.075 ± 0.017	0.078 ± 0.032	0.078 ± 0.011	**>3** -	0.166 ± 0.056	**1.712** ± 0.105	0.051 ± 0.011
2C1	**0.798** ± 0.041	0.020 ± 0.022	0.100 ± 0.030	0.049 ± 0.052	**2.025** ± 0.090	0.068 ± 0.045	**1.480** ± 0.089	0.047 ± 0.036
6C3	**0.715** ± 0.058	0.179 ± 0.034	0.112 ± 0.008	0.135 ± 0.025	**1.982** ± 0.077	0.175 ± 0.036	**1.635** ± 0.020	0.114 ± 0.047
3D8	**0.812** ± 0.019	0.101 ± 0.075	0.031 ± 0.035	0.089 ± 0.018	**>3** -	0.104 ± 0.074	**1.845** ± 0.075	0.132 ± 0.065
3H1	**0.801** ± 0.026	0.112 ± 0.042	0.203 ± 0.028	0.089 ± 0.045	**2.865** ± 0.078	0.112 ± 0.056	**1.815** ± 0.039	0.098 ± 0.058
2B8	**0.781** ± 0.178	0.089 ± 0.028	0.040 ± 0.045	0.130 ± 0.052	**2.312** ± 0.032	0.077 ± 0.059	**1.278** ± 0.049	0.053 ± 0.103
1F2	**0.695** ± 0.029	0.068 ± 0.009	0.031 ± 0.039	0.058 ± 0.033	**2.242** ± 0.072	0.089 ± 0.045	0.061 ± 0.023	0.058 ± 0.058
3C10	**0.917** ± 0.045	0.051 ± 0.064	0.042 ± 0.025	0.047 ± 0.018	**2.780** ± 0.039	0.51 ± 0.036	0.089 ± 0.095	0.094 ± 0.024
4F12	**0.710** ± 0.035	0.035 ± 0.011	0.025 ± 0.024	0.060 ± 0.014	**1.812** ± 0.045	0.101 ± 0.032	0.017 ± 0.028	0.037 ± 0.036
3H9	**0.265** ± 0.085	0.051 ± 0.022	0.048 ± 0.037	0.065 ± 0.045	**0.205** ± 0.019	0.076 ± 0.019	0.068 ± 0.055	0.120 ± 0.054
1G5	**0.290** ± 0.078	0.075 ± 0.047	0.042 ± 0.018	0.072 ± 0.039	**0.201** ± 0.039	0.092 ± 0.010	0.020 ± 0.014	0.054 ± 0.011
5F4	**0.310** ± 0.039	0.033 ± 0.035	0.049 ± 0.080	0.048 ± 0.033	**0.510** ± 0.042	0.093 ± 0.042	0.221 ± 0.028	0.135 ± 0.010

a - Refer to table 1 for a description of isolates. Leaf extract (1w/ 10v in citrate buffer).

b - Absorbance at 405 nm values after 1 hour substrate - enzyme - incubated period for ACP-ELISA and 2 hours for IDAS-ELISA (standard deviation for 2 repetitions and 2 replications). Absorbance at 405 nm values equal or above two times the healthy control absorbance at 405 nm value is considered as positive.

Table 6. Detection of BaYMV isolates with MAbs in ACP- DAS- and IDAS-ELISA

		Isolates[a]									
	MAbs	BaYMV 1(F2)[a1]	BaYMV 1(F3)[a1]	BaYMV 2(F1)[a1]	BaYMV 2(F2)[a1]	BaYMV 2(G.B.)[a2]	BaYMV 2(B)[a2]	BaYMV I -1(J)[a2]	BaYMV II -1(J)[a2]	WYMV(F1)[a1]	Healthy control
	6C3	0.188 ± 0.047[b]	0.178 ± 0.042	0.199 ± 0.038	0.170 ± 0.030	**0.344** ± 0.119	**1.158** ± 0.061	**0.776** ± 0.052	**0.786** ± 0.077	**0.746** ± 0.075	0.210 ± 0.047
	3D8	0.059 ± 0.014	0.057 ± 0.018	0.102 ± 0.009	0.133 ± 0.011	0.169 ± 0.059	0.150 ± 0.068	**0.628** ± 0.039	**0.619** ± 0.055	**0.723** ± 0.045	0.089 ± 0.017
	3H1	0.067 ± 0.018	0.068 ± 0.009	0.091 ± 0.010	0.074 ± 0.040	0.166 ± 0.028	0.238 ± 0.142	**1.365** ± 0.088	**1.254** ± 0.070	**1.126** ± 0.059	0.078 ± 0.013
	2B8	0.059 ± 0.012	0.059 ± 0.012	0.062 ± 0.024	0.070 ± 0.055	0.062 ± 0.012	0.162 ± 0.131	0.139 ± 0.059	0.119 ± 0.027	**0.812** ± 0.079	0.058 ± 0.016
IDAS ELISA	1F2	0.063 ± 0.053	0.054 ± 0.017	0.139 ± 0.067	0.095 ± 0.053	0.048 ± 0.060	0.078 ± 0.087	**0.654** ± 0.065	**0.919** ± 0.045	**0.951** ± 0.079	0.089 ± 0.047
	3C10	0.061 ± 0.017	0.056 ± 0.015	0.118 ± 0.031	0.106 ± 0.037	0.053 ± 0.009	0.109 ± 0.111	**0.825** ± 0.066	**0.882** ± 0.076	**1.009** ± 0.082	0.058 ± 0.016
	4F12	0.071 ± 0.038	0.060 ± 0.042	0.065 ± 0.025	0.095 ± 0.064	0.053 ± 0.071	0.218 ± 0.099	0.058 ± 0.076	0.060 ± 0.034	**0.897** ± 0.058	0.058 ± 0.037
	3H9	0.063 ± 0.025	0.064 ± 0.033	0.057 ± 0.082	0.083 ± 0.042	0.059 ± 0.049	0.087 ± 0.065	**0.699** ± 0.054	0.080 ± 0.074	**0.725** ± 0.065	0.078 ± 0.039
	1G5	0.057 ± 0.014	0.053 ± 0.045	0.077 ± 0.057	0.065 ± 0.003	0.060 ± 0.057	0.168 ± 0.129	**0.266** ± 0.089	**0.258** ± 0.067	**0.323** ± 0.078	0.065 ± 0.029
	6C3	**1.348** ± 0.099	**>3** -	**0.730** ± 0.091	**0.830** ± 0.061	**0.457** ± 0.067	**0.340** ± 0.071	**1.954** ± 0.109	**2.054** ± 0.119	**2.555** ± 0.108	0.076 ± 0.015
	3D8	**0.280** ± 0.032	**0.854** ± 0.089	**0.238** ± 0.079	**0.241** ± 0.067	**0.167** ± 0.045	**0.147** ± 0.012	**0.987** ± 0.036	**0.897** ± 0.009	**2.893** ± 0.079	0.064 ± 0.010
	3H1	**0.198** ± 0.070	**0.643** ± 0.043	**0.251** ± 0.097	**0.264** ± 0.086	**0.173** ± 0.075	**0.241** ± 0.092	**0.963** ± 0.011	**0.987** ± 0.069	**2.692** ± 0.098	0.073 ± 0.029
	2B8	**0.939** ± 0.058	**1.077** ± 0.039	**0.960** ± 0.074	**0.715** ± 0.0118	0.100 ± 0.025	0.095 ± 0.031	**0.898** ± 0.107	**0.994** ± 0.099	**2.895** ± 0.128	0.085 ± 0.003
ACP ELISA	1F2	0.078 ± 0.032	0.089 ± 0.019	0.099 ± 0.035	0.089 ± 0.067	NT -	NT -	0.128 ± 0.141	0.088 ± 0.058	**2.694** ± 0.195	0.053 ± 0.033
	3C10	0.071 ± 0.041	0.119 ± 0.058	0.069 ± 0.036	0.087 ± 0.037	0.118 ± 0.065	0.057 ± 0.012	**0.721** ± 0.091	**0.740** ± 0.063	**2.775** ± 0.155	0.068 ± 0.014
	4F12	0.078 ± 0.023	0.098 ± 0.019	0.122 ± 0.055	0.010 ± 0.028	NT -	NT -	0.144 ± 0.069	0.073 ± 0.039	**1.950** ± 0.088	0.050 ± 0.025
	3H9	0.169 ± 0.037	0.157 ± 0.092	0.140 ± 0.083	0.163 ± 0.058	0.150 ± 0.032	0.147 ± 0.058	0.145 ± 0.082	0.113 ± 0.036	**0.760** ± 0.087	0.069 ± 0.017
	1G5	0.127 ± 0.066	0.138 ± 0.053	0.148 ± 0.043	0.129 ± 0.023	0.132 ± 0.075	0.122 ± 0.032	0.178 ± 0.021	0.092 ± 0.017	**0.259** ± 0.036	0.069 ± 0.099
	PAb[c]	**1.983** ± 0.058	**2.110** ± 0.071	**1.615** ± 0.082	**1.876** ± 0.149	**0.780** ± 0.011	**1.258** ± 0.072	**1.912** ± 0.100	**1.956** ± 0.049	**1.950** ± 0.044	0.067 ± 0.015
DAS ELISA	PAb[c]	**1.880** ± 0.045	**1.999** ± 0.035	**1.980** ± 0.105	**1.794** ± 0.065	**1.954** ± 0.082	**1.730** ± 0.011	**1.792** ± 0.074	**1.453** ± 0.058	**1.244** ± 0.059	0.078 ± 0.012

a - Refer to table 1 for a description of isolates. Leaf extract (a1= 1w/10v, a2= 1w/ 100v in citrate buffer).

b - Absorbance at 405 nm values after 1 hour substrate - enzyme - incubated period for ACP-ELISA and 2 hours for IDAS-ELISA (standard deviation for 2 repetitions and 2 replications).

c - Polyclonal antibodies to BaYMV used to test BaYMV samples and healthy barley and to WYMV to test WYMV and healthy wheat.

Absorbance at 405 nm values equal or above two times the healthy control absorbance is considered as positive.

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