Comparison of DAS-ELISA and Enzyme Amplified ELISA for Detection of *Wheat Dwarf Virus* in Host Plants and Leafhopper Vectors*

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Abstract

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The sensitivity of double-sandwich ELISA (DAS-ELISA) and enzyme amplified ELISA (AMP-ELISA) to detect wheat and barley strains of *Wheat dwarf virus* (WDV) in host plants and its vector *Psammotettix alienus* Dahlb. was compared. The end-points of virus dilution in the sap from leaves proved that AMP-ELISA had a higher sensitivity to detect both virus strains in wheat and barley leaves. DAS-ELISA with short-term (2.5 h) substrate incubation failed to detect the WDV strains in the extract from vector nymphs of the 1st to 2nd instar. A lower sensitivity of this method was also observed after long-term (17 h) incubation. AMP-ELISA was a reliable technique for detection of virus strains in some young nymphs. DAS-ELISA with short-term substrate incubation had a lower sensitivity to detect both strains in the leafhopper nymphs of the 4th and 5th instar. Virus detection in these nymphs was successful using DAS-ELISA with long-term incubation and by AMP-ELISA. Both methods reliably detected WDV strains in the extract from *P. alienus* adults. As indicated by absorbance values, AMP-ELISA appeared to be more sensitive than DAS-ELISA only with short-term substrate incubation.

Key words: wheat dwarf virus; detection; DAS-ELISA; AMP-ELISA; wheat; barley; leafhoppers

A double-sandwich immunoenzymatic assay (DAS-ELISA), originally described by CLARK and ADAMS (1977), is frequently used as a reliable method for the detection of plant viruses. However, it is not sensitive enough for virus detection in materials with a low virus concentration such as vectors. Enzyme amplified immunoenzymatic assay (AMP-ELISA), described by SELF (1985) and STANLEY *et al.* (1985), is one of the modifications increasing its sensitivity. This modification was used for the first time by TORRANCE (1987), who reported its higher sensitivity for *Barley yellow dwarf virus* (BYDV) detection in plant material and aphid vectors. Detection of BYDV, *Beet mild yellowing virus* (BMYV) and *Beet western yellows virus* (BWYV) using AMP-ELISA was studied by RABENSTEIN and SCHLIEPHAKE (1996), who stated that it was more sensitive by one or two times than DAS-ELISA. Similar results with BMYV detection in aphids *Myzus persicae* (Sulz.) were reported by POLÁK (1998). LINDSTEN (1985) successfully detected WDV in sap of host plants and in leafhoppers of *Psammotettix alienus* by conventional DAS-ELISA.

The aim of this paper is to compare sensitivity and reliability of DAS-ELISA and AMP-ELISA for detection of wheat and barley WDV strains in host plants and the vector *P. alienus*.

MATERIAL AND METHODS

A wheat strain (RuW) and a barley strain of WDV (RuB) were used in comparative trials. The wheat strain was maintained and multiplied in the spring wheat cv. Jara, the barley strain in spring barley cv. Heran. Plants used for ELISA were infected at the stage of two leaves by leafhoppers of *P. alienus*. Younger leaves were sampled for WDV detection 4 weeks after inoculation. The samples were frozen to –20°C and stored until use. The sap from leaves was extracted with a jaw press, centrifuged at 7000 g for 10 min and diluted with extraction buffer using an arithmethical series at ratios 1:16 to 1:262 144.

Virus-free leafhoppers of *P. alienus* were reared on healthy plants of the winter wheat cv. Vlasta under greenhouse conditions. Acquisition feeding of the vectors on

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a source of infection lasted 3 d. Leafhopper adults were used in the first trial (Table 2). Tests of their infectivity were carried out with young plants of spring wheat after 3 d of acquisition and 5 d of inoculation feeding. Then the vectors were frozen to –20°C and stored until immunoenzymatic assays. Only infectious individuals were selected for tests. In the second trial, WDV was detected in nymphs of the 1^{st}–2^{nd} and the 4^{th}–5^{th} instar and in young adults. Following a 3 d acquisition feeding, leafhoppers were caged on young winter wheat plants for 5 d. Then they were frozen and stored at –20°C. The individual leafhoppers were homogenized in china mortars with 0.3 ml of extraction buffer. The homogenate was centrifuged at 7000 g for 10 min. One half of the extract was used for DAS-ELISA, the other half for AMP-ELISA.

All trials were carried out with commercial polyclonal antibodies to WDV (Sanofi) and GIBCO BRL ELISA amplification system (Life Technologies). Routine procedures and techniques were used for DAS-ELISA (CLARK & ADAMS 1977). Procedures according to STANLEY *et al.* (1985) were employed in trials with AMP-ELISA. Short intervals (1 h, 2.5 h) and long intervals (17 h) of substrate incubation were applied for WDV detection by DAS-ELISA. The amplified variant consisted of substrate incubation for 15 and 20 min, and amplification for 5 and 20 min (Tables 1–3). The extracts were tested in pairs of wells.

RESULTS AND DISCUSSION

WDV Detection in Host Plant Sap

Comparative trials were conducted to study the sensitivity of DAS- and AMP-ELISA for the detection of wheat and barley WDV strains in the leaf sap of their hosts; the dilution end point of both virus strains determined in a dilution series was used as a criterion of sensitivity of these methods (Table 1). Wheat and barley strains of the virus were detected in the sap of their hosts by DAS-ELISA after 1 h substrate incubation at a dilution ratio of 1:4096. Absorbance values of the wheat and barley strains were 0.110 and 0.099, respectively. After 17 h substrate incubation both strains were reliably detected at a sap dilution of 1:32 768 (absorbance 0.129 and 0.096). The dilution end point of wheat and barley sap was 1:131 072 in the AMP-ELISA system. These results indicate a higher sensitivity of AMP-ELISA than of DAS-ELISA to detect both WDV strains.

When DAS-ELISA (variant A) was used, the absorbance value of initial dilution levels for the sap from leaves of healthy wheat and barley plants approximated zero. But an increased absorbance level was recorded in AMP modification while assaying leaf saps of both cereals. The respective values for wheat and barley were 0.089 and 0.055. A similar result was reported by TORRANCE (1987) for BYDV detection in oat sap.

Table 1. Comparison of DAS-ELISA with AMP-ELISA in detection of WDV wheat and barley strains in sap of infected host plants

Sap dilution	Wheat strain						Barley strain					
	DAS-ELISA				AMP-ELISA		DAS-ELISA				AMP-ELISA	
	A		B				A		B			
	I	H	I	H	I	H	I	H	I	H	I	H
16	1.641	0.013	1.861	0.053	1.217	0.089	0.826	0.017	1.851	0.064	1.252	0.055
64	1.637	0	1.899	0	1.251	0.010	0.773	0	1.843	0	1.236	0.014
256	1.265	0	1.868	0	1.365	0	0.542	0	1.845	0	1.234	0
1 024	0.479	0	1.869	0	1.253	0	0.358	0	1.869	0	1.443	0
2 048	0.240	0	1.823	0	1.269	0	0.194	0	1.695	0	1.241	0
4 096	0.110	0	1.289	0	1.034	0	0.099	0	1.203	0	1.288	0
8 192	0.055	0	0.680	0	0.675	0	0.052	0	0.660	0	0.842	0
16 384	0.019	0	0.275	0	0.464	0	0.026	0	0.323	0	0.405	0
32 768	0.012	0	0.129	0	0.192	0	0	0	0.096	0	0.213	0
65 536	0	0	0.035	0	0.147	0	0	0	0.010	0	0.078	0
131 072	0	0	0.041	0	0.153	0	0	0	0	0	0.107	0
262 144	0	0	0.008	0	0.046	0	0	0	0	0	0.013	0

DAS-ELISA: A 405 values measured after incubation of substrate for 1 h in variant A, 17 h in variant B

AMP-ELISA: A 490 values, substrate added for 20 min, amplifier for 20 min

H = sap of healthy plants; I = sap of infected host plants

Higher absorbance values were determined in sap from wheat leaves than in sap from barley leaves at all levels of the dilution series after short- and long-term substrate incubation by DAS-ELISA as well as by AMP-ELISA. This appears to indicate a higher relative concentration of virus protein in wheat leaves, although it is possible that the affinity of commercial antibodies and the barley WDV strain might be lower.

WDV Detection in Vectors

Table 2 shows the results of comparative trials aimed at WDV detection in adults of *P. alienus* with biologically verified infectivity. They indicate that wheat and barley strains can reliably be detected in the vectors by both methods. This is shown by the relatively high absorbance values recorded in all experimental variants, although the values for extracts from individual leafhoppers fluctuated within a wide range. AMP-ELISA appeared to be more sensitive than conventional DAS-ELISA only after short-term (2.5 h) substrate incubation, but not with long-term (17 h) incubation. These conclusions are based on average absorbance values recorded in 20 vectors. Absorbance values for detection of the wheat strain by DAS-ELISA were 0.645 after short incubation and 0.861 after long incubation, and 1.094 for detection by AMP-ELISA. The respective average values of absorbance for detection of the barley strain were 0.465, 1.519 and 0.888. These data also document that vectors which were able to transmit the wheat strain had higher absorbance values than leafhoppers which transmitted the barley strain in the biological assay. This might be caused by a lower affinity of the barley strain to commercial antibodies, or by the lower relative virus content in leafhoppers.

Detection of WDV at different developmental stages of *P. alienus* by DAS and AMP-ELISA was studied in an-

Table 2. Comparison of DAS-ELISA with AMP-ELISA in detection of WDV wheat and barley strains in infectious individual adults of *Psammotettix alienus*

No. of *P. alienus*	Wheat strain			Barley strain		
	DAS		AMP	DAS		AMP
	A	B		A	B	
1	0.619	2.030	1.240	0.361	1.378	0.962
2	0.434	1.542	0.775	0.224	0.909	0.602
3	0.235	0.955	0.477	0.549	1.845	1.106
4	1.302	2.125	1.449	0.416	1.546	0.876
5	0.729	2.192	1.118	0.742	2.032	1.271
6	0.483	1.674	1.099	0.650	1.969	1.135
7	0.639	1.964	1.180	0.889	2.215	1.377
8	0.832	2.185	1.362	0.331	1.279	0.944
9	0.360	1.373	0.607	0.546	1.856	0.962
10	0.843	2.111	1.351	0.905	2.202	1.242
11	0.896	2.185	1.455	0.179	0.686	0.437
12	0.598	1.914	1.033	0.513	1.782	1.013
13	0.798	2.144	1.192	0.311	1.211	0.521
14	0.406	1.497	0.801	0.429	1.566	0.863
15	0.824	2.138	0.990	0.182	0.733	0.388
16	0.391	1.465	0.914	0.404	1.498	1.015
17	0.529	1.779	1.053	0.682	2.034	1.236
18	0.599	1.914	1.047	0.469	1.634	0.890
19	0.783	2.134	1.362	0.397	1.481	0.660
20	0.599	1.905	1.175	0.122	0.528	0.257
Control [b]	0.013	0.051	0.016	0.015	0.056	0.017

DAS-ELISA: incubation substrate for 2.5 h in variant A or 17 h in variant B
AMP-ELISA: substrate added for 15 min, amplifier for 5 min

[b]mean of 10 virus-free *P. alienus*

Table 3. Comparison of DAS-ELISA with AMP-ELISA in the detection of WDV wheat and barley strains in individual *Psammotettix alienus* of different development stages

Stage of *P. alienus*	No.	Wheat strain			Barley strain		
		DAS		AMP	DAS		AMP
		A	B		A	B	
Nymphs 1st–2nd instar	1	0.010	0.039	0.050	0.024	0.090	0.196
	2	0.020	0.032	0.037	0.002	0.008	–0.019
	3	0.003	0.013	0.020	0.014	0.013	0.012
	4	0.010	0.028	0.024	0.012	0.063	0.106
	5	0.007	0.012	0.024	0.011	0.023	0.045
	6	0.005	0.009	0.001	0.033	0.080	0.113
	7	0.028	0.134	0.232	0.008	0.033	0.075
	8	0.015	0.057	0.111	0	0	–0.012
	9	0.001	0.008	0.004	0.027	0.115	0.192
	10	0	0	0.011	0	0	0
	11	0	0	0	0.009	0.018	0.028
	12	0	0.007	0.011	0	0.016	0.035
Nymphs 4th–5th instar	1	0.172	0.907	1.096	0.078	0.525	
	2	0.069	0.114	0.123	0.055	0.313	0.403
	3	0.044	0.224	0.363	0.023	0.116	0.127
	4	0.021	0.133	0.123	0.062	0.332	0.458
	5	0.209	1.099	1.178	0.150	0.811	1.028
	6	0.419	1.857	1.352	0.146	0.770	1.282
	7	0.254	1.390	1.244	0	0.022	0.048
	8	0.233	1.269	1.283	0.084	0.480	0.673
	9	0.123	0.683	0.894	0.046	0.275	
	10	0.045	0.285	0.524	0.105	0.632	0.477
	11	0.605	2.074	1.370	0.015	0.099	0.138
	12	0.222	1.160	1.321	0.031	0.207	0.119
Imago	1	0.149	0.830	1.038	0.125	0.744	0.709
	2	0.400	1.741	1.336	0.164	0.921	1.112
	3	0.134	0.777	0.820	0.173	0.918	1.024
	4	0.115	0.640	0.754	0.137	0.844	0.912
	5	0.294	1.404	1.332	0.065	0.355	0.497
	6	0.203	1.124	1.272	0.049	0.253	0.248
	7	0.107	0.601	0.743	0.239	1.340	1.078
	8	0.484	1.923	1.359	0.166	0.953	1.086
	9	0.266	1.415	1.296	0.541	2.009	1.283
	10	0.256	1.301	1.227	0.216	1.200	1.249
	11	0.171	0.964	0.847	0.386	1.826	1.346
	12	0.294	1.442	1.131	0.246	1.406	1.332
	13	0.406	1.867	1.366	0.328	1.638	1.291
	14	0.090	0.477	0.328	0.451	1.898	1.316
Control[b]		0.013	0.051	0.016	0.015	0.056	0.017

DAS-ELISA: incubation substrate for 2.5 h in variant A or 17 h in variant B
AMP-ELISA: substrate added for 20 min, amplified for 20 min

[b]mean of 10 virus-free *P. alienus*

other comparative trial (Table 3). The results indicate that the two virus strains were not detected in any nymph of the 1st and 2nd instar by DAS-ELISA with short-term substrate incubation. Virus concentrations in young nymphs of the vector are obviously so low that the virus is hardly detectable by this method. The virus was detected in some nymphs by DAS-ELISA with long-term incubation. The use of AMP-ELISA allowed to detect the virus even in those individuals where DAS-ELISA had failed. In this case AMP-ELISA appeared to be more sensitive than DAS-ELISA, even at longer substrate incubation. Reliable detection of the wheat strain was feasible in 67% of the 4th–5th instar nymphs by DAS-ELISA with short substrate incubation, while with the barley strain it succeeded in 25% of the individuals. The wheat strain was detected in all nymphs by DAS-ELISA with long substrate incubation, and the barley strain in 92% of the individuals. Both strains were detected in all vectors by AMP-ELISA. When *P. alienus* imagos were assayed, the virus was detected by both methods in all cases. Similar to the results of the preceding trial (Table 2), AMP-ELISA appeared to be more sensitive than DAS-ELISA only with short-term substrate incubation. When long-term substrate incubation was used, DAS-ELISA was more sensitive than AMP-ELISA.

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Souhrn

VACKE J., CIBULKA R. (2000): **Srovnání DAS-ELISA a enzymem amplifikované ELISA při detekci viru zakrslosti pšenice v hostitelských rostlinách a v přenašečích**. Plant Protect. Sci, **36**: 41–45.

Byla srovnávána citlivost ELISA dvojitého sendviče (DAS-ELISA) s enzymem amplifikovanou ELISA (AMP-ELISA) při stanovení pšeničného a ječného kmene viru zakrslosti pšenice (WDV) v hostitelských rostlinách a vektorech *Psammotettix alienus* Dahlb. Větší citlivost detekce obou kmenů viru byla zjištěna v listech pšenice a ječmene pomocí AMP-ELISA. Dokládají to stanovené konečné body zředění viru ve šťávě z listů těchto obilnin. V extraktu z vektorů 1.–2. instaru se nepodařilo pomocí DAS-ELISA s krátkodobou (2,5h) inkubací substrátu detekovat žádný z kmenů WDV. Nižší citlivost této metody byla zaznamenána i v případě dlouhodobé (17h) inkubace. Spolehlivě byly kmeny viru v některých mladých nymfách stanoveny pomocí AMP-ELISA. Menší citlivost detekce kmenů v nymfách 4.–5. instaru byla zaznamenána v DAS-ELISA s krátkodobou inkubací substrátu, při dlouhodobé inkubaci a v AMP-ELISA byla detekce viru úspěšná. V extraktu z dospělých jedinců *P. alienus* byly kmeny WDV spolehlivě stanoveny oběma metodami. Podle naměřených absorbancí se amplifikovaná ELISA jevila jako citlivější než DAS-ELISA pouze v případě krátkodobé inkubace substrátu.

Klíčová slova: virus zakrslosti pšenice; DAS-ELISA; AMP-ELISA; detekce; pšenice; ječmen; *Psammotettix alienus*

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