Reaction of Wheat Varieties to Infection with Barley Yellow Dwarf Virus and Prospects for Resistance Breeding

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Abstract: The reaction of winter and spring wheat to infection with barley yellow dwarf virus (BYDV-PAV) was evaluated in three-year small-plot field trials on 71 wheat varieties registered in the Czech Republic and at two locations for two years on 63 selected potential sources of resistance. Disease symptoms (VSS) were visually recorded using a 0-9 scale and the percent reduction of grain weight per spike (GWS-R) was measured on twenty plants per plot. The evaluation showed that among the registered varieties of winter and spring wheat no variety had a high resistance to BYDV (with VSS lower than 3.5). GWS-R ranged between 24% and 60%. Higher variability in VSS was detected for the registered varieties of spring wheat compared to winter wheat. Among the registered varieties of winter wheat, Saskia, Rialto, Meritto, Rexia, and Svitava, as well as the spring wheat Leguan, received the best long-term evaluations. The highest level of resistance was detected for the PSR 3628 line (a hybrid of wheat and couch-grass), but in connection with a low agronomic value. The WKL91-138 line of spring wheat and some varieties (lines) with the detected moderate level of resistance, in particular, could offer good prospects for use in breeding. The presence of the Bdv2 gene was expressed only in the reduction of virus content on the 11th day after inoculation. Nevertheless, genotypes carrying this gene were evaluated in field trials as susceptible or very susceptible to infection with the Czech PAV isolate. Similarly, the presence of the Bdv1 gene detected with the help of WMS130 marker was no assurance of an increased level of resistance to BYDV. Hybridological analyses of crosses with the WKL91-138 line showed a polygenic nature of inheritance. Thus, the marker-assisted selection does not obviously promise success without a focus on detecting a larger number of QTLs.

Keywords: barley yellow dwarf; BYDV; Bdv1; Bdv2; resistance sources; breeding; wheat

The barley yellow dwarf virus (BYDV) is a serious threat to the cultivation of cereals in many parts of the world, including Central Europe. It is a luteovirus localised in the phloem and it is transmitted persistently, with variable effectiveness, by many kinds of aphids (SLYKHUIS 1967), of which Rhopalosiphum padi L., Sitobion avenae F. and Metopolophium dirhodum Walker are the most important in the Czech Republic. BYDV infects wheat with varying intensity each year, and the last mass outbreak was recorded in the

Czech Republic in 2002 (according to data from the State Phytosanitary Administration).

The barley yellow dwarf disease is caused by a group of related single-stranded RNA viruses assigned to the genus luteovirus (Barley yellow dwarf virus spp. PAV, PAS, MAV, GAV) or polerovirus (Cereal yellow dwarf virus-RPV) or unassigned to a genus (BYDV-SGV, BYDV-RMV, and BYDV-GPV) in the *Luteoviridae* family. Previously, only PAV isolates were identified in the Czech Republic on the basis of serological detection, but the presence

of PAS species has recently been detected in this territory (Kundu 2008).

The most effective and sustainable control method is the use of genetic resistance/tolerance to the virus complex (Henry et al. 2002). Singh et al. (1993) reported that the tolerance of wheat varieties such as Condor (Anza sister line) and nine other wheats was due to Bdv1, a common, partially effective and partially dominant gene. Tolerance to BYDV based on *Bdv1* might originate from the Brazilian spring wheat variety Frontana (parent of the cultivar Maringá). In field studies, this gene was found to be linked to genes Lr34 and Yr18 conferring adult plant resistance to leaf and yellow rust, respectively, and it was later located on the short arm of chromosome 7D (SINGH et al. 1993). However, Ayala et al. (2002) found only minor QTL (quantitative trait loci) explaining just 7% of the phenotypic variation for this 7D chromosome region. The large number of QTLs having mostly small effects found in this study and the continuous distribution of all evaluated traits confirmed the polygenic nature and complexity of BYDV tolerance in wheat. The BYDV resistance gene on 7Ai1(7X) coming from *Thinopyrum intermedium* was first identified in the disomic addition line L1 and was located on the long arm (Brettell et al. 1988). This BYDV resistance gene was designated Bdv2. Using L1 as the resistance source, a series of wheat - Th. intermedium 7D-7Ai1 translocation lines carrying the *Bdv2* gene (called TC lines) was developed (BANKS et al. 1995) and later PCR markers linked to this BYDV resistance gene were developed (Zhang et al. 2004). The expression of Th. intermedium-derived BYDV resistance in elite bread wheat backgrounds was studied by AYALA et al. (2001). It was found that some materials resistant to virus multiplication were sensitive to virus infection in the field.

The aim of this work was to assess the level of resistance to BYDV in varieties of winter and spring wheat currently registered in the Czech Republic and to assess the possibility of using detected sources of resistance in breeding wheat for resistance to this disease.

MATERIAL AND METHODS

The reaction of winter and spring wheat varieties to infection with BYDV was evaluated in small-plot field trials during 2004–2008. At the

Prague-Ruzyně location, a total of 55 winter wheat varieties and 16 spring wheat varieties registered in the Czech Republic were evaluated in three-year trials. Characteristics of the examined varieties are available on the website of the Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic: http://www.ukzuz.cz/.

Potential sources of resistance were evaluated in field infection trials over a two-year period at two locations (Prague-Ruzyně and Stupice). They partly included lines obtained from abroad (USA, Poland, Chile) with resistance documented for natural occurrences of BYDV, as well as current varieties and advanced breeding lines of the company Selgen a.s. that had shown an acceptable level of resistance in previous trials on BYDV tolerance. Moreover, lines created by crossing translocated lines and cultivated wheat (wheat \times Thinopyrum intermedium) that are carriers of the Bdv2 resistance gene (CIMMYT-ALME2YDRES materials and TC14-290E and TC14-290J lines) were included. The presence of these genes was confirmed on a molecular level. In total, 22 sources of resistance for winter wheat and 41 sources of resistance for spring wheat were evaluated.

Field infection trials carried out according to methods described by VACKE et al. (1996) had both infection variants and control (uninfected) variants. The plants were grown on two-row plots 1 m long with two replications (plant spacing: 6 × 22 cm). Infection and control variants were arranged in two separate blocks. To facilitate the evaluation of disease attack, the infected tworow plots (randomly arranged within a block) were placed in close proximity to uninfected plots (Figures 1 and 2). At the beginning of the tillering stage, infection with the PAV strain of BYDV was carried out by means of Rhopalosiphum padi aphids obtained from greenhouse rearings. Their inoculation suction periods lasted 5-7 days; the aphids were then killed by an insecticide. Applications of insecticides and fungicides during the vegetation period provided protection against undesirable viral and fungal diseases. To determine whether plants were infected with BYDV, enzyme-linked immunosorbent assays (ELISA) were performed according to CLARK and ADAMS (1977), when needed. The evaluation of disease attack was performed at the phase of full flowering (visual symptom score - VSS), with the use of a 0-9 scale (0 being without symptoms) developed by Schaller and Qualset (1980). After harvest,

20 randomly selected plants from close stands in each plot of the infected (I) and control variants were evaluated for grain weight per spike (GWS) to determine the percent reduction of GWS due to infection (GWS-R). The UNISTAT 5.0 package (UNISTAT, Ltd., London, UK) was used for statistical analyses of original data and STATISTICA package (StatSoft, Inc., Tulsa, USA) for graphics.

The presence of the *Bdv1* gene was detected indirectly using a WMS130 marker (Suenaga *et al.* 2003), which detects the presence of the *Lr34* resistance gene. The reason for applying this marker was the fact that, as reported by Singh *et al.* (1993), the *Bdv1* gene is closely linked with the *Lr34* and *Yr18* genes.

The *Bdv2* gene was detected using three published markers: BYAgi (STOUTJESDIJK *et al.* 2001), SCgpl (ZHANG *et al.* 2004) and Xgwm37 (AYALA *et al.* 2001).

The effect of the resistance gene Bdv2 was also verified using a semi-quantitative ELISA technique, following the basic method described in detail by Šíp et~al.~(2006). Seedling plants were inoculated at the 3-leaf stage by means of Rhopalosiphum~padi aphids, and a sample of the youngest sections of plant leaves was taken on the 11^{th} day after infection for immunochemical analysis. Nine genotypes carrying the Bdv2 gene (TC14290E, TC14290J, CIMMYT 227, CIMMYT 231, CIMMYT 234, CIMMYT 229, CIMMYT 225, CIMMYT 228, CIM

MYT 233) and seven non-*Bdv2* genotypes (Jara, Anza, Leguan, SG-S26-98, SG-S45-98, SG-S604-96, WKL91-138) were evaluated. Each genotype was evaluated in six replications. The relative virus content was assessed according to the measured absorbance (405 nm).

The study also included an analysis of progenies of crosses with the highly resistant line WKL91-138 and TC14-290E (carrier of *Bdv2* gene). The analysis was conducted for three crosses of spring wheat: Jara × WKL91-138, Leguan × WKL91-138 and Leguan × TC14-290E. The progeny of randomly selected F2 plants (for crosses with the line WKL91-138) and *Bdv2* gene carrying plants (for the cross with TC14-209E) were tested in F₃ generation for resistance to BYDV-PAV in field infection trials according to the method described above. In the case of Leguan/TC14-290E, the dominant DNA markers BYAgi (STOUTJESDIJK *et al.* 2001) and SCgp1 (ZHANG *et al.* 2004) were used in selecting plants.

RESULTS AND DISCUSSION

Evaluation of resistance to BYDV for registered varieties of winter and spring wheat

The attack of BYDV (VSS) related especially to colour changes of leaves (yellowing) and reduction

Table 1. *F* values from analyses of variance and % variation (%var) for attack of BYDV (VSS) and reduction of grain weight per spike (GWS-R) in 2 types of experiments with the registered winter and spring wheat varieties

Source of variation —		Series 1		Series 2				
	df	F value*	%var	df	F value*	%var		
VSS								
Year (Y)	2	267.83	33.62	2	31.60	10.92		
Variety (V)	55	7.55	26.08	13	30.33	68.14		
$Y \times V$	110	4.31	29.75	26	3.04	13.67		
Residual	168		10.55	42		7.26		
GWS-R								
Year (Y)	2	232.01	42.93	2	30.30	15.70		
Variety (V)	55	4.05	20.63	13	13.26	44.63		
$Y \times V$	110	2.05	20.88	26	4.28	28.83		
Residual	168		15.54	42		10.88		

Series 1 = winter wheat varieties - Ruzyně location, 2004-2006

Series 2 = spring wheat varieties –Ruzyně location, 2004, 2006 and 2008

^{*}All F values are statistically significant at P = 0.001

in straw length and tillering in the infected plants. By determining the percent reduction of grain weight per spike (GWS-R), the effect of infection on this yield character was assessed. The effects of variety and environment on the variability of the VSS and GWS-R in trials with winter and spring wheat varieties are shown in Table 1. The significant effects of genotype, environment and interaction of genotypes with the environment on both traits are evident for both winter and spring

wheat from the ANOVA results. The analysis of the share of individual sources of variability in overall variability in the trial showed a highly predominant role of year for winter wheat, while for spring wheat the effect of genotype on both traits significantly outweighed both the year and the genotype by year interaction components. Genotype by year interaction was also a significant source of variation (in winter wheat this effect was similar to the effect of variety).

Table 2. Average attack of BYDV (VSS: 0-9; 0 = without symptoms), reduction of grain weight per spike (GWS-R) and grain weight per spike after infection (GWS-I) in the registered winter wheat varieties obtained from three-year trials (Prague-Ruzyně: 2004-2006)

Variety	Origin	NGG	GWS-R GWS-I		37		N.C.C.	GWS-R	GWS-I
		VSS	(%)	(g)	Variety	Origin	VSS	(%)	(g)
Saskia	CZE	3.9 a	43	1.03	Bill	DEU	5.0 abc	49	1.14
Rialto	GBR	3.9 a	48	1.28	Vlasta	CZE	5.0 abc	54	1.12
Corsaire	FRA	4.0 ab	40	1.27	Biscay	GBR	5.1 abc	41	1.32
Sulamit	CZE	4.0 ab	46	1.17	Ludwig	AUT	5.1 abc	48	1.11
Athlet	DEU	4.0 abc	58	0.97	Globus	DEU	5.1 abc	53	1.20
Simila	CZE	4.2 abc	60	0.97	Trend	DEU	5.2 abc	49	1.20
Svitava	CZE	4.2 abc	36	1.38	Alibaba	DEU	5.2 abc	59	1.10
Clarus	DEU	4.2 abc	44	1.17	Ilias	NLD	5.3 abc	57	0.98
Karolinum	CZE	4.3 abc	42	1.26	Niagara	CZE	5.3 abc	36	1.31
Windsor	DEU	4.3 abc	43	1.28	Bruneta	CZE	5.3 abc	45	1.05
Astela	SVK	4.3 abc	44	1.18	Brea	CZE	5.3 abc	46	1.09
Meritto	CZE	4.3 abc	47	1.36	Batis	DEU	5.3 abc	53	1.04
Rexia	SVK	4.4 abc	32	1.35	Boka	CZE	5.4 abc	35	1.41
Clever	GBR	4.5 abc	44	1.12	Raduza	CZE	5.4 abc	56	0.90
Sparta (resistant check)	CZE	4.5 abc	46	1.05	Alka	CZE	5.4 abc	56	0.88
Sultan	CZE	4.6 abc	55	1.05	Semper	NLD	5.4 abc	60	0.84
Sepstra	DEU	4.6 abc	51	1.01	Ebi	DEU	5.5 abc	53	1.07
Caphorn	GBR	4.8 abc	44	1.26	Šárka	CZE	5.5 abc	57	0.94
Nela	CZE	4.8 abc	46	0.99	Estica	NLD	5.6 abc	55	1.00
Ritmo	NLD	4.8 abc	50	0.97	Rapsodia	GBR	5.6 abc	55	0.88
Record	DEU	4.8 abc	50	1.08	Mladka	CZE	5.6 abc	56	1.05
Complet	DEU	4.8 abc	56	1.07	Contra	DEU	5.7 abc	58	0.87
Darwin	GBR	4.8 abc	56	1.11	Hedvika	NLD	5.8 bc	38	1.24
Akteur	DEU	4.9 abc	50	1.24	Vlada (susceptible check)	CZE	5.8 bc	52	0.88
Solara	SVK	4.9 abc	51	0.98	Elpa	DEU	5.8 bc	54	1.05
Rheia	CZE	4.9 abc	56	1.01	Drifter	DEU	5.8 bc	60	0.99
Banquet	CZE	5.0 abc	43	1.25	Apache	FRA	5.8 c	57	0.89
Cubus	DEU	5.0 abc	47	1.15	Average		4.9	49	1.11

The differences in the response of winter and spring wheat varieties registered in the Czech Republic to artificial inoculation with BYDV are shown in Tables 2 and 3. For winter wheat, the average VSS value ranged between 3.9 and 5.8 and GWS-R from 32% to 60%. It is evident from Table 2 that differences in the resistance of registered varieties of winter wheat are difficult to demonstrate in view of yearly variations. Based on the results of a multiple comparison, only the Saskia and Rialto varieties differed significantly from the relatively more susceptible Hedvika, Vlada, Elpa, Drifter and Apache varieties in their degree of resistance judged on the basis of the symptomatic evaluation. There was a significant positive correlation between VSS and GWS-R both in winter wheat (r = 0.37, P = 0.0019) and spring wheat (r = 0.55, P = 0.0038), but differences between the display of symptoms and the level of tolerance were found for some varieties

(Athlet and Simila varieties showed higher resistance according to symptoms, but lower tolerance to infection; Niagara, Boka and Hedvika showed relatively higher tolerance and lower resistance as measured by VSS). The Rexia and Svitava varieties reacted to infection quite well (VSS = 4.4 and 4.2, respectively) and also had lower GWS-R (36% and 32%, respectively). The Saskia and Rialto varieties showed the best VSS (3.9) but only average GWS-R (43% and 48%, respectively). As documented in Table 3, higher susceptibility was recorded on average for spring wheat compared to winter wheat. The causes may be the longer growth and development cycle of winter wheat and the more pronounced negative effect of stresses, e.g. drought and higher temperatures for spring crops than for winter crops. Average VSS values for registered varieties of spring wheat ranged between 4.5 and 6.6 and GWS-R from 24% to 60%. A symptomatic reaction at a level equivalent to

Table 3. Average attack of BYDV (VSS: 0-9; 0 = without symptoms), reduction of grain weight per spike (GWS-R) and grain weight per spike after infection (GWS-I) in the registered spring wheat varieties obtained from three-year trials (Prague-Ruzyně: 2004, 2006, 2008)

Variety	Origin	VSS	GWS-R (%)	GWS-I (g)
WKL 91-138 (resistant check)	SYR	2.9 a	23	1.23
Leguan	CZE	4.5 bc	36	1.09
Anza (Bdv1)	BRA	4.6 bc	32	0.96
Aranka	CZE	4.7 bcd	38	1.18
Sirael	CZE	4.9 bcde	24	0.96
Vánek	CZE	5.3 bcdef	28	1.10
Bruncka	DEU	5.3 bcdef	39	1.22
Zuzana	CZE	5.4 bcdef	28	1.05
Septima	CZE	5.5 bcdef	43	1.18
Granny	CZE	5.5 bcdef	40	1.10
Tercie	CZE	5.5 bcdef	38	1.05
Kronjet	SWE	5.8 cdef	37	0.87
Vinjet	SWE	6.0 def	42	0.92
Munk	DEU	6.1 cdef	46	0.71
Swedjett	SWE	6.1 ef	47	0.89
Triso	DEU	6.2 ef	60	0.74
Sandra	CZE	6.2 ef	30	1.11
Corso	DEU	6.3 ef	49	1.10
Jara (susceptible check)	CZE	6.6 f	48	0.85
Average		5.4	38	1.03

that of the Anza variety (*Bdv1* gene carrier) was detected for Leguan. This is in agreement with findings in previous years (Šíp *et al.* 2005). The Zuzana, Vánek and Sirael varieties showed belowaverage GWS-R. The Triso, Corso and Swedjett varieties, which were symptomatically equivalent to Jara, the susceptibility control, and at the same time showed higher GWS-R, can be assessed as susceptible. The Sandra variety, evaluated as tolerant in previous years (VACKE *et al.* 1996), in our trials showed an only relatively lower GWS-R, but its symptomatic evaluation was on par with the Jara variety.

Evaluation of selected sources of resistance for winter and spring wheat

The results of evaluating 63 potential sources of resistance for winter and spring wheat at the Ruzyně and Stupice locations in 2006 and 2008 are presented in Tables 4 and 5. The average VSS of winter wheat was 4.0. Sparta, the moderately resistant control variety, had an average VSS of 3.3, while Vlada, the susceptible control variety, had an average VSS of 5.8. The PSR3628 line, which showed only moderate symptoms (VSS = 1.6) and a very low GWS-R (4%), was evaluated

Table 4. Average attack of BYDV (VSS: 0–9; 0 = without symptoms), reduction of grain weight per spike (GWS-R) and grain weight per spike after infection (GWS-I) in potential winter wheat sources of BYDV resistance obtained from trials performed in two years at two locations (Prague-Ruzyně, Stupice 2006, 2008)

Variety/line	Origin	VSS	GWS-R (%)	GWS-I (g)
PSR 3628	USA	1.6 a	4	1.32
SG-S17-03	CZE	3.3 b	26	1.59
Sparta (resistant check)	CZE	3.3 b	28	1.49
SG-U3097	CZE	3.6 bc	34	1.65
Meritto	CZE	3.6 bc	40	1.61
SG-RUH26-01	CZE	3.7 bcd	35	1.64
McCormic	USA	3.9 bcd	42	1.01
SG-S1517-05	CZE	3.9 bcd	34	1.46
Rexia	SVK	4.0 bcd	35	1.44
Roane	USA	4.0 bcd	41	1.07
SG-S1039-05	CZE	4.1 bcde	34	1.51
SG-S50-04	CZE	4.1 bcde	46	1.27
SG-S1825-05	CZE	4.1 bcdef	32	1.78
Svitava	CZE	4.1 bcdef	36	1.55
SG-KM554	CZE	4.2 bcdef	36	1.43
Tribute	USA	4.3 bcdefg	20	1.02
SG-S1333-05	CZE	4.3 bcdefg	27	1.54
SG-U3078	CZE	4.3 bcdefg	32	1.43
Niagara	CZE	4.5 cdefg	46	1.26
Sisson	USA	4.7 defgh	48	0.98
Avalanche	USA	5.1 fghi	50	0.88
Vlada (susceptible check)	CZE	5.8 i	45	1.04
Average		4.0	35	1.38

Table 5. Average attack of BYDV (VSS: 0-9; 0 = without symptoms), reduction of grain weight per spike (GWS-R) and grain weight per spike after infection (GWS-I) in potential spring wheat sources of BYDV resistance obtained from trials performed in two years at two locations and the results of molecular marker analyses using WMS130 (Bdv1) and BYAgi, SCgpl and Xgwm137 (Bdv2) (Prague-Ruzyně, Stupice 2006, 2008)

Variety/line	Origin \	VSS	GWS-R (%)	GWS-I (g)	WMS130	BYAgi	SCgpl	Xgwm137
WKL 91-138 (resistant check)	SYR	2.6a	25	1.10	+	_	_	_
SOA217/02	POL	3.7ab	44	1.15	_	_	_	_
Maringá 1	BRA	3.8bc	39	0.96	_	_	_	_
SG-S80-04	CZE	3.8bc	32	1.10	_	_	_	_
Bárbaro-B	CHL	3.9bc	19	1.20	+	_	_	_
Anza	MEX	4.1bcd	31	0.92	+	_	_	_
Bonbony	POL	4.2bcde	37	0.92	_	_	_	_
SG-S26-98	CZE	4.2bcde	24	1.16	_	_	_	_
SG-S884-04	CZE	4.2bcde	34	1.19	_	_	_	_
MVZ 1105-04	CZE	4.3bcde	28	1.19	_	_	_	_
Leguan	CZE	4.4bcde	39	1.02	+	_	_	_
SG-S604-96	CZE	4.4bcde	29	0.98	_	_	_	_
SG-S45-98	CZE	4.5bcde	30	1.00	_	_	_	_
MVZ 1108-04	CZE	4.6bcdef	26	1.16	_	_	_	_
Waluta	POL	4.6bcdef	46	0.86	_	_	_	_
Costero-B	CHL	4.8bcdef	41	0.83	_	_	_	_
MVZ 974-04	CZE	4.8bcdef	30	1.31	+	_	_	_
Katoda	POL	4.9cdefg	42	0.83	_	_	_	_
Quino-Baer	CHL	4.9cdefg	48	0.85	_	_	_	_
Bingo-Baer	CHL	5.3defgh	38	0.91	_	_	_	_
TC-14 290E	AUS	5.3efghi	44	0.84	_	+	+	+
CIM 0232-297	MEX	5.8fghij	52	0.77	+	+	+	_
TC-14 290J	AUS	5.9ghijk	42	0.86	_	+	+	+
CIM 0235-351	MEX	6.0ghijk	49	0.76	+	+	+	_
CIM 0231-296	MEX	6.1ghijk	35	0.91	+	+	+	+
CIM 0234-350	MEX	6.1hijk	50	0.81	+	+	+	+
CIM 0227-292	MEX	6.2hijk	41	0.84	+	+	+	+
CIM 0230-295	MEX	6.3hijk	48	0.75	+	+	+	+
CIM 0228-293	MEX	6.4hijkl	52	0.72	+	+	+	+
CIM 0236-352	MEX	6.4ijkl	41	1.26	_	+	+	_
CIM 0223-344	MEX	6.5ijkl	35	0.87	+	+	+	+
CIM 0229-294	MEX	6.5ijkl	39	0.87	+	+	+	_
CIM 0233-299	MEX	6.5jkl	43	0.88	+	+	+	+
Jara (susceptible check)	CZE	6.5jkl	49	0.82	+	_	_	_
CIM 0220-287	MEX	6.6jkl	51	0.85	+	+	+	+
CIM 0226-291	MEX	6.6jkl	45	0.90	+	+	+	+
CIM 0225-349	MEX	6.8jkl	41	0.71	+	+	+	+
CIM 0237-126	MEX	6.9jkl	52	0.86	+	+	+	_
CIM 0221-289	MEX	7.1kl	51	0.82	+	+	+	+
CIM 0224-345	MEX	7.2kl	47	0.63	+	+	+	+
CIM 0222-290	MEX	7.81	56	0.69	+	+	+	+
Average		5.4	40	0.93				

best in the tested sampling. This tall line was randomly selected in a cross of wheat and couch-grass (A. Lukaszewski, personal communication). It is very late in its transition to the generative phase (it is reckoned to be a perennial wheat). It exhibits a low agronomic value (GWS is particularly low, at only 1.37 g) and susceptibility to yellow rust. The McCormick, Roane, Sisson, Tribute and Avalanche varieties were included in the resistance tests on the basis of the level of resistance documented above for natural occurrence of BYDV (GRIFFEY et al. 2001, 2003, 2005a, 2005b; HALEY et al. 2003). In trials with artificial inoculation, moderate resistance was confirmed for the McCormick, Roane and Tribute varieties. The McCormick and Roane varieties exhibited a lower level of visual symptoms (VSS = 3.9 and 4.0, respectively) but higher GWS-R (42% and 41%, respectively). In comparison, the Tribute variety had only moderate GWS-R (20%) but just an average visual symptom score (VSS = 4.3). Among sources of mild resistance we can also include the widely grown Czech variety Meritto (Šíp et al. 2005), which was evaluated at a level equivalent to Sparta, the control variety. Its relative GWS-R, however, was higher. Moderate resistance was confirmed for the Rexia and Svitava varieties (Šíp et al. 2005) as well as for the advanced breeding lines SG-S17-03 (SG-S411-91/ CWW93/58), SG-U3097 (893316-a/SG-S159-94), SG-RUH26-01 (PBIS-95-92/Šárka) and SG-S1517-05 (Svitava/Raleigh).

In spring wheat (Table 5), a high level of resistance was in accordance with previous results (Šíp *et al.* 2005) established for the WKL-91-138 line (coming from ICARDA in Syria) and for the

Figure 1. Symptoms of BYDV infection in three spring wheat varieties grown on two row plots; from left to right: susceptible Jara, medium resistant Leguan and resistant WKL 91-138 (uninfected control variant in the rear)

Brazilian variety Maringá (*Rht-B1b* isogenic line). Moderate resistance was detected in the Anza variety. None of the tested sources had a lower visual symptom score than the WKL91-138 resistant line (VSS = 2.6); the average reduction of grain weight per spike was 25%. The Bárbaro-B variety from Chile exhibited slight symptoms (VSS = 3.9) as well as low GWS-R (19%). The new breeding lines of the company Selgen, a.s. - SG-S26-98 (SANDRA/ST1197-87), SG-S80-04 (SAXANA//ESTICA/SG-S8-93), and MVZ1105-04 (SG-S26-98/SG-S113-98) – as well as genotypes from Poland - Bombona and SOA217/02 - were also found moderately resistant to BYDV. Overall, genotypes carrying the Bdv2 resistance gene were evaluated as susceptible to very susceptible. It is evident that *Bdv2* gene carriers (the CIMMYT set and the line derived from TC14) detected using molecular markers (see Table 5) showed a susceptible reaction equivalent to that of the control variety Jara (Figures 1 and 2). These lines similarly had medium or high GWS-R.

A statistically significant difference was determined, however, in absorptions (405 nm) on the 11th day after infection among the groups of genotypes with and without the *Bdv2* gene. As seen in Figure 3, *Bdv2* carriers had a lower virus content in inoculated seedling plants. This finding is in accordance with previous information on the function of the *Bdv2* gene (Banks *et al.* 1995) and confirms the effect of this gene on a certain reduction of the virus content (virus multiplication) in plants after infection. Based on evaluation of the averages of 9 selected *Bdv2* gene carriers and 7 non-*Bdv2* genotypes, for which the evalu-



Figure 2. Susceptible symptomatic reaction of the lines carrying Bdv2 gene (uninfected control variant in the rear)

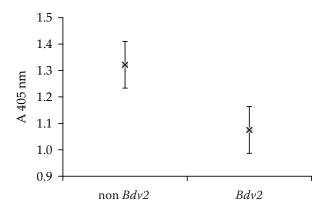


Figure 3. Average absorbances in infected plant leaves on the $11^{\rm th}$ day after inoculation with BYDV-PAV (ELISA, 405 nm) and 95.0% Tukey's HSD test for groups of Bdv2 and non-Bdv2 spring wheat lines

ation as to the content of the virus was done (see Material and Methods), it is evident, however, that the field resistance of these genotypes was evaluated on average as low (average VSS = 6.4). The average VSS of the 'non- Bdv2' genotypes was two grades better (4.3). Similarly, GWS-R was also higher on average for *Bdv2* gene carriers (46%) than for 'non-Bdv2' genotypes (34%). Only TC14-290E was evaluated at the average level of the tested line set. The effect of the *Bdv2* gene on increasing field resistance to the Czech isolate of BYDV-PAV was not shown. This finding is not in agreement with some previously published results (Banks et al. 1995; Henry et al. 2001). Undoubtedly, however, it is necessary to take into account that the cited results were obtained using a Mexican isolate of BYDV-PAV. AYALA et al. (2001) pointed out differences in the expression of the Bdv2 gene. CHAIN et al. (2006) showed a possibility of the adaptation of BYDV to a gene for partial resistance. These authors reported that isolates issued from serial passages on a host carrying a gene for partial resistance caused increased damage in field conditions when compared with the initial isolate.

As was expected, the presence of the *Bdv1* gene was detected using the WMS130 marker in the Anza variety, and also in the resistant line WKL 91-138, moderately resistant Leguan variety, breeding line MVZ 974-04 and Bárbaro-B variety. Its presence was demonstrated in the majority of genotypes that carry *Bdv2* gene as well as in the susceptible Jara variety. Considering high variability in the visual symptom score of the above '*Bdv1*' materi-

als (VSS = 2.6-7.8), practical use of this marker proves unsubstantiated.

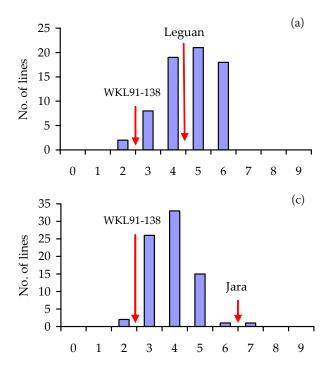
The BYAgi and SCgpl markers proved to be reliable for detecting the Bdv2 gene, the presence of which was expected in the set of 'CIMMYT' lines and lines derived from TC14, while the presence of the Bdv2 gene was detected only in 15 out of 20 genotypes (75%) using the Xgwm 137 marker.

Opportunity for using the identified sources in breeding for resistance to BYDV

The detection of the PSR 3628 line, which represents a source of high resistance, can be considered as highly beneficial. However, the agronomic value of this line is very low, which limits its use in practical breeding programmes. Increasing the level of resistance through the use of detected sources with moderate resistance (McCormick, Roane and Tribute varieties or varieties grown in the Czech Republic such as Meritto, Rexia, Rialto and Svitava) is evidently limited, when considering the assumed polygenic nature of inheritance and the considerable variations in the level of resistance owing to the impact of environmental conditions.

The sources detected for spring wheat are obviously more promising to be used in breeding for resistance. In addition to WKL91-138, with the highest demonstrated level of resistance, the Bárbaro-B, Bombona, SG-S80-04 and SOA217/02 varieties (lines) were newly detected as sources of moderate resistance. An advantage of these genotypes is their high agronomic value, which enables their efficient use in breeding. These varieties (lines) of spring wheat could be used even for improving the resistance of winter wheat varieties. It seems promising to cross these materials with e.g. SG-S17-03 or McCormick and Tribute.

To examine the prospect of using some sources of resistance in practical breeding, the level of resistance of F₃ lines derived from crosses of Leguan/WKL91-138, Leguan/TC14 290E and Jara/WKL91-138 was assessed in field infection trials after artificial inoculation with BYDV-PAV. Figure 4 depicts the frequency distribution for VSS, while Figure 5 shows the average level of VSS and GWS-R in comparison with parental varieties. The frequency distribution for VSS in the populations of lines derived from the crosses with WKL91-138 is indicative of the quantitative (polygenic) nature of BYDV resistance in this line.



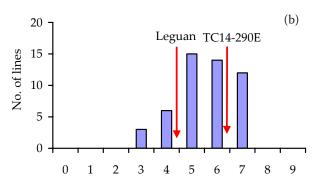
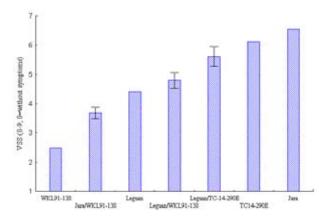


Figure 4. Attack of BYDV (0–9 scale; 0 = without symptoms) for F3 lines of three spring wheat crosses: (a) – Leguan × WKL91-138; (b) – Leguan × TC14-290E; (c) – Jara × WKL91-138

Despite achieving the highest level of resistance, the use of this line in breeding can be complicated, particularly due to its inferior agronomic quality. The results, however, show that the genetic gain, as far as resistance to BYDV is concerned, can be relatively strong especially for some crosses. After crossing of the WKL91-138 line with the Jara variety, for example, 26% of lines showed VSS in the class '3' while only 8% of lines of the Leguan/WKL91-138 cross exhibited this level of resistance. These results indicate that the crossing of parents showing a higher resistance level may not always be the most promising. AYALA et al. (2002) stated that both parents differing in resistance level might carry genes for reduced

symptom expression. Considering the diversity of the contribution from various "complementary" parents, analysing various F_2 's in infection trials and selecting the crosses that are more promising from these aspects can be advantageous. From a breeding point of view, the selection of a "complementary" parent with high yielding potential and many other desirable properties should speed up the breeding process.

The results obtained for the Leguan/TC14 cross did not show a significant effect from the *Bdv2* gene on increasing field resistance to the Czech isolate of BYDV-PAV. As shown in Figures 4 and 5, the average VSS for this cross was 5.62, significantly higher than for Jara/WKL91-138 (3.69) and



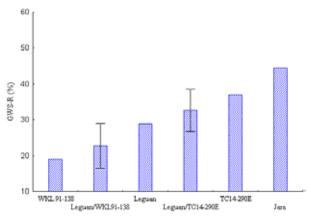


Figure 5. Average attack of BYDV (VSS) and reduction of grain weight per spike (GWS-R) in parental varieties and randomly selected F_3 lines of three spring wheat crosses (bars indicate standard error of the mean)

Leguan/WKL91-138 (4.80), and genotypes with VSS ≥ 5 were highly prevalent (82%). Average GWS-R for the Leguan/TC14-290E cross was 10% higher (32.6%) compared to the Leguan/WKL91-138 hybrid. This, however, does not represent a statistically significant difference.

The present analyses suggest that the nature of BYDV resistance for wheat is clearly polygenic on the basis of the analysis of available sources. Analyses of populations segregating for the *Bdv1* gene (AYALA *et al.* 2002) brought similar results. It is evident that the introduction of a single gene is not sufficient for achieving the required level of resistance. Based on these findings, we can conclude that the marker-assisted selection, for the time being, is not very well applicable without a focus on detecting a larger number of QTLs. The analyses in segregating populations, however, have shown that significant progress can be achieved by using the WKL91-138 line when crossed with a suitable 'complementary' parent

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