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Response of wheatgrasses and wheat × wheatgrass hybrids to barley yellow dwarf virus*

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Summary. Resistance to barley yellow dwarf virus (BYDV), manifested by low enzyme-linked immunosorbent assay (ELISA) values in plants exposed to viruliferous aphids, was identified in several wheatgrasses (*Agropyron* spp.). ELISA results were similar for root and leaf extracts of infested plants. No difference in reaction to BYDV was found between plants grown in the field and those in the growth chamber. Interspecific hybrids were generated using pollen from single resistant plants of *Agropyron* spp. to pollinate soft red winter wheat spikes. Resistance in hybrids appeared to be at the level of virus replication rather than at the level of vector inoculation. The hybrids varied in their reaction to BYDV. Expression of BYDV resistance in hybrids was influenced not only by wheat genotype and *Agropyron* species but, in some cases, reaction varied even among hybrids between the same wheat genotype and *Agropyron* plant. Implications of these results are discussed.

Key words: Barley yellow dwarf virus (BYDV) – Wheatgrasses – Wheat × *Agropyron* hybrids – ELISA

Introduction

Barley yellow dwarf (BYD) is one of the most serious viral diseases of wheat worldwide. Although some wheat cultivars and germplasm lines show less severe symptoms than others after infection by barley yellow dwarf virus (BYDV), yield of even these genotypes can be severely reduced (Carrigan et al. 1981; Baltenberger et al. 1987). Differences among wheat lines for symptom severity are likely due to differences in tolerance rather than resistance, because the concentration of virus in infected plants is not consistently correlated with symptom severity (Skaria et al. 1985). Tolerance in wheat is not controlled by a single gene (Qualset et al. 1973).

Sharma et al. (1984) and Shukle et al. (1987) reported high levels of resistance to BYDV isolates in several wheatgrasses (*Agropyron*, broad sense; for revised classification of *Agropyron*, Barkworth and Dewey 1985) in growth chamber experiments. Eweida and Ryden (1984) reported low concentration of BYDV in *A. repens* (L.) Beauv. inoculated in a greenhouse. Shukle et al. (1987) also found that resistance was largely due to failure in virus replication, although in some cases a major component of resistance was the inability of the aphid vector to penetrate leaf phloem. The BYDV resistance of *Agropyron* species is of special interest. However, *Agropyron* is notorious for disharmonious segregation and genes from wheatgrasses have been transferred to wheat with difficulty. Thompson and Grafius (1950) noticed that half of the wheat × *A. trichophorum* hybrids became infected with leaf rust and that the resistance of the hybrids depended on the *A. trichophorum* selection in its parentage. A stable disomic addition line conferring stripe rust resistance was obtained from one cross but not from others (Cauderon and Rhind 1976). Shukle et al. (1987) reported that some plants grown from the seed lots of varieties of *Agropyron* spp. were susceptible to BYDV. It follows, therefore, that individual resistant plants within an *Agropyron* species need to be identified and used for a precise gene transfer. Many wheat × *Agropyron* hybrids have been produced (Sharma and Baenziger 1986), but most of the literature deals with morphology and

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Table 1. Number of plants of each *Agropyron* species tested and found negative or positive for reaction to two strains of barley yellow dwarf virus by ELISA. Infected controls = positive; healthy controls = negative; wheat = positive; positive = ELISA value exceeded twice the control; negative = ELISA value did not differ or exceed the control

Species	Variety	No. plants tested		No. plants negative		No. plants positive	
		PAV	RPV	PAV	RPV	PAV	RPV
A. intermedium	SD	6	10	6	10	0	0
	Oahe	6	3	5	3	1	0
A. trichophorum	Luna	9	7	9	7	0	0
A. elongatum	Jose	23	15	18	15	5	0
A. smithii	Arriba	8	5	8	5	0	0
	Barton	9	8	7	7	2	1
	Rosanna	5	9	5	9	0	0
A. sibiricum	–	10	0	4	–	6	–
A. riparium	Sodar	2	1	2	1	0	0
A. spicatum	–	1	1	1	1	0	0
Total		79	59	65	58	14	1

Table 2. I/N ratios for individual *Agropyron* plants and for some of wheat cultivars selected for crosses, after inoculation with the PAV isolate of barley yellow dwarf virus; I/N = ELISA value for inoculated (sample)/ELISA value for non-inoculated (healthy control)

Agropyron species/Wheat	Variety	*Agropyron* Plant No.	PAV I/N ratio	
			Field	Growth chamber**
A. intermedium	SD	17*	–	1.01, 0.83
		58	1.09	0.83
		113*	–	0.70, 1.05
	Oahe	80*	1.50	0.82, 0.82
		81*	1.14	0.93, 0.92
A. trichophorum	Luna	74	0.90	–
		75	0.92	0.92
		76*	1.01	0.80
		77A*	–	0.83, 0.80, 1.49
		106*	–	1.08
		37C	–	1.24
A. elongatum	Jose	71*	0.85	0.86, 0.86, 1.22
		112*	–	1.86
		E1	–	0.78
		E4	–	0.93
		E6	–	0.89
Infested wheat	Caldwell	–	7.90, 3.55, 4.79	3.24, 6.57
	Elmo	–	2.58, 4.76	–
	Florida 302	–	–	5.89
	Pioneer 2550	–	–	3.12
	Pioneer 2551	–	–	3.12
	WI 1349-6	–	–	2.29
Infested control, wheat	Abe	–	–	13.40
Infested control, oat	Clintland 64	–	–	4.41–49.35
Healthy control, wheat	Caldwell	–	–	0.83
Healthy control, *Agropyron*	SD	–	0.83	–
	Oahe	–	0.80	–
	Luna	–	0.93	–
	Jose	–	1.00	–
ELISA value of healthy control, oat	Clintland 64	–	–	0.04–0.27

* Tested for RPV isolate also and were found resistant
** More than one value indicates multiple tests

Table 3. Number of F_1 seedlings tested for reaction to the PAV isolate of barley yellow dwarf virus and their I/N ratios

Cross		No. of F_1 seedlings tested	I/N ratio of F_1 seedlings	
			Ratio < 2	Ratio > 2
Caldwell	× *A. intermedium*, SD, Pl. no. 17	5	1.23, 1.38	3.06, 4.28, 9.23
OH256-1	× *A. intermedium*, SD, Pl. no. 17	8	1.38	2.28, 2.66, 4.32, 5.15, 7.04, 8,30, 8,57
Caldwell	× *A. intermedium*, SD, Pl. no. 58	1		5.30
Elmo	× *A. intermedium*, SD, Pl. no. 58	1		22.19
Compton	× *A. intermedium*, SD, Pl. no. 113	2	1.33, 1.45	
Caldwell	× *A. intermedium*, Oahe, Pl. no. 80	1		8.81
Caldwell	× *A. intermedium*, Oahe, Pl. no. 81	8	1.74, 1.83	2,79, 3.02, 6.02, 7.04, 9.66, 14.94
Pioneer 2550	× *A. intermedium*, Oahe, Pl. no. 81	1		11.64
78447RK1	× *A. trichophorum*, Luna, Pl. no. 37C	2	1.49	2.16
76791RA7	× *A. trichophorum*, Luna, Pl. no. 37C	1		2.87
Auburn	× *A. trichophorum*, Luna, Pl. no. 37C	3	1.10, 1.26, 1.86	
Caldwell	× *A. trichophorum*, Luna, Pl. no. 37C	1	1.42	
WI1349-6	× *A. trichophorum*, Luna, Pl. no. 74	1	1.07	
Florida 302	× *A. trichophorum*, Luna, Pl. no. 74	1	1.76	
Pioneer 2550	× *A. trichophorum*, Luna, Pl. no. 75	1	1.16	
OH256-1	× *A. trichophorum*, Luna, Pl. no. 75	1	1.04	
Elmo	× *A. trichophorum*, Luna, Pl. no. 75	3	0.75, 1.06, 1.20	
861046E	× *A. trichophorum*, Luna, Pl. no. 75	3	0.77, 0.95	2.07
Caldwell	× *A. trichophorum*, Luna, Pl. no. 75	1	0.94	
WI1349-6	× *A. trichophorum*, Luna, Pl. no. 75	2	1.19, 1.38	
Caldwell	× *A. trichophorum*, Luna, Pl. no. 76	1		3.13
Caldwell	× *A. trichophorum*, Luna, Pl. no. 77A	1		4.30
8138I1	× *A. trichophorum*, Luna, Pl. no. 77A	1	1.50	
WI1349-6	× *A. trichophorum*, Luna, Pl. no. 77A	1	1.29	
PS840026-1	× *A. trichophorum*, Luna, Pl. no. 77A	1	1.25	
Caldwell	× *A. trichophorum*, Luna, Pl. no. 106	1		8.83
Caldwell	× *A. elongatum*, Jose, Pl. no. 71	5		2.70, 3.51, 5.11, 7.21, 10.64
Pioneer 2550	× *A. elongatum*, Jose, Pl. no. 71	3		3.60, 4.43, 9.00
Florida 302	× *A. elongatum*, Jose, Pl. no. 71	2	1.74	6.08
77531	× *A. elongatum*, Jose, Pl. no. 71	9	1.13, 1.74	2.20, 3.61, 3.78, 5.72, 7,39, 7,76, 8.21
Caldwell	× *A. elongatum*, Jose, Pl. no. E1	3	1.86	7.35, 16.59
Caldwell	× *A. elongatum*, Jose, Pl. no. E4	2		2.19, 2.73
Caldwell	× *A. elongatum*, Jose, Pl. no. E6	3		6.70, 13.94, 15.00
Pioneer 2551	× *A. elongatum*, Jose, Pl. no. 112	2		3.08, 5.51
Wheat	× *A. elongatum*, Jose, Pl. no. –	15*		8.20, 8.96, 9.54, 10.95, 11.22, 11.28, 11.89, 13.54, 14.00, 14.78, 17.26, 18.28 23.72, 27.78, 34.62

Infected controls = 11.49–24.53, Healthy oat ELISA value = 0.027–0.047
* Seedlings obtained from seed received from Dr. J. Nelson. I/N ratio < 2 = Uninfected (Negative reaction), I/N ratio > 2 = Infected (Positive reaction)

cytology of the hybrids. Expression of disease resistance at the F_1 stage has been rarely reported (Reitz et al. 1945; Love and Suneson 1945), and we are not aware of any report for resistance to BYDV.

Here we report the expression of BYDV resistance in *Agropyron* plants grown in growth chamber and field, and in their hybrids with soft red winter wheats (SRWW), *Triticum aestivum* L.

Materials and methods

The *Agropyron* species evaluated for resistance to BYDV are listed in Table 1. The seed was obtained from Curtis & Curtis Seed, New Mexico, and cytologically confirmed for specific chromosome number. SD is a line from S. Dakota.

Some of the resistant plants of *A. intermedium*, *A. elongatum* and *A. trichophorum* (Table 2) were crossed with a number of SRWW cultivars and breeding lines (Table 3). Pollen from single resistant *Agropyron* plants was used to pollinate individual wheat spikes and a record of hybrid plants was kept. Most of the hybrid seedlings were raised by embryo culture as described by Sharma and Baenziger (1986); only a few were obtained from mature shrivelled seed. Some hybrid seeds of wheat × *A. elongatum* var. Jose were received from Dr. J. Nelson, United Agriseeds, Urbana, Illinois, from which 15 seedlings were produced (Table 3). The hybrids were confirmed cytologically and/or morphologically. Eleven of the 16 *Agropyron* plants used for hybridization with wheat were progeny tested.

Agropyron plants, wheat × *Agropyron* hybrids, and some of the wheat parents and controls were infested at seedling stage

with the P-PAV isolate of BYDV (Hammond et al. 1983), the most common isolate using *Rhopalosiphum padi* L. as the vector. Vegetative clones of some of the *Agropyron* plants were infested with the Rochow RPV isolate of BYDV also using the same vector. Viruliferous aphids carrying the PAV or RPV isolate were obtained from cultures maintained on susceptible oat, *Avena sativa* L. cv Clintland 64. Ten aphids were released on each plant. The plants were infested and maintained in growth chambers (20 ± 1 °C, 14 h light) for one week. Aphid survival was recorded and plants without live aphids were reinfested. Aphids were killed by malathion and 14 days later plant samples were collected. Some of the *Agropyron* plants were also grown in the field. Noninfested control plants and infested Clintland 64 oat were treated similarly. A random sample of eight *Agropyron* plants and 24 hybrids resistant to PAV was reinfested one to three times to confirm resistance.

Tissue samples were prepared and virus in the resulting extracts was measured by ELISA procedure as described by Hammond et al. (1983). Samples were recorded positive (infected, susceptible) when their ELISA values exceeded twice the control value, i.e., when I/N ratio [ELISA value for inoculated (sample)/ELISA value for noninoculated (healthy control)] exceeded two.

To determine whether resistance to BYDV in hybrids was due to failure in virus increase or to the inability of the vector to inoculate plants, aphid feeding behavior was electronically monitored for leaf phloem contact and phloem ingestion according to the method described by Shukle et al. (1987).

Results

ELISA results were similar for leaf and root extracts of *Agropyron* spp. Out of ten *A. elongatum* and ten *A. sibiricum* plants tested, results differed only in one plant of *A. elongatum* for which the ELISA value of the leaf extract was negative but the ELISA value of the root extract was positive. Only leaf tissue was utilized in subsequent analyses.

Seventy-nine plants of seven *Agropyron* species were tested for PAV and 59 for RPV (Table 1). The frequency of resistant plants was much higher for RPV than for PAV, showing the more virulent nature of PAV on *Agropyron* spp. Only one plant was positive to RPV (I/N ratio = 2.15); all others were negative (I/N ratio = 0.66 to 1.86). For PAV, 65 plants were negative (I/N ratio = 0.62 to 1.86) and 14 plants were positive (I/N ratio = 2.01 to 22.37).

No differences in reaction to BYDV were found between field-grown *Agropyron* plants and their clones grown in a growth chamber. However, when tissue was harvested a second time 2 weeks after the first harvest, from a random sample of ten field-grown plants (five resistant and one susceptible to PAV, and four resistant to RPV), there was an increase in I/N ratio in all, and out of nine plants resistant in the first analysis, four had I/N values exceeding 2 (2.07 to 2.17). This increase could be due to contamination by other related BYDV isolates in the field.

Of *Agropyron* plants which were negative for PAV in multiple tests in the field as well as in the growth chamber, five *A. intermedium*, six *A. trichophorum* and five *A. elongatum* were selected for crosses with wheat (Table 2). Nine of these were also tested for RPV isolate and all were negative. Eleven of the 16 plants were progeny-tested. Progeny size varied from 3–11 plants. Eighty-three of the 84 progeny plants tested were negative (I/N ratio = 0.15 to 1.83). One out of eight plants in the progeny of plant no. 112 was positive (I/N ratio = 2.24).

The hybrids varied in their response to PAV as determined by ELISA (Table 3). The expression of resistance in hybrids was affected by *Agropyron* species, wheat genotype, and individual *Agropyron* plants. Out of 97 hybrid plants between various wheat lines and *Agropyron* plants, 66 were positive and 31 were negative. Among 18 cross combinations where more than 1 hybrid seedling between a wheat variety and an *Agropyron* plant were tested, reaction varied in 8 of those crosses. Out of 18 crosses, 6 had all positive, 4 had all negative, but 8 had both positive and negative reaction to BYDV (Table 3). The three hybrids between Elmo and plant no. 75 of *A. trichophorum* were negative, whereas the hybrid between Elmo and plant no. 58 of *A. intermedium* was positive. Hybrids of Caldwell and Pioneer 2550 with plant no. 71 of *A. elongatum* gave a positive reaction, but those of Florida 302 and 77531 gave variable reactions. Hybrids of Caldwell with plant E6 were positive, those with plant E4 had suppressed virus titer, and those with plant E1 were variable.

Phloem ingestion by aphids was greatest from Clintland 64, a susceptible oat variety. Phloem ingestion took place in plant no. 71 of *A. elongatum* and its hybrid with Florida 302, but at relatively low levels. Because the aphids contacted the phloem, resistance in the hybrids lies at the level of virus replication rather than at the level of vector inoculation. This conclusion is based on study of only one hybrid.

Discussion

Resistance to BYDV in *A. intermedium*, *A. elongatum*, *A. smithii*, and *A. sibiricum* reported here has previously been reported (Sharma et al. 1984; Shukle et al. 1987), but resistance in *A. riparium*, *A. trichophorum*, and *A. spicatum* is reported for the first time. The testing was done not only on growth chamber-grown material but also on field-grown material. The results confirm the growth chamber (Sharma et al. 1984; Shukle et al. 1987) and greenhouse (Eweida and Ryden 1984) experimental findings that wheatgrasses, perhaps across all *Agropyron* species, may be the best source of resistance to BYDV for wheat improvement. The transfer of this resistance will be facilitated if it is conferred by one or a few genes.

Investigations on the genetic control of this resistance in *Agropyron* are currently underway in our laboratory.

Some of the hybrids having negative reaction gave positive response on reinfestation. The second test was done after vernalization at a later stage of plant development. Plant age and growth pattern could have affected the viral antigen content. There may be accumulation of the virus with repeated infestation. Chromosome instability may also be the reason that some hybrids show a positive reaction in repeated tests at later stages of growth.

According to the most accepted model for resistance to viral diseases derived from a related species, the resistant host plant may produce an inhibitor of the virus replicative cycle and the susceptible host may have a null allele which does not produce a functional inhibitor (Fraser 1986). This kind of resistance can be dominant but could also be gene-dosage dependent and sensitive to environment. Reactions of wheat × *Agropyron* hybrids do not indicate dominance as both negative and positive values were obtained. A large number (68%) of hybrids had positive ELISA values, indicating that the *Agropyron* genes expressed poorly in the hybrids or were suppressed in the presence of the wheat genome. Since the expression of resistance in several hybrids was suppressed (I/N ratio approximately 2), there seems to be evidence for a possible gene-dosage dependence of resistance. Variation from resistant to susceptible suggests that resistance in the *Agropyron* species used may not be monogenic. Resistance to wheat streak mosaic virus was conditioned by more than one factor in *A. intermedium* and was hemizygous-ineffective in *A. elongatum* (Stoddard et al. 1987).

Variation among hybrid plants between a wheat genotype and an *Agropyron* plant could be due to heterozygosity in the *Agropyron* plants used. This notion is supported by the fact that some *Agropyron* plants were susceptible. Chromosome pairing is not perfect in *Agropyron* (Cauderon 1958) and sometimes the critical chromosome(s) may not be included in the gamete. Thus, all the gametes produced by an *Agropyron* plant might not have been alike, resulting in variable progeny in crosses with wheat. Intraplant variation has been reported in grass species, and differences in plants propagated asexually from the same clone were striking (Nielsen 1968). Progeny tests indicated some evidence of heterozygosity but heterozygosity may not explain the whole variation and variation may also be due to rapid genome changes; these can include gross chromosomal alterations or chromosome instability as well as changes in absolute genome size or DNA content, and can lead to non-uniform progeny of interspecific hybrids (Walbot and Cullis 1985).

Non-uniformity among *Agropyron* plants and among the F_1s for BYDV expression observed here, and variation among different plants of the same variety of *Agropyron* for morphology and crossability with wheat (unpub. data) indicate great diversity in *Agropyron* seed lots. This underscores the importance of using individual resistant plants and cautions against using bulk pollen from different plants for gene transfer into wheat. Variable response among hybrid plants between a wheat genotype and an *Agropyron* plant cautions against producing only a few hybrids and backcross plants. Influence of wheat genotype and variation among hybrids dictate that a broad spectrum of wheat genotypes and a number of wheatgrass plants should be used in crosses to enhance the chances of success in gene transfer. The dosage-dependent nature of resistance to BYDV should be kept in mind during the transfer of this resistance to wheat, and hybrids showing suppressed virus titer or intermediate reaction should not be discarded.

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