Resistance to barley yellow dwarf luteovirus in *Aegilops* species

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Makkouk, K. M., Comeau, A. and Ghulam, W. 1994. **Resistance to barley yellow dwarf luteovirus in *Aegilops* species.** Can. J. Plant Sci. **74**: 631–634. One thousand and ninety-seven *Aegilops* accessions were evaluated for their reaction to a PAV serotype of barley yellow dwarf luteovirus (BYDV). The accessions tested belong to the species *bicornis*, *biuncialis*, *caudata*, *crassa*, *columnaris*, *comosa*, *cylindrica*, *kotschyi*, *longissima*, *mutica*, *neglecta* (= *triaristata* 4 ×), *ovata*, *peregrina*, *searsii*, *sharonensis*, *speltoides*, *tauschii* (= *squarrosa*), *triuncialis*, *umbellulata*, *uniaristata*, *vavilovii* and *ventricosa*. The first evaluation of virus levels in the different accessions was conducted at International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, using double antibody sandwich ELISA (DAS-ELISA). Accession reaction ranged from highly resistant to highly susceptible. Thirty-eight *Aegilops* accessions resistant at ICARDA, were evaluated at Sainte-Foy, Quebec, Canada, by tissue-blot immunoassay. Diversity of response to BYDV infection was again observed in this elite group. Seven accessions belonging to the species *biuncialis*, *caudata*, *neglecta* and *triuncialis* were highly BYDV resistant at both locations; five of these originated from Bulgaria.

Key words: Introgression, interspecific, *Triticum aestivum*, BYDV, ELISA, immunoassay, tissue blot

Makkouk, K. M., Comeau, A. et Ghulam, W. 1994. **Résistance au virus de la jaunisse nanisante de l'orge chez les espèces d'Aegilops.** Can. J. Plant Sci. **74**: 631–634. Mille quatre vingt dix sept lignées d'Aegilops furent évaluées pour leur réaction au sérotype PAV de la jaunisse nanisante de l'orge (VJNO). Ces lignées appartenaient aux espèces *bicornis*, *biuncialis*, *caudata*, *crassa*, *columnaris*, *comosa*, *cylindrica*, *kotschyi*, *longissima*, *mutica*, *neglecta* (= *triaristata* 4 ×), *ovata*, *peregrina*, *searsii*, *sharonensis*, *speltoides*, *tauschii* (= *squarrosa*), *triuncialis*, *umbellulata*, *uniaristata*, *vavilovii* et *ventricosa*. La première évaluation des diverses lignées fut faite à ICARDA, Alep, Syrie, par le "double antibody sandwich ELISA" DAS-ELISA. Les réactions furent très diverses, entre la forte résistance et la forte sensibilité. Trente-huit lignées d'*Aegilops* classées résistantes à ICARDA furent réévalués à Ste-Foy, Canada par un essai sérologique sur l'empreinte de tissu. Une certaine diversité de réponse à l'infection virale fut encore observée parmi ces sélections. Sept lignées, appartenant aux espèces *biuncialis*, *caudata*, *neglecta* et *triuncialis*, furent très résistantes au VJNO aux deux sites d'essai, et cinq de ces lignées étaient originaires de Bulgarie.

Mots clés: Introgression, interspécifique, *Triticum aestivum*, VJNO, ELISA, essai sérologique, empreinte de tissu

Barley yellow dwarf luteovirus (BYDV) is an important disease of cereals, and the search for resistance to this virus in wild relatives is economically justified. Several researchers (Sharma et al. 1984; Brettell et al. 1988; Ceoloni et al. 1988) have reported desirable traits in the wild relatives of cereals. Those traits constitute an important genetic resource for cereal improvement (Cauderon 1979). *Aegilops* species are often used in interspecific hybridization with durum (*Triticum durum*) and bread wheat (*Triticum aestivum*). The F_1 hybrids can be rescued and backcrossed with relative ease (Comeau and St-Pierre 1992; Comeau et al. 1993). There are no previous reports of BYDV resistance in this genus.

The Gene Bank of the International Center for Agricultural Research in the Dry Areas (ICARDA) has a large collection of *Aegilops* species. In this study we evaluated 1097 *Aegilops* accessions for their reaction to BYDV.

MATERIALS AND METHODS

Germplasm Evaluated

During the 1988–1992 growing seasons the following *Aegilops* accessions were evaluated at ICARDA in Syria: 10 *Ae. bicornis*, 123 *Ae. biuncialis* (syn. *macrochaeta*), 15 *Ae. caudata* (syn. *dichasians*), 16 *Ae. columnaris*, 3 *Ae. comosa*, 7 *Ae. crassa*, 60 *Ae. cylindrica*, 30 *Ae. kotschyi*, 4 *Ae. longissima*, 5 *Ae. mutica*, 223 *Ae. ovata*, 83 *Ae. peregrina*, 25 *Ae. searsii*, 1 *Ae. sharonensis*, 50 *Ae. speltoides*, 40 *Ae. tauschii* (syn. *squarrosa*), 75 *Ae. triaristata* 4× (syn. *neglecta* for tetraploids, syn. *recta* for hexaploids), 249 *Ae. triuncialis*, 25 *Ae. umbellulata*, 4 *Ae. uniaristata*, 22 *Ae. vavilovii*, 27 *Ae. ventricosa*. The most BYDV-resistant accessions were evaluated during the 1992–1993 growing season in an elite *Aegilops* nursery at Sainte-Foy, Quebec, Canada.

Artificial Inoculation

In field tests at ICARDA, two 1-m rows with 10 plants/meter were grown for each *Aegilops* accession. At the 4–6 leaf stage, 10–15 viruliferous *Rhopalosiphum padi* aphids were placed on each plant and after 48 h these aphids were killed with the insecticide Pirimor. At Sainte-Foy, the accessions

Abbreviations: BYDV, barley yellow dwarf luteovirus

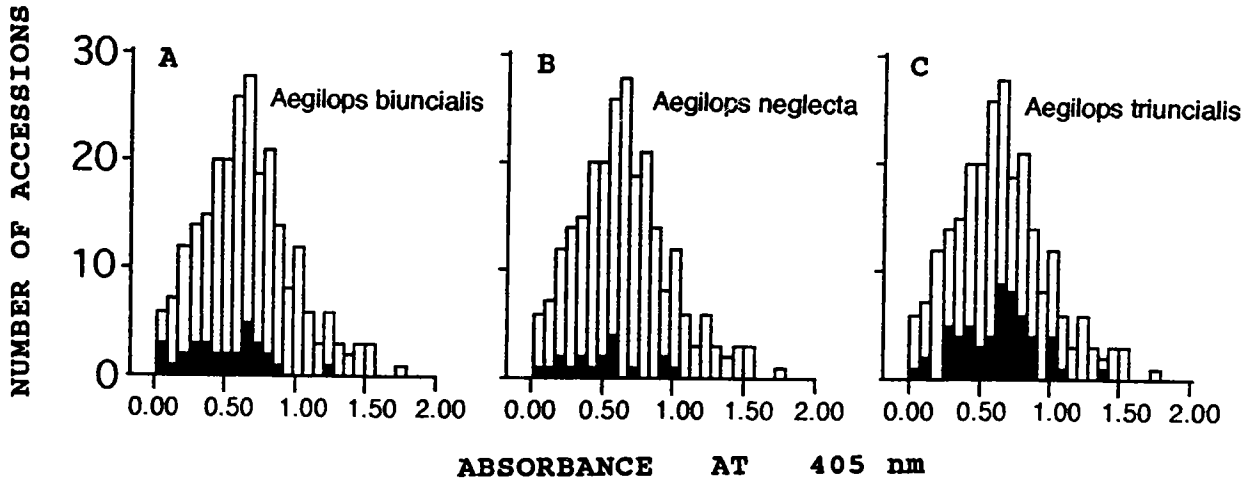

Fig. 1. Distribution of the ELISA values for the *Aegilops* accessions tested in 1992 (empty bars) as compared to the distribution of the values obtained for the accessions of *Ae. biuncialis* (A), *Ae. neglecta* (B) and *Ae. triuncialis* (C) (solid bars). The average healthy reading of all the *Aegilops* species tested was 0.16.

were grown in pots and placed in growth chambers operating at 16 h light and 8 h of darkness. Temperature of incubation was 22°C during the light period and 18°C during the dark period. The plants were inoculated at the 4–6 leaf stage with a Quebec isolate of PAV-BYDV using a method similar to that described for the ICARDA tests.

Virus Detection

At ICARDA, evaluation of *Aegilops* plants for BYDV was carried out using a variation of the double antibody sandwich ELISA (DAS-ELISA) procedure of Clark and Adams (1977); the leaves were extracted (1 g leaf/1 mL buffer) in 0.2 M phosphate buffer, pH 6. ELISA plates (Dynatech) were coated with 1 μg mL^{-1} immunoglobulins (IgG) and the alkaline phosphatase-conjugated IgG was used at a dilution of 1:1000. IgG, used for both coating and conjugate, was purified by the caprylic acid method (Steinbuch and Audran 1969) from a BYDV-PAV antiserum produced earlier in the virology laboratory at ICARDA. Test reactions were quantified spectrophotometrically by measuring absorbance values at 405 nm.

Plants evaluated at Sainte-Foy were tested by tissue-blot immunoassay (TBIA). The TBIA procedure used the chromogenic substrate, nitro blue tetrazolium (NBT)/5-bromo-chloro-3-indolyl phosphate (BCIP) (Lin et al. 1990; Hsu and Lawson 1991; Makkouk et al. 1993). Test reaction was evaluated by counting the number of phloem bundles stained with a distinctive blue-purple colour. At the time of the ICARDA trial, the TBIA method for detection of BYDV was not available, hence DAS-ELISA was the logical choice.

RESULTS

Virus content in the *Aegilops* accessions at ICARDA varied from almost zero to a relatively high level (ELISA value of 1.875). The data were interpreted using a positive–negative threshold, calculated as the healthy mean plus 3 SD. The thresholds varied slightly among the species tested and among the growing seasons (0.110–0.225). The 1991–1992 data were easy to interpret, because the difference between the resistant and susceptible entries was large and the distribution was close to normal (Fig. 1, empty bars). Most accessions of *Ae. biuncialis* and *Ae. neglecta* occupied the left side of the distribution curve, suggesting that virus multiplication was lower than in the other species tested. In 1992, some accessions of these two species had very little virus; a few *Ae. triuncialis* accessions were also very resistant (Fig. 1, A, B,C).

A group of *Aegilops* accessions that demonstrated a low level of virus multiplication at ICARDA was tested against a Quebec BYDV-PAV isolate. The TBIA method revealed a variability in the extent of virus invasion of the phloem vessels as measured by the number of stained bundles (Fig. 2). In comparison to that of the BYDV-tolerant wheat cultivar Maringa, virus invasion in all of these accessions was reduced. A number of *Aegilops* accessions showed a very low level of virus 5, 7, 11, 21 and 60 d after virus inoculation (Table 1).

DISCUSSION

There was a wide difference in BYDV titer among the *Aegilops* accessions tested reflecting differences in their genetic response to BYDV. None was immune to BYDV infection as shown with some *Thinopyrum* accessions (Comeau and Plourde 1987). However, a high level of BYDV resistance was found in a few accesssions of *Ae. caudata*, *Ae. biuncialis*, *Ae. neglecta* and *Ae. triuncialis*.

As a diagnostic tool for detecting BYDV, TBIA has been proven to be a very sensitive and simple procedure (Makkouk et al. 1994). This technique detects low levels of this virus in the plant tissue, avoiding false positives because the shape and localization of the stained dots has a high diagnostic value; therefore it does not require a positive threshold value.

Trials in Quebec in 1981 on 100 accessions of *Aegilops*, mostly *Ae. tauschii* (= *squarrosa*) and on 25 accessions of

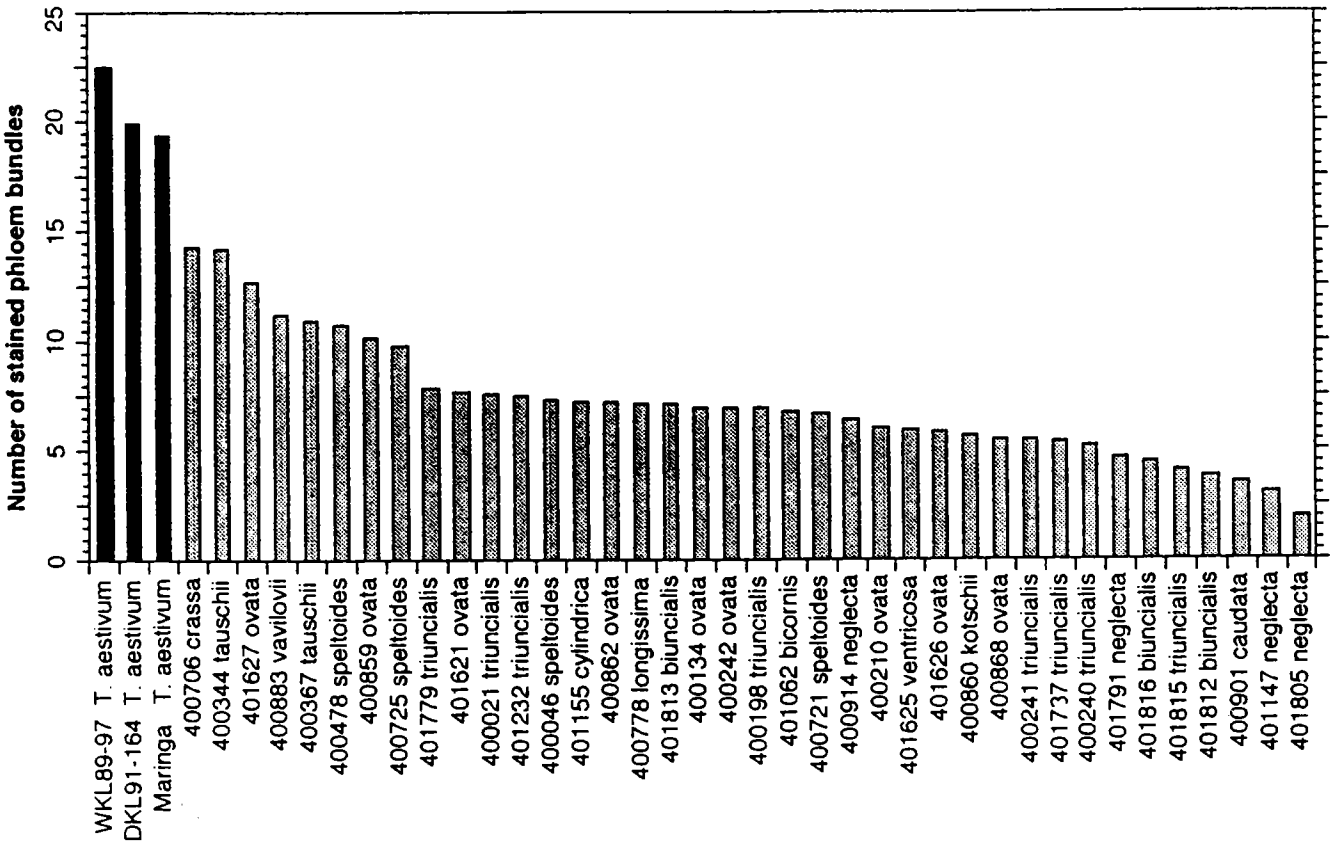

Fig. 2. Average number of stained phloem bundles per stem section of selected accessions from 14 *Aegilops* species when tested by the tissue-blot immunoassay. Each value represents the average of four readings at 5, 7, 11 and 21 d after BYDV-PAV inoculation, with four replications for each date.

Table 1. Comparative resistance to BYDV-PAV and origin of the best performing *Aegilops* accessions and of *Triticum aestivum* "Maringa"

Germplasm	ICARDA accession number	Geographical origin	Number of stained phloem bundles[z] Days after inoculation 5	7	11	21	60	Mean
Aegilops								
caudata	400901	Syria	2	6	3	3	7	4.2
neglecta	401147	Turkey	1	2	9	1	11	4.8
	401791	Bulgaria	5	2	6	7	–	5.0
	401805	Bulgaria	2	0	1	4	7	2.8
biuncialis	401812	Bulgaria	6	2	4	4	7	4.6
	401816	Bulgaria	7	4	4	3	6	4.8
triuncialis	401815	Bulgaria	6	4	2	5	3	4.0
Triticum aestivum "Maringa"		Brazil	32	17	10	18	30	21.4

[z] Each value represents the average number of stained phloem bundles of four stem (including leaf sheath) sections. The stain deposit occurs specifically around virus particles adsorbed on the membrane, so the absence of stained bundles means resistance.

Triticum monococcum and *T. urartu* showed that diploid A and D genome species were highly susceptible to BYDV. The trials at ICARDA (1991 and 1992) revealed that accessions *Ae. neglecta* 401147 and *Ae. triuncialis* 400240 were moderately resistant. These two lines were confirmed as moderately resistant in Sainte-Foy trials (Fig. 2), and have been used in crosses with wheat.

The species identified as resistant possess genomes UM (*Ae. biuncialis* and *Ae. neglecta*), CU (*Ae. triuncialis*) and C (*Ae. caudata*). Chromosome counts are needed to distinguish tetraploid from hexaploid lines within accessions labeled as *Ae. triaristata*. According to some taxonomists, these are different species, *Ae. neglecta* (4 ×) and *Ae. recta* (6 ×), but there is no consensus. The crossing and backcrossing should

be relatively easy with *Ae. triuncialis* and *Ae. caudata*. We are aware of slight difficulties in creating the F_2 with *Ae. neglecta*. This cross was more difficult than the *T. aestivum*/*Ae. tauschii* cross. The rate of F_1 plant regeneration was about 1% in our previous studies (Comeau et al. 1992). The backcrossing difficulty was reduced at Sainte-Foy by the use of bread wheat cultivars AC Mimi and AC Pollet as initial mother plants. Chromosome pairing between A, B, D and the C, U, M genomes may be very low (Riley and Law 1965). Introgression of genes into wheat from resistant accessions of the four species reported in this study should therefore be greatly improved by the use of the high-pairing promoter phlb (Ceoloni et al. 1988), or by other methods aiming at the same goal.

The present study indicates that some *Aegilops* accessions may provide useful sources of BYDV resistance.

ACKNOWLEDGMENT

The BYDV isolate used in this study at Sainte Foy, Quebec, was isolated earlier by C. Cloutier, Laval University, Sainte Foy, Quebec, and identified as a PAV serotype by W.F. Rochow, Cornell University, New York.

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