desired F_1 hybrid that has the *Ph* gene and produce BC_1 *Phph* heterozygote derivatives by crossing the *Ph* F_1 with the Chinese Spring *phph* genetic stock. A PCR-based protocol then allows us to identify the progeny that possesses the *ph* recessive locus, and this becomes a source for multiple homoeologous transfers.

Agrotricum (2n = 8x = 56): potential for barley yellow dwarf virus resistance and its cytogenetics.

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Testing of various germ plasms for resistance to BYDV has been done over the past few years in CIMMYT both in the field and in controlled greenhouse conditions. The germ plasm was comprised of elitc cultivars, Triticeae species of the three gene pools, amphiploids from some intergeneric hybrids, BC_1 self-fertile derivatives of the intergeneric hybrids, and some partial amphiploids. One such partial amphiploid is *Agrotricum* (2n = 8x = 56), which was identified in Canada as being resistant to BYDV. In Mexico, we have obtained similar data to support the resistance and, after studying the cytogenetics of this genetic stock, have initiated a program to produce addition lines, identify addition lines that show resistance, and transfer the resistance genes to our spring wheat cultivars. We report here the cytogenetic progress and BYDV resistance data on the original 56-chromosome stock and its initial backcross derivatives. Appropriate controls were included in the study.

Cytology of *Agrotricum*. Somatic cytology of *Agrotricum* indicated some aneuploidy in the seedlings analyzed where 56-chromosome normal derivatives were present, but plants with 54, 55, and 57 chromosomes and some with telocentrics also were present. Plants with 56 chromosomes were Giemsa C-banded, and the stable lines were analyzed further by FISH. The 56-chromosome, partial amphiploid possessed 14 *Thinopyrum* chromosomes, 40 normal wheat chromosomes, and a pair of wheat chromosomes with a translocation between chromosome 3D of wheat and *Thinopyrum*. The exchange is at the terminal end of 3DL. Such plants were analyzed at meiosis, and all were normal with 29 bivalents at metaphase I and a normal anaphase separation. These plants were seed increased and tested for BYDV resistance. They were all resistant.

The euploid stock then was backcrossed to the susceptible wheat cultivars Prinia and Bagula, which resulted in BC_1 progeny with 2n = 7x = 49 chromosomes. These progenies also tested positive for BYDV resistance. The BC_1 progeny was advanced further by backcrossing to each of the two parental wheat cultivars and also by selfing to eventually identify plants with 43 chromosomes (21 bivalents +1 univalent). From these, 44 chromosome (22 bivalent) derivatives were obtained by selfing of the 43-chromosome plants or by producing 22-chromosome haploids after crosses with maize and then doubling these haploids.

The 44-chromosome progeny was C-banded, and five disomic additions were identified. One of these addition derivatives has a very low BYDV concentration when tested by ELISA, and further work is in progress to introgress the resistance into wheat. Backcross derivatives with 42 chromosomes (possessing the translocated pair) did not possess BYDV resistance.

BYDV screening–virus isolates and BYDV inoculation. The BYDV-PAV isolate used was collected in Mexico and maintained in CIMMYT's greenhouse through transmission by aphids. Inoculation was by infesting 7-day-old seedlings from the *Agrotricum* germ plasm, parental wheat cultivars, and a resistant check (TC14) with 10 viruliferous aphids (*Rhopalosiphum padi*) that had acquired BYDV by feeding on infected plants for 48 hours. The seedlings were isolated from each other by transparent plastic tubes. After a 2- to 5-day period, aphids were killed with Metasystox (Bayer). In each entry, two plants were kept free of aphids to serve as the uninoculated controls.

Enzyme-linked immunosorbent assay (ELISA). The flag-1 leaf was collected at different dates after inoculation for the evaluation of the virus titer by ELISA. Double antibody sandwich ELISA (DAS ELISA) was used with a few modifications. Polystyrene microtiter plates (NUNC) were incubated at 37°C for 3 hours with coating polyclonal antibodies directed against the U.S. isolates provided by K. Perry (Purdue University, W. Lafayette, IN). Plant sap (1:10, in 0.1M phosphate buffer pH 7.0) was incubated for 3 hours at 37°C. Alkaline phosphatase-labeled, polyclonal, anti-PAV antibodies (1:1000) were incubated overnight at 4°C. P-nitrophenyl phosphate substrate (Sigma) was added at a concentration of 1 mg/ml, and the mixture was incubated for 1 to 2 hours at room temperature. Optical density (OD) was measured at 405 nm. A plant was considered infected when the OD was higher than twice that obtained for the

uninfected control. The resistant line had low virus titers, which were equivalent to or slightly less than that obtained with TC14, the resistant check entry.

Summary of results. 1. The average OD was much lower in *Agrotricum* (OK 7211542) than in the susceptible and resistant wheat cultivars used in backcrosses. The values for *Agrotricum* were significantly lower than those of the resistant check (TC14 line) and those of the two susceptible cultivars, Prinia and Bagula (Table 3).

Table 3. Comparison of virus titers in ELISA after inoculation with BYDV-PAV in test wheat germ plasms.

Germ plasm	Average OD
TC14/2*Spear	0.243 ± 0.108
OK7211542	0.113 ± 0.095
Bagula	0.834 ± 0.309
Prinia	0.766 ± 0.301

2. In the backcross derivatives, the low virus titers were conserved. Titers were not significantly different from each other in *Agrotricum* and its backcrosses to Prinia and Bagula but were different compared to those of Prinia and Bagula. The trend is elucidated in Fig. 1.

3. Analysis of advanced-backcross, selfed derivatives has identified one 44-chromosome line possessing low virus titers. All plants of this line are being characterizedcytologically, and seed is being increased. They will be tested further and subjected to cytogenetic manipulation to effect the resistance transfer in order to recover a euploid wheat with 2n = 6x = 42 chromosomes.

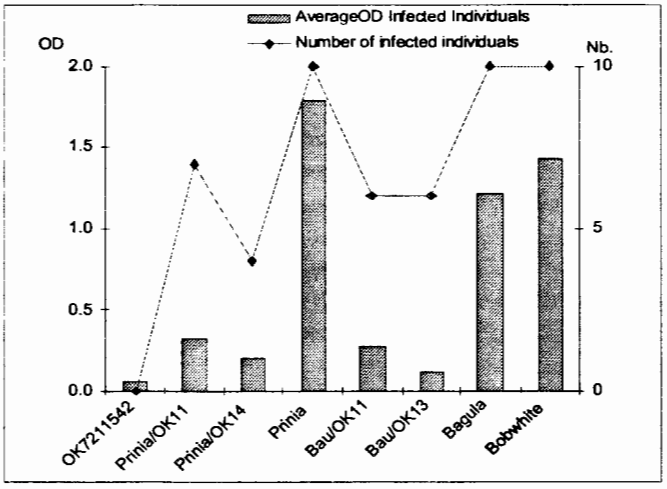

Fig. 1. Virus titers obtained in ELISA for OK7211542 and the BC_1F_1 lines from its backcross to the susceptible wheat Bagula and Prinia. Bau = Bagula, Bow = Bobwhite, OK11 and OK14 = OK7211542 plant numbers 11 and 14 where each had 2n = 8x = 56 chromosomes.

Thinopyrum bessarabicum (2n = 23x = 14, JJ) disomic chromosome addition lines in bread wheat: current germ plasm status.

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Thinopyrum bessarabicum is a self-fertile, maritime grass possessing salinity tolerance and resistance to wheat scab. These important abiotic and biotic characteristics make *Th. bessarabicum* an important Triticeae species to exploit for wheat improvement. We have been producing addition lines of the species in bread wheat. The addition lines were made in a mixed-wheat background (Chinese Spring/*Th. bessarabicum*//Genaro). For the homoeologous group 3, a homozygous 3JL, disomic addition line also was extracted.

The above disomic addition lines having *Th. bessarabicum* (J or Eb) chromosomes in a *T. aestivum* background (2n = 44; 21 II ABD + 1 II J) were analyzed using both AFLPs and RAPDs. Among the J-specific, AFLP fragments amplified from 32 selective amplification primer pairs, 195 fragments were single-chromosome specific. These included 44 AFLP markers for 1J, 46 for 2J, 39 for 4J, 37 for 5J, and 29 for 7J. Although no AFLP markers were specific for 3JL alone, we identified two RAPD markers specific to this chromosome arm. In addition, there were two RAPD markers for 1J, two for 2J, six for 4J, one for 5J, and two for 7J. Fifty-nine AFLP and two RAPD J-specific markers were present in the amphiploid but absent in all tested CIMMYT disomic addition lines making them potential putative markers for 6J or 3JS. The 50 AFLP markers and four RAPD markers were present in all (or at least five) J chromosomes. CIMMYT-derived 2J and 5J addition lines are distinguishable from those originating from the U.K. by 22 and 27 genotype-specific AFLP markers, respectively. All these molecular markers, whether genotype-, chromosome- or genome-specific, are useful in monitoring the introgression of J-chromosomal segments into wheat chromosomes.

Field testing of these addition lines for scab in particular posed a constraint associated with lateness of the germ plasm and also was a constraint for the salinity tests. Some instability of the lines also was encountered. Hence, an elite