Barley Chromosome Location and Expression of Dwarf Bunt Resistance in Wheat Addition Lines

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ABSTRACT

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Barley (*Hordeum vulgare*) is resistant to dwarf bunt of winter wheat (*Triticum aestivum*) caused by *Tilletia controversa*. Nine wheat-barley addition lines were utilized to determine which barley chromosomes and chromosome arms carry resistance genes. The lines included six disomic addition lines, WB1, WB2, WB3, WB4 WB6, and WB7, and three ditelosomic addition lines, WB5S (containing the short arm of barley chromosome 5), WB6S (containing the short arm of barley chromosome 6), and WB6L (containing the long arm of barley chromosome 6). These lines, their parent cultivars, and susceptible winter wheat cv. Wanser were inoculated with spores of *T. controversa* at the two-leaf stage. The barley parent, Betzes, showed no infection and only 5 of 401 heads of addition line WB6 were infected. Lines WB1, WB2, WB3, WB4, and WB7, and cvs. Wanser and Chinese Spring showed a high incidence of infection. WB6S had significantly lower bunt incidence (0 to 1%) than WB6L or WB5S. Lines WB6L and WB5S had a high incidence of infection. This suggests that the short arm of barley chromosome 6 carries the dwarf bunt resistance gene(s). The incidence of bunt infection in wheat-barley hybrid plants was examined. Hybrids were regenerated from embryo rescue followed by F_1 inflorescence culture of wheat-barley crosses and by embryo rescue followed by induction of somatic embryos directly from culture of the immature embryos. Both methods resulted in production of amphiploid plants as well as haploid hybrids (n = 28). Infection incidence in crosses with zero, one, and two copies of the barley genome in a wheat background was 64, 30, and 1%, respectively, suggesting a dosage effect associated with the resistance gene(s). The female wheat parent influenced the expression of barley resistance gene(s) in these wheat-barley hybrids.

Dwarf bunt of winter wheat, *Triticum aestivum* L., caused by *Tilletia controversa* Kühn in Rabenh., is an economically damaging disease (19). Winter barley (*Hordeum vulgare* L.) may be a source of resistance for winter wheat. It would be desirable to have new sources of resistance genes. All current resistant wheat cultivars owe their resistance mainly to the wheat introduction, PI178383 (4,5). Several studies have described genetic resistance in wheat (7,11,12,16), but none has addressed the genetics of dwarf bunt resistance in barley. Identifying the barley chromosome(s) carrying resistance and demonstrating expression of this resistance in a wheat background would aid efforts to transfer it to winter wheat. Alien addition lines of wheat have been used to locate the chromosomes that carry genes of interest in a variety of plant species (2,6,9,10). Most of these lines have enabled determination of the chromosomal location of genes encoding various isozymes. Dhaliwal et al. (6) investigated the chromosomal location of a gene for Karnal bunt resistance, but there has been no work identifying barley chromosomes that may contain resistance genes for dwarf bunt.

The objectives of this study were to determine the chromosomal location of genes that confer resistance to dwarf bunt in barley, to determine if the genes would be expressed in a wheat background, to determine if there is a gene dosage effect to expression, and to determine if the expression of the resistance is influenced by the wheat parent background.

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MATERIALS AND METHODS

Six wheat-barley disomic addition lines, obtained from K. J. Kasha, University of Guelph, Canada, and A. K. M. R. Islam, University of Adelaide, Australia, were grown in the greenhouse. Single seeds were planted to 100-mm-square pots containing a mixture of one part vermiculite, one part peat moss, and one part perlite. Susceptible winter wheat cv. Wanser, the barley parent, Betzes, and spring wheat parent Chinese Spring served as controls in 1992. In 1993, ditelosomic wheat-barley addition lines WB6S (short arm), WB6L (long arm), and WB5S (short arm) were also grown in the greenhouse. Disomic addition line WB6 and Chinese Spring were used as controls. Ditelosomic addition lines were obtained from N. Lapitan, Colorado State University, Fort Collins, and from A. K. M. R. Islam, University of Adelaide, Australia. Addition line WB5 could not be obtained since the long arm of chromosome 5 of the barley parent Betzes contains a genetic element that causes abnormalities in meiosis resulting in sterile plants (14). This chromosome could not be evaluated for the presence of the resistance gene(s).

Gene dosage effects were determined by studying the expression of resistance in haploid wheat (n = 21), euploid wheat (2n = 42), wheat-barley hybrids (2n = 28), and wheat-barley amphiploids (2n = 56). Haploid and euploid wheat lacked barley chromosomes, but the hybrids and amphiploids had one and two sets of barley chromosomes, respectively. Wheat and barley cultivar effects were determined by studying bunt reaction in four wheat × barley cross combinations in both 1991 and 1992: Fukohokamuji × Luther, Fukohokamuji × Boyer, Wanser × Luther, and Wanser × Boyer. Spring wheat cv. Fukohokamuji, and winter barleys Boyer and Luther, were utilized as female and male parents, respectively, because of their cross compatibility (15). Later, Wanser was also utilized as a female parent. Wheat-barley hybrids and amphiploids were generated by two separate methods. One method was F_1 embryo rescue (15) followed by immature inflorescence culture of the rescued F_1 plants. Somatic regeneration was also accomplished by culture of embryonic calli from rescued F_1 embryos.

Embryo culture. Sixteen days after pollination, immature kernels were collected, surface sterilized with 20% (vol/vol) commercial bleach (1.05% $NaOC1_2$) for 12 min, and rinsed in sterile, distilled water four times. Immature embryos were then excised and placed on each of the following media: Murashige-Skoog (MS) salts + 0.5 mg liters^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid) + 0.5 mg liters^{-1} kinetin; orchid medium; orchid medium + 2 mg liters^{-1} kinetin and MS salts + kinetin 2 mg liters^{-1}. All media contained 3% sucrose and 0.75% agar and were adjusted to a pH of 5.7. As embryogenic callus was induced, it was subcultured for proliferation of embryos or regeneration of plants. All culture conditions and transplant methods were the same as for inflorescence culture.

Inflorescence culture. F_1 seedlings from embryo rescue, as described by Lizarazu

(15), were grown in the greenhouse. Unemerged 10- to 20-mm-long inflorescences (spikes) were removed and kept at 4°C in the refrigerator for 3 to 5 days. The spikes were surface sterilized with 70% ethanol for 1 min, followed by a 40% (vol/vol) solution of commercial bleach (2.1% $NaOCl_2$) for 12 min, and rinsed four times in sterile, distilled water. The spikes were excised aseptically under a stereomicroscope and cut into sections. The sections were placed on media containing MS salts, 1 mg liters^{-1} 2,4-D, 0.1 mg liters^{-1} kinetin, and 2% (wt/vol) sucrose, and incubated at 26/23°C day/night temperatures with a 14-h photoperiod. The medium was replaced after 4 weeks, and the sections with developing callus were maintained for another 4 weeks. At the end of 8 weeks, selected calli were moved to maintenance and regeneration media for the development of embryos and plant regeneration. Regenerants without roots, or those too weak to be transplanted into the soil, were transferred to rooting or growth medium. Calli with poor shoots were subcultured on MS shoot multiplication medium A (Sigma Chemical Co., St. Louis, MO) to improve shoot regeneration; these regenerants often required one more subculture on rooting medium for satisfactory rooting. All other aspects of the rooting medium were the same. Finally, regenerants were transplanted into pots containing equal parts peat moss, perlite, and vermiculite. Pots were covered with plastic film and placed in the greenhouse. As plants became established, the plastic film was removed.

Regenerants that were to have chromosomes doubled were treated with colchicine very soon after root initiation. Regenerants were treated by transferring them to rooting media with 0.02% colchicine added. They were exposed to colchicine in the medium for 3 to 5 days.

Chromosome counts were made on five root tips of each plant from all addition lines and regenerant lines. Counts were also made on 10 root tips from each plant treated with colchicine. Mitotic squashes were prepared according to Hsiao et al. (13).

One week after transplanting, the regenerants were inoculated as described. A suspension of non-germinating spores was used for all of the experiments. Plants were inoculated at the one- or two-leaf stage of growth (stages 11 to 12) (20). Spores of *T. controversa* were collected from the dwarf bunt nursery at the Utah State University, Greenville Farm. A mixture of races, as determined by infection of bunt-resistant differential lines, was used. Spores were applied in suspension (0.5 g of spores + 20 ml of water). Sufficient soil in the pot was removed to expose plant crowns, 0.05 ml of the spore suspension was injected into a point 5 mm above the crown, and the remainder of the suspension was sprayed on the crown area. The soil was then replaced around the base of the plants.

Table 1. Comparison of mean bunt incidence in wheat-barley disomic addition lines in two experiments

Addition line/cultivar[y]	Total heads	Bunted heads (%)[z]	Total plants	Bunted plants (%)[z]
1991				
WB6	32	0 a	7	0 a
Wanser	47	20 b	11	36 b
WB4	24	21 b	7	29 b
WB2	140	29 b	10	50 b
WB3	84	51 b	14	50 b
WB1	38	58 b	6	67 b
WB7	46	59 b	7	57 b
1992				
WB6	38	3 a	7	14 a
WB7	19	48 b	3	100 b
WB4	26	62 bc	3	100 b
WB2	20	63 bc	4	75 b
Wanser	47	78 bc	10	80 b
Chinese Spring	110	95 c	10	100 b
WB3	39	99 c	4	100 b
WB1	6	100 c	1	100 b

[y] WB# = wheat-barley disomic addition line, the number is the barley chromosome pair that is added to the wheat line.
[z] Numbers within columns and years that are followed by a letter in common are not statistically different ($P = 0.05$) as calculated by the Ryan-Einot-Gabriel-Welsch multiple range test.

Table 2. Comparison of mean dwarf bunt incidence in wheat-barley ditelosomic addition lines WB6S, WB6L, WB5S, and cv. Chinese Spring

Addition line/cultivar[y]	Total heads	Bunted heads (%)[z]	Total plants	Bunted plants (%)[z]
Colorado				
WB6S	89	1 a	11	9 a
WB6	72	1 a	7	14 a
WB6L	130	95 b	11	100 b
Australia				
WB6S	44	0 a	8	0 a
Chinese Spring	103	59 b	12	100 b
WB6L	91	72 b	10	100 b
WB5S	127	86 b	11	91 b

[y] WB# = wheat-barley disomic addition line, the number is the barley chromosome pair that is added to the wheat line.
[z] Numbers within columns and source location that are followed by a letter in common are not statistically different ($P = 0.05$) as calculated by the Ryan-Einot-Gabriel-Welsch multiple range test.

Table 3. Comparison of mean dwarf bunt incidence for wheat and wheat-barley hybrids with different barley genome doses

Wheat/barley cross regenerants	Chromosomes (no.)	Copies of barley genome	Total heads	Bunted heads (%)[z]	Total plants	Bunted plants (%)[z]
1991						
Wheat/barley amphiploids	56	2	95	1 a	7	14 a
Wheat/barley hybrids	28	1	452	37 b	19	90 b
Wheat	42	0	24	54 bc	1	100 b
Haploid wheat	21	0	253	64 c	9	100 b
1992						
Wheat/barley amphiploids	56	2	167	1 a	14	7 a
Wheat/barley hybrids	28	1	529	27 b	24	71 b
Wheat	42	0	160	61 c	12	92 bc
Haploid wheat	21	0	389	65 c	18	92 c

[z] Numbers within columns and years that are followed by a letter in common are not statistically different ($P = 0.05$) as calculated by the Ryan-Einot-Gabriel-Welsch multiple range test.

Plants were scored for disease infection at maturity. Because dwarf bunt is a systemic disease, tillers that are infected usually produce uniformly bunted heads. Each tiller must be infected separately, however. A plant was considered infected if a single head of the plant was bunted. In addition, separate measurements were made of numbers of individual heads that were bunted.

RESULTS

Only plants in wheat-barley addition line WB6 remained free of bunt in the 1991 experiment (Table 1). In 1992, one of seven plants of WB6 was slightly infected but the other five addition lines, Wanser, and Chinese Spring were all heavily infected. The barley parent Betzes showed no infection. WB6S displayed high resistance to dwarf bunt, whereas WB6L was susceptible in subsequent tests (Table 2). Results for WB6S and WB6L from Australia were similar to results for materials from Colorado. No WB6S plants were infected, whereas WB6L, WB5S, and Chinese Spring were highly infected. Bunt incidence for WB6L, WB5S, and Chinese Spring ranged from 59 to 86% for head counts and from 91 to 100% for plant counts, which was higher than the incidence for WB6S.

Time required for immature F_1 embryo culture was shorter than that required for F_1 inflorescence culture. Embryo culture also produced more hybrid progenies. Dwarf bunt incidence was highest in haploid wheat and was slightly lower in euploid wheat in both 1991 and 1992 (Table 3). Barley-wheat hybrids (n = 28), were less susceptible than haploid wheat, and amphiploids (2n = 56), were highly resistant to dwarf bunt (Fig. 1). Only 1 out of 95 heads in 1991 and 1 out of 167 heads in 1992 were infected. Root chromosome counts from the amphiploid plants indicated that many of the plants were mixoploids with some tillers of the plant having 56 chromosomes and some having 28 chromosomes. In 1991, 21% of the hybrids were mixoploids, while in 1992 70% were mixoploids. All of the heads of these plants, with the exception of a single head in 1991 and in 1992, were uniformly resistant.

There were differences in the level of resistance for crosses depending on the wheat cultivar used as a female in the cross (Table 4). Crosses involving Wanser as female parent were more resistant than crosses involving Fukohokamuji. No significant differences were detected between the two barley cultivars, Boyer and Luther, that were used as the male parents.

DISCUSSION

Our results suggest that a major dwarf bunt resistance gene is located on the short arm of barley chromosome 6. In addition, bunt infection depended upon the number of copies of the barley genome present in the hybrid. These results suggest that there was a dosage effect associated with resistance. Head count data from both years indicate a nearly additive effect. Plant counts indicate that at least two doses of the barley genome are required for the most effective resistance (no bunted kernels). We have presented data on bunt incidence both on plant and head counts. Plants that are susceptible are expected to have all tillers uniformly bunted. We have found that this is not always the case. Tillers that are bunted do produce completely bunted heads. Only rarely did we observe partially bunted heads as is somewhat more common in the field even in completely susceptible cultivars. Plants that have a low percentage of bunted tillers result from either tiller escapes or from some partial resistance. We have been unable to determine which of these possibilities is more probable, but both of these possibilities must be kept in mind when interpreting the data.

Some of the amphiploid plants had varying numbers of chromosomes. Frequently only part of an amphiploid plant had the full complement of chromosomes (2n = 56) as measured by root tip counts. Such plants still expressed high resistance to dwarf bunt throughout all tillers. This may be an indication that gene(s) products are mobile and systemic, thus conferring resistance to the entire plant. This is only inferential, and no other evidence exists to confirm this, though the possibility is intriguing. It is also possible that root tip chromosome counts did not adequately reflect chromosome numbers throughout the entire plant. To verify these numbers, chromosome counts would need to be made in the leaves and heads of the tillers.

Our crosses between wheat and barley with different parents indicated that the expression of the resistance gene(s) from the barley parents was influenced by the wheat genetic background. This was only observed in the barley-wheat hybrids (n = 28) when one copy of the barley and wheat genomes was present. Only two wheat parents were used but it is not unexpected that different wheat parents might affect the expression of the barley gene(s) for resistance to dwarf bunt, as Chen and Line (3) reported that epistatic effects between genes for resistance to stripe rust in wheat were dependent on the wheat genetic background. At the same time, the lack of any observable differences in the resistance conferred by the two barley parents cannot be construed to indicate that there are no cultivar differences for the resistance genes, although we detected none.

Genes for resistance to leaf rust, stem rust, and Hessian fly have been successfully transferred to and expressed in hexaploid wheat from *Secale cereale*, *Aegilops ventricosa*, and *Agropyron elongatum* (1,8,

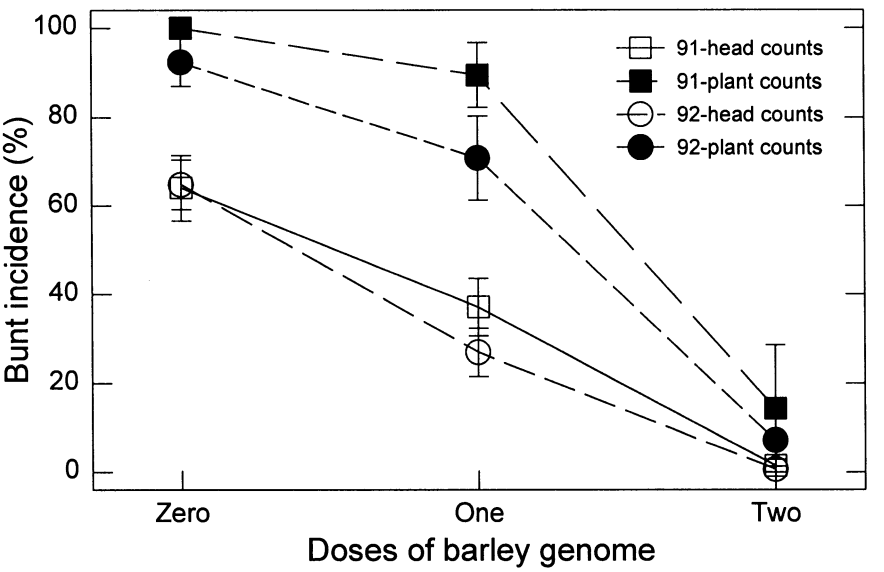

Fig. 1. Barley chromosome dosage effects on dwarf bunt infection.

Table 4. Comparison of mean bunt incidence in different cross combinations

Wheat/barley cross combination	Total heads	Bunted heads (%)[z]	Total plants	Bunted plants (%)[z]
Fukohokamuji × Boyer	909	50 a	39	82 a
Fukohokamuji × Luther	912	46 ab	37	87 a
Wanser × Boyer	398	32 bc	19	58 a
Wanser × Luther	295	22 c	14	71 a

[z] Numbers within columns that are followed by a letter in common are not statistically different (P = 0.05) as calculated by the Ryan-Einot-Gabriel-Welsch multiple range test.

17,18). The barley resistance gene(s) to dwarf bunt represent a potentially valuable source to expand the genetic base of resistance genes in wheat.

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