Chromosomal Location of the Powdery Mildew Resistance Gene of Amigo Wheat

M. Heun, B. Friebe, and W. Bushuk

First and second authors, plant geneticist and cytogeneticist, respectively, Institute of Agronomy and Plant Breeding, D-8050 Freising-Weihenstephan, West Germany. Third author, NSERC research professor, Food Science Department, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

Present address of first author: Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853-1902.

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ABSTRACT

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Powdery mildew resistance of Amigo wheat has been analyzed and mapped by combining F_2 segregation data with cytogenetic and storage protein analyses. It is concluded that the powdery mildew resistance of Amigo is regulated by one gene with conditioned dominance. Cytogenetic and electrophoretic analyses revealed that the complete wheat chromosome arm 1AS is missing in Amigo and has been replaced by the rye chromosome arm 1RS. No susceptible recombinant was found among 1,034 F_2 plants of crosses of Amigo with *Pm3* resistant lines; *Pm3* is known to be located on 1AS. Thus, the powdery mildew resistance gene of Amigo is assumed to be located on the rye chromosome 1RS translocated to wheat chromosome 1AL. The resistance pattern of Amigo is different from that of lines carrying gene *Pm8*. Free segregation of the Amigo resistance gene and *Pm8* (located on 1RS of 1RS·1BL translocations) occurred. On the basis of these results, we propose the gene symbol *Pm17* for the Amigo powdery mildew resistance gene.

Additional keywords: Triticum aestivum, wheat-rye translocations.

Amigo is a hexaploid wheat (*Triticum aestivum* L.) cultivar known to carry an 1AL·1RS wheat-rye chromosome translocation (13,19,21). This cultivar (selected by Sebesta and co-workers) was developed by crossing advanced hexaploid wheat lines with the octoploid triticale Gaucho, treating the obtained seed with X-rays and reselecting for a wheat phenotype (19). Amigo expresses resistance both to insects (e.g., greenbug) and diseases (e.g., powdery mildew and leaf rust) (22).

TABLE 1. Characterization of the wheat powdery mildew isolates used to identify *Pm1* to *9* and *Mlk*[a]

Isolate no.	Virulence//avirulence formula													
6	1	2	(3a)*	3c	4a	4b	5*	6	7	k	//	3b	8	9
9a	2	3a	3c	4a	4b	5	6	7	8	//	1	3b	k*	
85063	1	4a	4b	5	(6)	7	8	//	2	3a	3b	3c	k	
85135	1	2	4a	4b	(5)	6	7	9	//	3a	3c	3c	8	k
W72/27	1	2	(5)	(6)	7*	9	//	3a	3b	3c	4b	4b	8	k

[a] Intermediate reactions (marked by *) indicate incomplete virulence of the isolate against the respective resistance gene. All five powdery mildew isolates are avirulent to the Amigo powdery mildew resistance gene.

Lowry et al (14) found that the resistance of Amigo to *Erysiphe graminis* D.C. ex. Merat f. sp. *tritici* Em. Marchal is conditioned by one dominant gene. In crosses of Amigo with a wheat line carrying gene *Pm3a* known to be on wheat chromosome 1AS (2,16), strong linkage was found since only two susceptible recombinants occurred among 375 F_2 plants (14). This result indicates that the powdery mildew resistance gene of Amigo is located on 1AS. However, Amigo is a 1AL·1RS translocation line (19) and lacks 1AS. To resolve this apparent discrepancy, we repeated the experiments of Lowry et al (14) on a broader basis. In addition, Giemsa C-banding and storage protein analyses were carried out in order to confirm the chromosomal constitution of this line.

MATERIALS AND METHODS

Seeds of the near-isogenic Chancellor lines Chul/8*Chancellor (Chul/8CC), Asosan/8*Chancellor (Asosan/8CC), Sonora/8*Chancellor (Sonora/8CC), developed by Briggle (1), were provided by J. G. Moseman, Beltsville, MD. Seeds of the West German winter wheat cultivars Disponent and Kanzler were obtained from the collection held by the Bundessortenamt, Hannover, West Germany. Seed of Amigo was taken from the collection of the Institute of Agronomy and Plant Breeding, Weihenstephan, West Germany.

Single-pustule-derived powdery mildew isolates were used to characterize host lines and they included: Nos. 6 and 9a collected in West Germany by the first author; isolates Nos. 85063 and 85135 provided by P. M. Fried, Zürich, Switzerland, and isolate W72/27 provided by W. Summers, Cambridge, UK. These powdery mildew isolates were characterized against *Pm1* to *Pm9* and *Mlk* as shown in Table 1. Genes *Pm10, 11, 14, 15* are not effective against *Erysiphe graminis tritici* (16; McIntosh, *personal communication*) and were not considered here. Gene *Pm12* (located on 6A) and *Pm16* (located on 4A) are part of the commercial activities of the Institute of Plant Science Research, Cambridge Laboratory (IPSR)/Agricultural Genetics Company Ltd. (agc) and thus were not considered here, too. Gene *Pm13* (located on 3B) has been developed recently and could not be considered either.

The methods used for inoculation and disease assessment are those described by Heun and Fischbeck (9,10). For the F_2 analysis, two identical experiments were prepared for each cross combination, and each experiment was arranged in six specific petri dishes (containing 10 ml of 0.5% agar and 5 ppm of benzimidazole). Each experiment contained one of the two 3-cm-long leaf segments cut from each F_2 plant. In addition, two 3-cm-long leaf segments were cut from single plants of both parents (25 plants each) and the F_1 (five plants) and placed into the two experiments per F_2 analysis. Leaves of the two experiments of each of the five cross combinations were inoculated with the same or with different powdery mildew isolates using a settling tower. The inoculation densities ranged from 230 to 450 spores/cm^2 of leaf area.

Ten days after inoculation, disease assessment was done by observing infection type (ranging from 0 to IV), infection grade (0–9), and pustule size (three classes). These data were combined to form three groups, i.e., r = resistant, i = intermediate, and s = susceptible host reactions (9,10).

Chromosome identification was carried out according to the Giemsa C-banding technique described by Giraldez et al (7). The composition of storage proteins was analyzed by polyacrylamide gel electrophoresis (PAGE) without and with sodium dodecyl sulfate (SDS-PAGE) according to Sapirstein and Bushuk (18) and Ng and Bushuk (17), respectively.

RESULTS

For verification of the presence or absence of 1AS and 1RS, C-banding and protein analyses were performed, as all chromosomes of wheat can be identified according to their characteristic C-banding patterns (Fig. 1). (Chromosomes 4A and 4B were rearranged as was approved at workshop I at the 7th International Wheat Genetics Symposium, Cambridge, 1988.) The rye chromosome segment 1RS can be distinguished from chromosomes of wheat by the presence of characteristic large terminal and subterminal C-bands. In addition, a faint band usually seen as two dots adjacent to the large subterminal C-band was observed in most chromosomes analyzed. The presented C-banding pattern of Amigo (Fig. 1) clearly shows that the rye chromosome segment 1RS is translocated to the long arm of wheat chromosome 1A which is only marked by a terminally located C-band. No evidence was obtained for the presence of the short arm of chromosome 1A in Amigo. The C-banding patterns of the chromosomes 7A and 7B indicate that these chromosomes are involved in a reciprocal translocation, the breakpoints being located in their short arms.

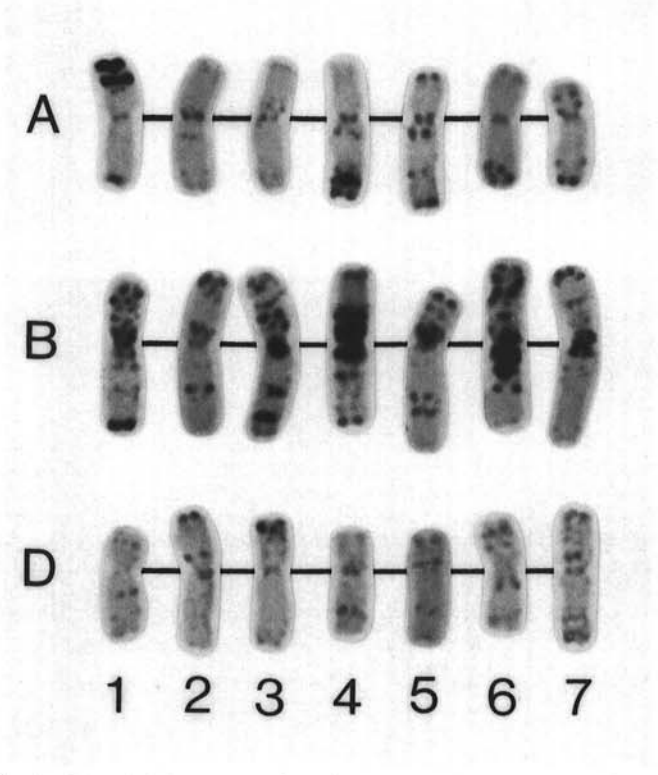

Fig. 1. C-banded karyotype of Amigo wheat. The short arm of rye chromosome 1R present as a 1AL·1RS wheat-rye translocation can be identified by large terminal and subterminal C-bands.

Analysis of group 1 storage protein composition of Amigo was carried out by PAGE and SDS-PAGE gel electrophoresis for confirming the chromosomal constitution of this line (Fig. 2). Whereas no evidence was obtained for the present of *Gli-A1* locus proteins, located on 1AS (16) (Figure 2, left three lanes), the SDS-PAGE electrophoregrams (Fig. 2, right three lanes) clearly show the presence of *Glu-A1* locus proteins (subunit 2*) located on 1AL (16) in Amigo.

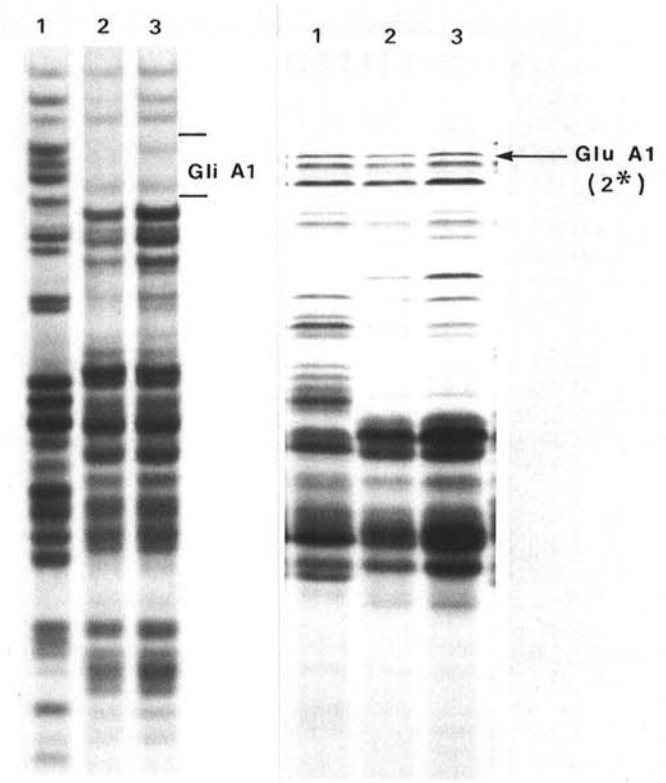

Fig. 2. Polyacrylamide electrophoregrams (left three lanes) are showing the absence of *Gli-A1* locus proteins in Amigo. The presence of *Glu-A1* locus proteins (subunit 2*) in Amigo is shown by the SDS-PAGE electrophoregrams (right three lanes). Lane 1 = Neepawa (standard reference); lanes 2 and 3 = Amigo.

TABLE 2. Disease reaction (r = resistant, i = intermediate, and s = susceptible) of P_1, P_2, F_1, and F_2 wheat plants of the cross Amigo × Kanzler after inoculation with powdery mildew isolate No. 9a

Cross	Generation	Disease reaction			
		r	i		s
Amigo × Kanzler	P_1	25[a]	0		0
	P_2	0	0		25
	F_1	0	→	5[b]	←
	F_2	65	→	190	←

[a] Number of plants in the respective class.
[b] Plants are combined into an intermediate, susceptible class.

The number of genes responsible for the powdery mildew resistance of Amigo was analyzed in the F_2 of the cross Amigo × Kanzler (Table 2); Kanzler is a West German winter wheat cultivar with no powdery mildew resistance gene (9).

These F_2 data indicate that the powdery mildew resistance of Amigo is conditioned by one gene with incomplete dominance for susceptibility. Since the F_1 and the P_2 reactions were very similar, a combined class (intermediate, susceptible) was formed. The observed segregation ratio is 65 (resistant):190 (intermediate, susceptible) and fits well the 1 r:3 i,s hypothesis ($\chi^2 = 0.03$) for this assumption.

For testing the relationship between the Amigo powdery mildew resistance gene and *Pm8* we analyzed the cross Amigo into Disponent; Disponent is a West German winter wheat cultivar carrying *Pm8* (9). The combined data of two experiments are shown in Table 3. Despite the complication caused by the incomplete resistance of Disponent (we formed a combined resistant/intermediate class to deal with this problem), the number of highly susceptible plants is in accordance with the hypothesis of two unlinked genes, i.e., with one gene of Amigo and the other of Disponent (*Pm8*). The observed segregations of 239 r,i:16 s and 243 r,i:17 s fit well the 15 r,i:1 s hypothesis ($\chi^2_1 = 0.00$, $\chi^2_2 = 0.04$) in both cases. The Amigo powdery mildew resistance is expressed as dominant in relation to the powdery mildew isolates Nos. 6, 85135, and W72/27, whereas it is not dominant in relation to powdery mildew isolate No. 9a as mentioned previously.

We continued these studies by analyzing the crosses of Amigo × Asosan/8CC (*Pm3a*) in two independent F_2 experiments, each consisting of two identical designs (Table 4). Among a total of 505 F_2 plants, a susceptible plant was not observed. The F_1 plants did not always show the very high degree of resistance of both parents (some were close to intermediate), but taking both assessments per plant into account, all F_1 and F_2 plants were clearly resistant.

The analyses were continued by checking the crosses of Amigo × Chul/8CC (*Pm3b*) and Amigo × Sonora (*Pm3c*) (Table 5). The crosses are similar; in both cases, the Amigo powdery mildew resistance gene is crossed with a *Pm3* allele, i.e., *Pm3b* in the first cross and *Pm3c* in the second cross, and no susceptible recombinants resulted. Together with the F_2 plants of the cross of Amigo × *Pm3a* not one susceptible recombinant was observed among 1,034 F_2 plants.

DISCUSSION

Cytogenetic and storage protein analysis of Amigo. The C-banding pattern of the A-, B-, and D-genome chromosomes of Amigo observed in the present study is similar to that described for other cultivars of hexaploid wheat (3,5,6,15). The rye chromosome arm 1RS can be distinguished easily from wheat chromosomes because it is marked by large terminal and subterminal C-bands (20). The rye chromosome arm 1RS of Amigo is identical in C-banding pattern with the rye segment present in the 1BL·1RS wheat-rye translocation lines (4). The C-banding analysis of

TABLE 3. Disease reaction of P_1, P_2, and F_2 wheat plants of the cross Amigo × Disponent (*Pm8*), determined in two experiments consisting of two designs[a]

Cross	Generation	Disease reaction with powdery mildew isolate no.							
		W72/27				6/85135			
		r[b]		i	s	r		i	s
Amigo × Disponent	P_1	25[c]		0	0	25		0	0
	P_2	→	25	←	0	→	25	←	0
	F_2	→	239	←	16	→	243	←	17

[a] The plants were classified according to their reaction with powdery mildew isolate W72/27 after two independent inoculations (left) and after two independent inoculations with powdery mildew isolate Nos. 6 and 85135 (right).
[b] r = resistant, i = intermediate, s = fully susceptible.
[c] Number of plants.

Amigo confirms earlier reports that this cultivar carries a 1AL·1RS translocation. By analyzing meiotic chromosome pairing in F_1 hybrids between Amigo and ditelocentric or double-ditelocentric lines of Chinese Spring and Chinese Spring/Imperial wheat-rye addition lines, Zeller and Fuchs (21) showed that the short arm of chromosome 1A of Amigo had been replaced by the rye chromosome arm 1RS. By using in situ hybridization of a dispersed rye DNA probe and Giemsa C-banding, Lapitan et al (13) showed that the breakpoint of the 1AL·1RS translocation of Amigo is located within the centromeric region. This was also confirmed by C-banding analysis of Schlegel and Kynast (19); however, their C-banding pattern of the translocated 1AL·1RS chromosome of Amigo differs from that reported here. Because the subterminal marker band of 1RS in the karyotype of Amigo published by Schlegel and Kynast (19) was only weakly stained in contrast to the large C-band reported here, the difference is probably caused by lower resolution of the C-banding technique applied by those authors.

Our cytological data concerning the presence of 1RS and the absence of 1AS also agree with the analysis of storage protein composition of Amigo. The protein band controlled by the *Glu-A1* locus known to be located on the long arm of chromosome 1A (16) is present, while the protein band controlled by the *Gli-A1* locus located on the short arm of chromosome 1A (16) is missing.

Two additional cytogenetic aspects require further comment. First, the C-banded karyogram of Amigo shows the presence of a reciprocal translocation involving the short arms of chromosomes 7A and 7B. Since the breakpoints of this translocation are located in interstitial regions of the short arms of these chromosomes, it is highly probable that the translocation was induced by the X-ray treatment used to produce Amigo. The presence of a translocation involving chromosomes 7A and 7B also was reported by Hollenhorst and Joppa (12) and Zeller and Fuchs (21).

TABLE 4. Disease reaction of P_1, P_2, F_1, and F_2 wheat plants of the cross Amigo × Asosan/8CC (*Pm3c*), determined in two experiments, and combined[a]

Cross	Generation	Disease reaction with powdery mildew isolate No. W72/27		
		r[b]	i	s
Amigo × Asosan/8CC	P_1	50[b]	0	0
	P_2	50	0	0
	F_1	12	0	0
	F_2	505	0	0

[a] The plants were classified according to their reaction with powdery mildew isolate W72/27 after four independent inoculations.
[b] r = resistant, i = intermediate, s = susceptible.
[c] Number of plants.

TABLE 5. Disease reaction of P_1, P_2, F_1, and F_2 wheat plants of the crosses Amigo × Chul/8CC (*Pm3b*) and Amigo × Sonora/8CC (*Pm3c*)[a]

Cross	Generation	Disease reaction		
		r[b]	i	s
Amigo × Chul/8CC	P_1	25[c]	0	0
	P_2	25	0	0
	F_1	5	0	0
	F_2	216	0	0
Amigo × Sonora/8CC	P_1	25	0	0
	P_2	25	0	0
	F_1	6	0	0
	F_2	313	0	0

[a] The plants were classified according to their reaction with powdery mildew isolate Nos. 6 and 85135 (upper cross) and Nos. 85063 and 85135 (lower cross) after independent inoculations with each isolate.
[b] r = resistant, i = intermediate, s = susceptible.
[c] Number of plants.

Second, the meiotic analyses of Hollenhorst and Joppa (12) and Zeller and Fuchs (21) indicated the presence of another wheat-rye translocation in Amigo involving the chromosomes 6A and 6B. Our results (Fig. 1) do not confirm the presence of a 6A·6B translocation in Amigo since neither chromosome 6A nor 6B differs in morphology and in C-banding pattern from that of the corresponding chromosomes of Chinese Spring. However, it should be pointed out that only structural rearrangements involving chromosome regions that carry marker bands can be detected by C-banding analyses. Therefore, rearrangements involving small unbanded chromosome regions can not be detected by this technique.

Segregation analyses of F_2 data. The genetic analyses show that the Amigo resistance is conditioned by one gene inherited independently from *Pm8*. This is in accordance with the results of Lowry et al (14). Our paper is the first report that the powdery mildew isolates influence the expression of dominance of the gene in Amigo, but this is not surprising, because conditioned dominance has already been reported for this host-pathogen system before (8). The results with powdery mildew isolate No. 9a are especially important. With this isolate, a 1 r:3 i,s segregation resulted from the cross Amigo × Kanzler. This complicated the analyses of the cross Amigo × Chul/8CC (*Pm3b*) when inoculated with powdery mildew isolate No. 9a: The five F_1 plants we analyzed (data not given) showed i,s reaction. Therefore, susceptible F_2 plants indicating homologous crossing (as suggested by the results of Lowry et al [14]) would be hard to distinguish from heterozygous plants with i,s reactions. Accordingly, we did not report the F_2 results of the cross Amigo × Chul/8CC obtained with powdery mildew isolate No. 9a. However, the variable expression of the Amigo gene may explain the discrepancy between our results and those of Lowry et al (14) concerning the crosses of Amigo × *Pm3* resistant lines. Here, no susceptible plants were observed among 1,034 F_2 plants (analyzed with different powdery mildew isolates), whereas Lowry et al (14) obtained two susceptible plants out of 375 F_2 plants of the cross Amigo × Asosan/8CC. It may be that these two plants were heterozygous plants and on the border between the two disease reaction classes formed by Lowry et al (14). A test of the progeny of these plants would determine whether recombination actually occurred. All 47 F_3 lines tested by Lowry et al (14) were resistant; unfortunately, the progenies of the two susceptible F_2 plants were not tested (Moseman, *personal communication*). Therefore, the reaction with powdery mildew isolate No. 9a does not complicate the clear-cut results obtained with all other powdery mildew isolates and provides an explanation for the discrepancy between our results and those of Lowry et al (14). The lack of susceptible F_2 plants in the crosses of Amigo × *Pm3* resistant lines reported here is not the result of an allelic relationship (between the *Pm3* alleles and the Amigo gene) but the result of the absence of homoeologous recombination between 1AS (carrying the *Pm3* alleles) and 1RS (carrying the Amigo gene).

The Amigo powdery mildew resistance is phenotypically different from *Pm8*; *Pm8* gives resistant reactions with isolates Nos. 6, 85135, and W72/27 and susceptible ones with isolates Nos. 9a and 85063, whereas all these isolates are avirulent for the Amigo resistance gene. The fact that both genes are segregating independently according to a 15:1 segregation in F_2 may imply that both resistances are located on the same chromosome arm but separated by some distance, allowing free recombination.

Summarizing all analyses, it is justified to assume that the Amigo powdery mildew resistance gene located on 1RS of the 1AL·1RS translocation is phenotypically and genotypically different from *Pm8* located on 1RS of the 1BL·1RS translocation. We propose the gene symbol *Pm17* for this powdery mildew resistance gene.

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