Genetics

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.

We would like to thank the Deutsche Forschungsgemeinschaft (DFG) for financial support.

Accepted for publication 30 April 1990.

ABSTRACT


Powdery mildew resistance of Amigo wheat has been analyzed and mapped by combining F2 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and electrophoretic analyses revealed that the complete wheat chromosome arm IAS is missing in Amigo and has been replaced by the rye chromosome arm IRS. No susceptible recombinant was found among 1,034 F2 plants of crosses of Amigo with Pm3 resistant lines; Pm3 is known to be located on IAS. Thus, the powdery mildew resistance gene of Amigo is assumed to be located on the rye chromosome IRS 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 IRS of IRS-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 triticae 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 I. Characterization of the wheat powdery mildew isolates used to identify Pml to 9 and Mlk

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>1</th>
<th>2</th>
<th>3a*</th>
<th>3c</th>
<th>4a</th>
<th>4b</th>
<th>5*</th>
<th>6</th>
<th>7</th>
<th>k</th>
<th>3b</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>2</td>
<td>(3a)*</td>
<td>3c</td>
<td>4a</td>
<td>4b</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>k</td>
<td>/</td>
<td>3b</td>
<td>8</td>
</tr>
<tr>
<td>9a</td>
<td>2</td>
<td>3a</td>
<td>3c</td>
<td>4a</td>
<td>4b</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>k</td>
<td>/</td>
<td>3b</td>
<td>8</td>
</tr>
<tr>
<td>85063</td>
<td>1</td>
<td>4a</td>
<td>4b</td>
<td>5</td>
<td>(6)</td>
<td>7</td>
<td>8</td>
<td>k</td>
<td>2</td>
<td>3a</td>
<td>3b</td>
<td>3c</td>
<td>8</td>
</tr>
<tr>
<td>85135</td>
<td>1</td>
<td>2</td>
<td>4a</td>
<td>4b</td>
<td>(5)</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>/</td>
<td>3a</td>
<td>3c</td>
<td>3c</td>
<td>8</td>
</tr>
<tr>
<td>W72/27</td>
<td>1</td>
<td>2</td>
<td>(5)</td>
<td>(6)</td>
<td>7*</td>
<td>9</td>
<td>/</td>
<td>/</td>
<td>3a</td>
<td>3b</td>
<td>3c</td>
<td>4b</td>
<td>8</td>
</tr>
</tbody>
</table>

*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 IAS (2,16), strong linkage was found since only two susceptible recombinants occurred among 375 F₂ plants (14). This result indicates that the powdery mildew resistance gene of Amigo is located on IAS. However, Amigo is a 1AL·1RS translocation line (19) and lacks IAS. 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 isolates W72/27 provided by W. Summers, Cambridge, UK. These powdery mildew isolates were characterized against Pml to Pm9 and Mlk as shown in Table 1. Genes Pml0, 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 Pml0 (located on 4A) are part of the commercial activities of the Institute of Plant Science Research, Cambridge Laboratory (IPSR)/Agricultural Genetics Company Ltd. (age) and thus were not considered here, too. Gene Pml13 (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 F₂ 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 F₂ plant. In addition, two 3-cm-long leaf segments were cut from single plants of both parents (25 plants each) and the F₂ (five plants) and placed into the two experiments per F₂ 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/cm² 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 Girald 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 Sapienzin and Bushuk (18) and Ng and Bushuk (17), respectively.

RESULTS

For verification of the presence or absence of IAS and IRS, 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 IRS 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 IRS 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 Gmu-A1 locus proteins (subunit 2*) located on 1AL (16) in Amigo.

The number of genes responsible for the powdery mildew resistance of Amigo was analyzed in the F2 of the cross Amigo × Kanzler (Table 2); Kanzler is a West German winter wheat cultivar with no powdery mildew resistance gene (9).

These F2 data indicate that the powdery mildew resistance of Amigo is conditioned by one gene with incomplete dominance for susceptibility. Since the F1 and the F2 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 (χ² = 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:16 s and 243 r:17 s fit well the 15 r:11 s hypothesis (χ² = 0.00, χ² = 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 F2 experiments, each consisting of two identical designs (Table 4). Among a total of 505 F2 plants, a susceptible plant was not observed. The F1 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 F1 and F2 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 F2 plants of the cross of Amigo × Pm3a not one susceptible recombinant was observed among 1,034 F2 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 IRS can be distinguished easily from wheat chromosomes because it is marked by large terminal and subterminal C-bands (20). The rye chromosome arm IRS of Amigo is identical in C-banding pattern with the rye segment present in the IBL-1RS wheat-rye translocation lines (4). The C-banding analysis of

---

**TABLE 2. Disease reaction (r = resistant, i = intermediate, and s = susceptible) of F1, F2, and F3 wheat plants of the cross Amigo × Kanzler after inoculation with powdery mildew isolate No. 9a**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Disease reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amigo × Kanzler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>P2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F1</td>
<td>0</td>
<td>5*</td>
</tr>
<tr>
<td>F2</td>
<td>65</td>
<td>190</td>
</tr>
</tbody>
</table>

*Number of plants in the respective class.

**TABLE 3. Disease reaction of P1, P2, and F2 wheat plants of the cross Amigo × Disponent (Pm8), determined in two experiments consisting of two designs**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Disease reaction</th>
<th>W72/27</th>
<th>6/85135</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>i</td>
<td>s</td>
</tr>
<tr>
<td>Amigo × Disponent</td>
<td>P1</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P2</td>
<td>25</td>
<td></td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>239</td>
<td>16</td>
<td>243</td>
<td>17</td>
</tr>
</tbody>
</table>

*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).

* Number of plants.
Amigo confirms earlier reports that this cultivar carries a 1AL·1RS translocation. By analyzing meiotic chromosome pairing in F₁ hybrids between Amigo and dicentrocenic or double-dicentrocenic 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 IRS. 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 IRS 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 IRS and the absence of IAS also agree with the analysis of storage protein composition of Amigo. The protein band controlled by the Glu- AI locus known to be located on the long arm of chromosome 1A (16) is present, while the protein band controlled by the Glu- AI 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 Holienhorst and Joppa (12) and Zeller and Fuchs (21).

Second, the meiotic analyses of Holienhorst 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 unbounded chromosome regions can not be detected by this technique.

Segregation analyses of F₂ 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 × Kanzer. This complicated analyses of the crosses Amigo × Chul/8CC (Pm3b) when inoculated with powdery mildew isolate No. 9a: The five F₂ plants we analyzed (data not given) showed i s reaction. Therefore, susceptible F₂ 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 F₂ 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 F₂ plants (analyzed with different powdery mildew isolates), whereas Lowry et al. (14) obtained two susceptible plants out of 375 F₂ 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 F₂ lines tested by Lowry et al. (14) were resistant; unfortunately, the progenies of the two susceptible F₂ 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 F₂ 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 homologous recombination between IAS (carrying the Pm3 alleles) and IRS (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 1:1 segregation in F₂ 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 IBL·1RS translocation. We propose the gene symbol Pm17 for this powdery mildew resistance gene.

LITERATURE CITED

2. Briggie, L. W., and Sears, E. R. 1966. Linkage of resistance to Erysiphe graminis f. sp. tritici (Pm3) and hairy glume (Hg) on chromosome...
IA of wheat. Crop Sci. 6:559-561.


