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LINKAGE BETWEEN THE *Rpg1* GENE FOR STEM RUST RESISTANCE AND THE *f5* LOCUS ON BARLEY CHROMOSOME 1

YUE JIN,* BRIAN J. STEFFENSON, AND JEROME D. FRANCKOWIAK

Abstract

Linkage studies can expedite the transfer of agronomically important genes in breeding programs. A study was conducted to determine the linkage relationship between loci segregating for stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) resistance (*Rpg1*) and a chlorina mutant (*f5*), and to confirm the linkage among *Rpg1*, *brl* (brachytic) and f_c (chlorina seedling). 'Bowman' barley (*Hordeum vulgare* L.) was crossed to genetic stocks possessing *brl*, f_c, and *f5*, respectively. Estimates of linkage distances were 9.6 ± 1.4% between *Rpg1* and *brl*, 13.6 ± 1.8% between *Rpg1* and f_c, and 25.9 ± 2.6% between *Rpg1* and *f5*. The linkage between *Rpg1* and *f5* was established.

THE *Rpg1* (or *T*) gene (9) in barley confers resistance to a number of pathotypes (races) of the wheat stem rust pathogen (3,4,15). This gene has protected barley from significant losses due to stem rust since the release of 'Kindred' barley in 1942. Nearly every barley cultivar grown in the North Central region of the USA has been bred with *Rpg1*. Although a pathotype (Pgt-QCC) virulent to *Rpg1* was recently detected in the Great Plains (5,10), the gene is still effective against some of the most prevalent pathotypes in the North American wheat stem rust population.

Genotypes possessing *Rpg1* can be easily identified in the field because they exhibit a low level of rust infection and primarily incompatible infection responses. The evaluation of resistance in the seedling stage, however, has been difficult because most barley genotypes display mesothetic reactions (mixture of compatible and incompatible infection types on the same leaf) to many wheat stem rust pathotypes (4,6,8,15). Pathotype MCC was found to be valuable in differentiating genotypes with *Rpg1* (14). Linkage between *Rpg1* and *brl* (brachytic), f_c (chlorina seedling) (2), *wx* (waxy endosperm) (12), and *Run1* [resistance to *Ustilago tritici* (Pers.) Rostr. Syn *Ustilago nuda* (C.N. Jersen) Rostr.] (1,11) were previously studied. Based on these studies, *Rpg1* was placed in the chromosome 1 linkage group (7). A chlorina-plant mutant gene, *f5*, was located on the short arm of chromosome 1 (16); however, the exact location of this locus is not known. This study was conducted to determine the linkage relationship between the *Rpg1* and *f5* loci, and to corroborate the linkage relationships between *Rpg1*, and *brl*, and f_c.

Materials and Methods

Three F_2 populations were developed from crosses between Bowman (PI 483237) barley, which possesses *Rpg1*, and the genetic marker stocks of DWS 1132, BGS 002, and BGS 018, which possess *brl*, f_c, and *f5*, respectively. For all three crosses, the genes were tested in the coupling phase. The morphological markers used in this study were readily recognizable at the seedling stage of growth. Both *f5* and f_c are chlorina mutants, and the homozygous recessive genotype of each locus produces light green seedlings; however, f_c chlorina seedlings change to green after the first leaf stage. Plants exhibiting the chlorina phenotype were tagged at the first leaf stage. The homozygous genotype of *brl* can be recognized by smaller and broader leaves at the seedling stage.

Seedlings of parental and F_2 plants from each cross were evaluated for their infection types to pathotype MCC of *P. graminis* f. sp. *tritici* (culture A-5), provided by Dr. J.D. Miller (Northern Crop Sciences Laboratory, USDA-ARS, Fargo, ND). F_1 plants were not available for this test. The ITs were assessed using the system of Miller and Lambert (6) in which IT 0; indicates necrotic flecks without visible sporulation, IT 1 indicates small sporulating pustules surrounded by distinct necrosis, IT 2 and IT 3 indicate progressively larger pustules with or without necrosis, and IT 4 describes large pustules. Most genotypes homozygous for *Rpg1* exhibit ITs ranging from 0; to 21 and those without the gene, 23- to 33- (14). In a preliminary experiment, Bowman exhibited ITs ranging from 0; to 21, but ITs of 0; and 1 predominated. The ITs on parents possessing the recessive allele for stem rust susceptibility ranged from 23- to 33+. In the classification of F_2 seedlings, plants with ITs of 23- or higher were considered susceptible (homozygous recessive for *Rpg1*), and those with ITs of 0;, 0;1, 12 or 21 were considered resistant (homozygous or heterozygous for *Rpg1*).

Seed was sown in super-cell containers (Stuewe & Sons, Corvallis, OR) filled with No. 1 Sunshine mix (Fisons Horticulture, Vancouver, BC), and grown at 21 to 25 °C in the greenhouse. Plants were inoculated with pathotype MCC 7 d after planting when the primary leaves were fully expanded. Inoculations were made with 6.0 mg urediniospores mL^{-1} Soltrol 170 oil (Phillips Petroleum Co., Borger, TX) applied at a rate ≈1 μL oil per plant. After inoculation, plants were incubated in a chamber where the humidity was maintained near saturation for 16 h at 21 °C in the dark. The leaf surfaces were kept moist by periodic misting (32 s misting every 16 min) from an ultrasonic humidifier (13). After the mist period, the plants were exposed to sunlight in a greenhouse and allowed to dry slowly. The plants were subsequently incubated in a growth chamber at 25 to 28 °C with a photoperiod of 12 h (115-W fluorescent bulbs emitting between 132.2 to 144.4 μmol photon m^{-2} s^{-1}). The ITs on each plant were assessed 12 d after inoculation. The chi-squared and maximum-likelihood methods were used to test the independence of segregation in the F_2 populations, and to estimate the recombination fraction, respectively.

Results and Discussion

The observed frequencies of each phenotypic class, calculated chi-squared values for independence of segregation, and maximum-likelihood estimates of recombination fractions for the three crosses are given in Table 1. All calculated chi-squared values for testing single pairs of genes were not significant, with the exception of the cross involving *Rpg1* and *f5*. In this cross, the number of susceptible plants was slightly higher than expected. This may have resulted from misclassification of the stem rust infection type.

The calculated values for linkages in all three crosses were significant, indicating linkage relationships between *Rpg1* and the three mutant loci. The maximum-likelihood estimate of the linkage distance was 25.9 ± 2.6% between *Rpg1* and *f5*. The estimated distances

Y. Jin and B.J. Steffenson, Dep. of Plant Pathology; and J.D. Franckowiak, Dep. of Crop and Weed Sciences, North Dakota State Univ., Fargo, ND 58105. Received 8 May 1992. *Corresponding author.

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Abbreviations: cM, centimorgan; IT, infection type.

Table 1. Segregation ratios of F_2 plants from barley crosses involving *Rpg1* and three morphological markers *brl*, f_c, and *f5* on chromosome 1.

Genes tested		Number of F_2 plants in each phenotype†				Chi-square test‡			Recombination fraction§	SE
A	*b*	*AB*	*Ab*	*aB*	*ab*	X^2_A	X^2_B	X^2_L		
									%	
Rpg1	*brl*	332	26	20	110	0.70	2.14	319.18	9.6	1.4
Rpg1	f_c	303	22	34	82	0.40	0.47	192.02	13.6	1.8
Rpg1	*f5*	246	50	52	71	4.24	3.36	88.90	25.9	2.6

† Phenotype *AB* represents F_2 plants with dominant alleles at both loci. The double recessive genotype (*aabb*) produces the phenotype of *ab*.
‡ $X^2_{0.05}$ (1) = 3.84 and $X^2_{0.01}$ (1) = 6.63. Expected ratio for X^2–testing is 3:1 for genes *A* (X^2_A) and *B* (X^2_B), and 9:3:3:1 for linkage (X^2_L).
§ Recombination fraction calculated using the method of maximum likelihood.

were 9.6 ± 1.4% between *Rpg1* and *brl*, and 13.6 ± 1.8% between *Rpg1* and f_c, which were slightly smaller than the previously reported values of 12.6 and 16.7%, respectively, (2). Unpublished data from the North American Barley Genome Mapping Project indicate that *Rpg1* is very close (≈3 cM) to a subtelomeric marker (*Tell1s*) on the short arm of chromosome 1. Therefore, *f5* is postulated to be proximal in relationship to *Rpg1*, *brl*, and f_c. This putative location of *f5* is in conflict with Tsuchiya (17), who placed the *f5* locus at the distal end of the short arm of chromosome 1. The exact location of *f5* can only be resolved with further linkage analysis.

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