

Genetic Analysis of Partial Resistance to Powdery Mildew in Bread Wheat Line Saar

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ABSTRACT

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Powdery mildew, caused by *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *tritici*, is an important disease of bread wheat (*Triticum aestivum*) in many countries. The CIMMYT bread wheat line Saar has exhibited a high level of partial resistance to powdery mildew in field trials conducted in Europe, Asia, and South America, and represents a valuable source of resistance in wheat breeding. A set of 114 random F₅ inbred lines from the cross Saar × Avocet-YrA (susceptible) were evaluated in replicated field trials at two locations in southeastern Norway to determine the number of genes involved in partial resistance to powdery mildew. Narrow-sense heritability estimates were high (0.83 to 0.92). Based on both quantitative and qualitative genetic analyses, the minimum number of genes with additive effects segregating for powdery mildew resistance in the population was four. Transgressive segregation indicated that Avocet-YrA might have contributed one minor gene for resistance. It is concluded that partial resistance to powdery mildew in Saar is controlled by at least three genes. Such resistance conferred by multiple genes having additive effects is expected to be durable.

Powdery mildew, caused by *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *tritici*, is an important disease of wheat in specific regions of the world, including parts of China, Africa, North and South America, and Europe (1,13). It is most severe under intensive production systems in temperate and maritime climates, and grain yield losses have been reported in the range from 5 to 34% (7,11,16). Resistance is an economical and environmentally safe means of controlling this disease (1,13). Race-specific resistance genes that confer a hypersensitive response and often give complete protection are frequently used in wheat breeding (13). However, the durability of such resistance is usually very short since it can easily be overcome by simple genetic changes in the pathogen (19,25, 26,29).

A more sustainable alternative to race-specific resistance genes is to develop and deploy germ plasm with partial or horizontal resistance. Partial resistance is known to reduce infection frequency and retard the growth and reproduction of the pathogen in adult plants (27). Such resistance has also been termed slow mildewing (25) or adult plant resistance (11). It is inherited

as a quantitative trait (8,10) and has been shown to be durable. Examples include the North American winter wheat cultivar Knox that had effective resistance against powdery mildew for more than two decades of commercial production (26) and continues to be resistant. Likewise, the partial resistance in its derived cultivar Massey has also remained effective over a similar duration of time (17).

Natural epidemics of powdery mildew do not occur in Mexico, where CIMMYT (International Maize and Wheat Improvement Center) has its main breeding operations, and hence most of the breeding material is susceptible when grown in powdery mildew-prone areas. However, international testing has facilitated identification of widely adapted genotypes that exhibit low levels of powdery mildew infection across many locations, and may provide potential sources of partial, race-non-specific resistance (M. Lillemo and M. van Ginkel, *unpublished*). From such global testing, sister lines of the CIMMYT wheat cross Saar were identified among the best lines for partial resistance to powdery mildew, and found to lack any known race-specific resistance at the seedling stage when tested against a differential set of 20 Chinese isolates of *B. graminis* f. sp. *tritici* (30).

The objective of this study was to determine the inheritance of resistance to powdery mildew in Saar under natural field epidemics, and determine the minimum number of genes involved in its resistance.

MATERIALS AND METHODS

Plant material. A set of 114 randomly selected F₅ inbred lines from the cross between the powdery mildew resistant CIMMYT line Saar and susceptible Avocet-YrA was developed and kindly provided by A. Navabi, the University of Alberta, Edmonton, Canada (22,23). The pedigree of Saar is Sonoita F81/Trap#1//Baviacora M92, and the population was developed from a pure inbred line with selection history CG25-099Y-099M-4Y-2M-3Y-0B. Avocet-YrA is a line selected from the heterogeneous Australian cultivar Avocet for lacking yellow rust resistance gene *YrA*. Seed of the population was multiplied in a net house at El Batan, Mexico, prior to field trials.

Test locations. Field evaluations were carried out at two locations in southeastern Norway: Vollebakk research farm at Ås (59°N, 90 m above sea level) with soil type classification Mollic Gleysol (14), and Staur research farm close to Hamar (60°N, 153 m above sea level) with soil type classification Eutri-Endostagnic Cambisol (14). Both locations experience severe natural epidemics of powdery mildew every year and are characterized by a different virulence composition (29). A stable snow cover during most winters in the Hamar area allows local populations of *B. graminis* f. sp. *tritici* to survive on winter wheat and has led to the buildup of very unique and locally adapted virulence combinations. In contrast, the region around Ås usually receives new inoculum every summer by winds from the south and has a virulence composition more like that observed in the UK, Germany, and Poland (6,29).

Experimental design. The parents and 114 F₅ lines were planted in a randomized complete block design with two replications at each location. Both trials were planted in the second week of June 2004, a month later than the commercially grown wheat, to ensure an ample source of natural inoculum from surrounding wheat fields. The lines were planted in small hill-plots to provide a favorable microclimate for mildew development, and the field was surrounded by susceptible spreader rows consisting of a mixture of very susceptible lines from the previous year's testing.

Powdery mildew assessment. Mildew severity was scored on the whole canopy by use of the modified Cobb scale (0 to

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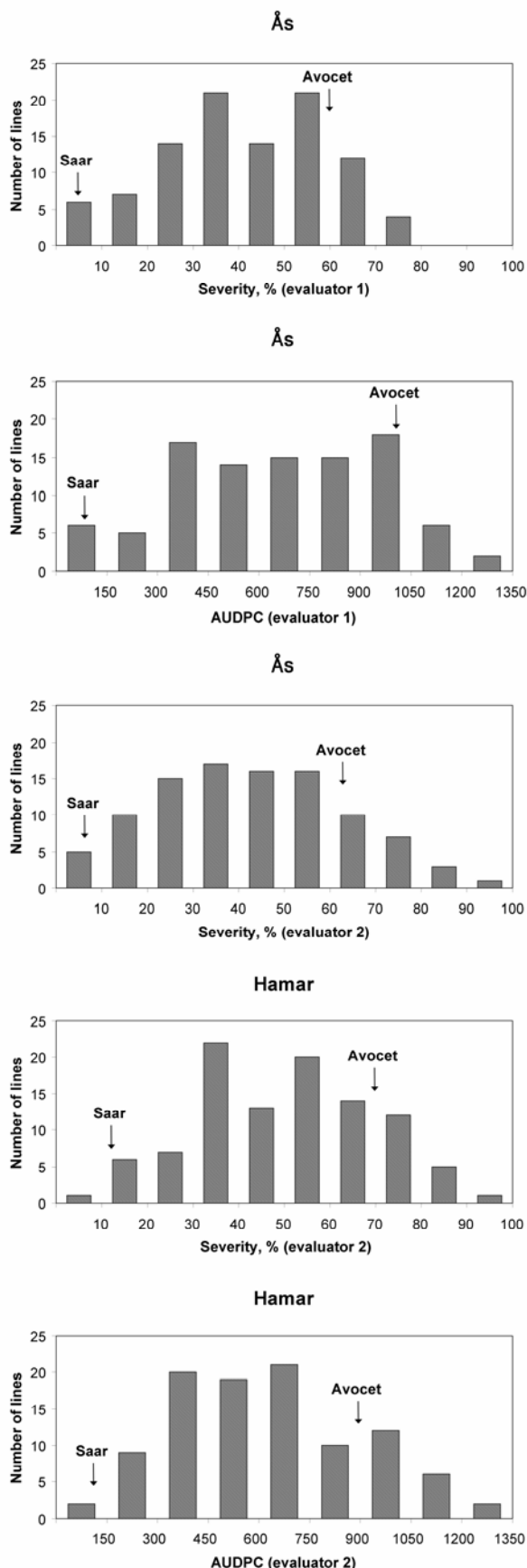


Fig. 1. Distributions of frequencies for final powdery mildew severity (GS 75) and area under the disease progress curve (AUDPC) of F_5 lines of the cross Saar \times Avocet-YrA at two locations in south-eastern Norway. Mean values for the parents are indicated by arrows.

100% infected leaf area) (24) three times at 10-day intervals during the season; the first scoring was done when the majority of lines were in the late booting stage (GS 45), and the last notes were taken when the most susceptible lines had reached maximum severity around GS 75. The disease scoring at Ås was done by evaluator one (H. Skinnes), and the scoring at Hamar was done by evaluator two (M. Lillemo). The last scoring at Ås was done independently by both researchers to provide comparison. The area under the disease progress curve (AUDPC) was calculated according to Bjarko and Line (2), and used for subsequent analysis together with the severity at the last date of scoring.

Statistical analysis. Analyses of variance were conducted to determine differences in mildew scores among the F_5 lines using SAS 8.1 (SAS Institute, Cary, NC). Narrow-sense heritabilities were obtained using the formula $h^2 = \sigma_g^2 / \sigma_p^2$; σ_g^2 and σ_p^2 were estimated from the ANOVA table as outlined in Singh et al. (28), $\sigma_g^2 = (\sigma_L^2 - \sigma_E^2) / r$, and $\sigma_p^2 = \sigma_g^2 + \sigma_E^2$; in this formula, h^2 = narrow-sense heritability, σ_p^2 = phenotypic variance, σ_g^2 = genetic variance, σ_L^2 = variance of the F_5 lines, σ_E^2 = error variance, and r = number of replications. The heritability estimate thus calculated can be considered to be mostly of the narrow-sense type since the dominance variance is relatively limited in the F_5 generation and the additive-by-additive genetic variance can be included in the heritability estimate at this level of inbreeding.

Gene number estimates. A quantitative estimate for the minimum number of genes controlling powdery mildew resistance was made according to Wright's method (31): $n = D^2 / [8\sigma_g^2 / (2 - 1/2^{(g-2)})]$ in which D = the genotypic range of the lines in generation F_g . Adjusted for the level of inbreeding in F_5 , the modified formula becomes $n = D^2 / 4.27\sigma_g^2$. The general assumptions are: no linkage, no epistasis, no dominance, equal effects of all loci, and no transgressive segregation. Failure to meet any of these criteria will lead to an underestimation of gene numbers, and n is thus a conservative estimate (3). In Wright's original formula, D is calculated as the difference between the two parents and assumes that all plus factors are contributed by one parent. This assumption can be dealt with by calculating D from extreme segregants, in cases where genes are contributed by both parents (21,31). Here, D was estimated as the range of F_5 line means multiplied by the heritability, which tends to eliminate the environmental influence and give more stable gene number estimates (28).

A qualitative approach for estimating the number of segregating genes was also taken by grouping the F_5 lines into resistant and intermediate-susceptible types. A line was considered resistant when it had a similar disease score as the resistant parent Saar \pm one standard deviation. The good-

ness-of-fit to the expected segregation ratios for three, four, and five independent genes was tested by chi-square analysis.

RESULTS AND DISCUSSION

Powdery mildew development was good at both locations, and the susceptible parent Avocet-YrA reached 60 to 70% leaf area affected by the last evaluation date. A few of the 114 F₅ lines either failed to produce a dense stand due to shortage of seed, or were very late in development and were excluded from the analysis. Thus, there were 99 and 101 lines included in the analysis at the two locations (Table 1).

Severity at the last evaluation date and AUDPC for the F₅ lines showed a continuous distribution close to normality at both locations (Fig. 1), and several lines exhibited higher levels of susceptibility than Avocet-YrA. The powdery mildew scores at the two locations were in good agreement, with an R² of 0.73 (Fig. 2). Transgressive segregation toward susceptibility was also evident when comparing the ranges and means of the F₅ lines with the scores of the parents (Table 1). The narrow-sense heritabilities were high at both locations (Table 1).

Gene number estimates, both quantitative and qualitative, are given in Table 2. Such estimates are often associated with uncertainties, and we therefore conducted the experiment in two different environments and let two evaluators score the powdery mildew independently of each other. The data were then analyzed by two different methods, giving a total of 10 different estimates that are given in Table 2. The estimates were largely in agreement across locations and evaluator, but Wright's method tended to give higher gene number estimates for evaluator two and for the Hamar location compared to Ås (Table 2). This could largely be explained by the differences in phenotypic range from the two evaluators and locations (Table 1). Estimates by the chi-square method were less influenced by evaluator and testing environment. Based on the two kinds of genetic analyses, it appears most likely that at least four genes segregated for powdery mildew resistance in the population, although the possibility of three genes could not be rejected by the chi-square analysis. Transgressive segregation of progeny more susceptible than Avocet-YrA indicated that the susceptible parent also

contributed some resistance, and if we assume that it contributed one resistance gene, Saar would have contributed at least three genes for powdery mildew resistance in this population. It should be noted that this is an estimate of the minimum number of genes since the assumption that all segregating genes for a quantitative trait have equal effects is very unlikely to be true, and thus the real number could be higher. Quantitative Trait Locus (QTL) studies of partial resistance to powdery mildew have also shown that different loci have different effects (15,17,20), but the gene number estimates could still be a good indicator for the number of important QTLs for the trait.

The means of the F₅ lines were higher than the midparent values at both locations (Table 1) and were not reduced by traditional transformation methods for percentage data (square root, arcsine, and arcsine square root, *data not shown*). Biometrical explanations for such differences are either dominance or epistasis (18). Partial dominance for quantitatively inherited powdery mildew resistance has been reported (8), but the effects are usually small (12), and not likely to play any important role at this level of inbreeding. Epistasis is a more likely explanation, since the susceptible

parent likely carried a minor gene for resistance that could confer a greater reduction in powdery mildew infection when acting alone in a susceptible background than when acting together with the other resistance genes from the resistant parent. Such additive-by-additive epistasis for powdery mildew resistance has also been reported in other studies (9,12).

The gene number estimates are in good agreement with other genetic studies of partial resistance to powdery mildew. Two to three genes with moderate to high heritabilities were reported for the winter wheat cultivars Knox 62, Massey, Redcoat, and Houser (8,10). Subsequently, a mapping study identified three QTLs in Massey (on chromosomes 1B, 2A, and 2B) that accounted for about half of the phenotypic variance for powdery mildew severity when crossed to the susceptible cultivar Becker (17). Several studies have been conducted on the powdery mildew resistance of the French winter wheat RE714 (4,5,20). The line carries the race-specific resistance genes *Pm4b* and *MIRE* but expresses adult plant resistance in the field when these genes are overcome by matching virulences in the pathogen. Molecular mapping in two different genetic back-

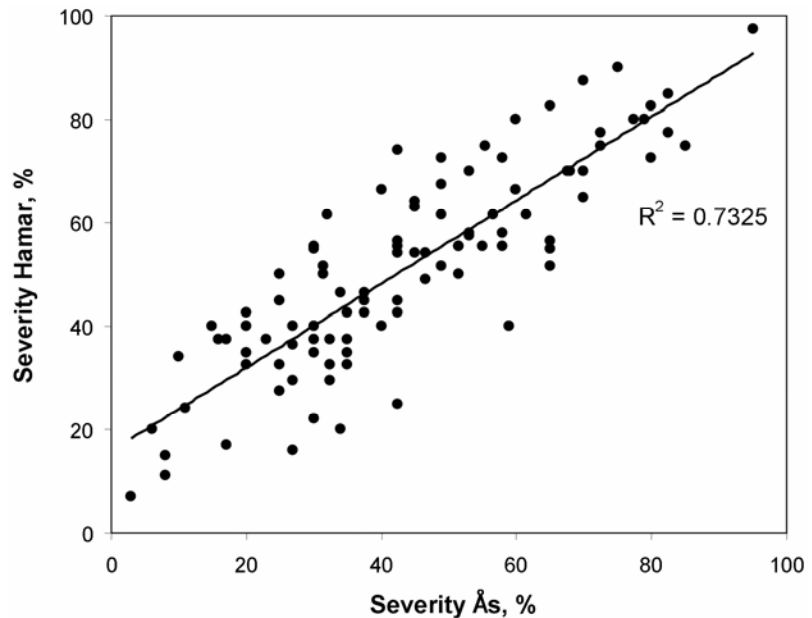


Fig. 2. Comparison of powdery mildew severities for 99 F₅ lines at the two trial locations Ås and Hamar. The plot shows average powdery mildew severities for two replications at the third assessment date for evaluator two.

Table 1. Range and mean powdery mildew score for parents and F₅ lines, and narrow-sense heritability estimates

Location	Evaluator	Score	Parents			F ₅ lines			SE ^a	h ² – narrow-sense heritability
			Saar	Avocet	Mean	No. of lines	Range	Mean		
Ås	1	Severity (%)	4.5	60.0	32.3	99	3.0-80.0	42.4	7.6	0.83
Ås	1	AUDPC	94.5	1,008.8	551.7	99	58-1,338	658.1	116.0	0.86
Ås	2	Severity (%)	7.0	62.5	34.8	99	3.0-95.0	43.9	7.3	0.87
Hamar	2	Severity (%)	12.0	70.0	41.0	101	7.0-97.5	51.1	5.5	0.92
Hamar	2	AUDPC	135.0	897.5	516.3	101	70-1,290	626.3	76.1	0.92

^a Standard error of the means of individual F₅ lines.

Table 2. Gene number estimates for powdery mildew resistance based on Wright's formula (19) and χ^2 analysis

Location	Evaluator	Score	Wright's method	Number of F ₅ lines		χ^2 and P values ^a		
				Resistant	Susceptible-intermediate	3 genes	4 genes	5 genes
Ås	1	Severity	3.40	6	93	1.93 P = 0.17	0.34 P = 0.56	6.23 P = 0.01
Ås	1	AUDPC	3.55	7	92	1.12 P = 0.29	1.12 P = 0.29	10.03 P < 0.01
Ås	2	Severity	4.12	6	93	1.93 P = 0.17	0.34 P = 0.56	6.23 P = 0.01
Hamar	2	Severity	4.58	5	96	3.13 P = 0.08	0.01 P = 0.94	3.16 P = 0.08
Hamar	2	AUDPC	4.20	6	95	2.08 P = 0.15	0.29 P = 0.59	5.96 P = 0.01

^a Expected segregation ratios used for chi-square analysis were 0.103:0.897, 0.048:0.952, and 0.023:0.977, respectively, for 3, 4, and 5 independent genes in F₅. This assumes only additive gene action and equal effects of all loci.

grounds detected two major QTLs that showed stable expression across environments; one QTL on 5D and the other corresponding to the location of the defeated *MIRE* gene on 6A (20). Much higher gene numbers were reported by Keller et al. (15), who detected 18 QTLs for powdery mildew resistance in a segregating wheat by spelt population. However, only two QTLs with major effect were consistent over environments. One was derived from the winter wheat cultivar Forno and located on 7B and the other from the spelt cultivar Oberkulmer and located on 5A.

The chromosomal locations of genes for powdery mildew resistance in Saar have yet to be determined, but the line has good adult plant resistance to leaf rust and stripe rust, which is controlled by the *Lr34/Yr18* gene complex on 7D and possibly two other minor resistance genes (22,23). Based on the phenotypic leaf tip necrosis marker, preliminary results indicate that *Lr34/Yr18* is also involved in resistance to powdery mildew (M. Lillemo, R. P. Singh, and J. K. M. Brown, *unpublished*). It therefore seems likely that Saar represents a new source of powdery mildew resistance that is different from those previously characterized. Incorporation of this resistance in other cultivars should be feasible in an environment conducive to powdery mildew, and could be facilitated by development of molecular markers linked to these genes.

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LITERATURE CITED

- Bennett, F. G. A. 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programmes. *Plant Pathol.* 33:279-300.
- Bjarko, M. E., and Line, R. F. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology* 78:457-461.
- Burton, G. W. 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). *Agron. J.* 43:409-417.
- Chantret, N., Mingeot, D., Sourdil, P., Bernard, M., Jacquemin, J. M., and Doussinault, G. 2001. A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. *Theor. Appl. Genet.* 103:962-971.
- Chantret, N., Sourdil, P., Roder, M., Tavaud, M., Bernard, M., and Doussinault, G. 2000. Location and mapping of the powdery mildew resistance gene *MIRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. *Theor. Appl. Genet.* 100:1217-1224.
- Clarkson, J. D. S. 2000. Virulence survey report for wheat powdery mildew in Europe, 1996 - 1998. *Cereal Rusts and Powdery Mildews Bull.* Vol. 28 Online publication 2000/1204clarkson.
- Conner, R. L., Kuziyk, A. D., and Su, H. 2003. Impact of powdery mildew on the yield of soft white spring wheat cultivars. *Can. J. Plant Sci.* 83:725-728.
- Das, M. K., and Griffey, C. A. 1994. Heritability and number of genes governing adult-plant resistance to powdery mildew in Houser and Redcoat winter wheats. *Phytopathology* 84:406-409.
- Das, M. K., and Griffey, C. A. 1995. Gene action for adult-plant resistance to powdery mildew in wheat. *Genome* 38:277-282.
- Griffey, C. A., and Das, M. K. 1994. Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. *Crop Sci.* 34:641-646.
- Griffey, C. A., Das, M. K., and Stromberg, E. L. 1993. Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. *Plant Dis.* 77:618-622.
- Haute, R. A., Coffman, W. R., Sorrells, M. E., and Bergstrom, G. C. 1987. Inheritance of partial resistance to powdery mildew in spring wheat. *Theor. Appl. Genet.* 73:609-615.
- Hsam, S. L. K., and Zeller, F. J. 2002. Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). Pages 219-238 in: *The Powdery Mildews, A Comprehensive Treatise*. R. R. Belanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver, eds. American Phytopathological Society, St. Paul, MN.
- ISSS, ISRIC, and FAO. 1998. World reference for soil resources. *World soil resources reports*. Vol. 84. ISSS, ISRIC, and FAO, Rome.
- Keller, M., Keller, B., Schachermayr, G., Winzeler, M., Schmid, J. E., Stamp, P., and Messmer, M. M. 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. *Theor. Appl. Genet.* 98:903-912.
- Lipps, P. E., and Madden, L. V. 1988. Effect of triadimenol seed treatment and triadimefon foliar treatment on powdery mildew epidemics and grain yield of winter wheat cultivars. *Plant Dis.* 72:887-892.
- Liu, S. X., Griffey, C. A., and Maroof, M. A. S. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. *Crop Sci.* 41:1268-1275.
- Mather, K., and Jinks, J. L. 1971. *Biometrical genetics*. 2nd ed. Chapman and Hall, London.
- McDonald, B. A., and Linde, C. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* 124:163-180.
- Mingeot, D., Chantret, N., Baret, P. V., Dekeyser, A., Boukhatem, N., Sourdil, P., Doussinault, G., and Jacquemin, J. M. 2002. Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. *Plant Breed.* 121:133-140.
- Multize, D. K., and Baker, R. J. 1985. Genotype assay and method of moments analyses of quantitative traits in a spring wheat cross. *Crop Sci.* 25:162-167.
- Navabi, A., Singh, R. P., Tewari, J. P., and Briggs, K. G. 2003. Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. *Plant Dis.* 87:1522-1529.
- Navabi, A., Singh, R. P., Tewari, J. P., and Briggs, K. G. 2004. Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. *Crop Sci.* 44:1156-1162.
- Peterson, R. F., Campbell, A. B., and Hannah, A. E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res. C.* 26:496-500.
- Roberts, J. J., and Caldwell, R. M. 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in Knox wheat. *Phytopathology* 60:1310.
- Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. *Phytopathology* 63:867-872.
- Shaner, G. 1973. Reduced infectability and inoculum production as factors of slow mildewing in Knox wheat. *Phytopathology* 63:1307-1311.
- Singh, R. P., Ma, H., and Rajaram, S. 1995. Genetic analysis of resistance to scab in spring wheat cultivar Frontana. *Plant Dis.* 79:238-240.
- Skinnes, H. 2002. Breakdown of race specific resistance to powdery mildew in Norwegian wheat. *Cereal Rusts and Powdery Mildew Bull.* Vol. 30 Online publication 2002/1201skinner.
- Wang, Z. L., Li, L. H., He, Z. H., Duan, X. Y., Zhou, Y. L., Chen, X. M., Lillemo, M., Singh, R. P., Wang, H., and Xia, X. C. 2005. Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. *Plant Dis.* 89:457-463.
- Wright, S. 1968. *Evolution and genetics of populations*. Vol. I. Genetic and Biometric Foundations. University of Chicago Press, Chicago.