Genetic Analysis of Resistance to Scab in Spring Wheat Cultivar Frontana

RAVI P. SINGH, HONG MA, and SANJAYA RAJARAM, International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

ABSTRACT

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.

The Brazilian spring wheat (*Triticum aestivum*) cv. Frontana is resistant to scab (caused by *Fusarium graminearum*) in Mexico and various other countries. The number of genes involved in resistance to this disease was estimated. This was done by evaluating random inbred F_6 lines and their parents for scab resistance. The lines were derived from crosses of Frontana with susceptible, or moderately susceptible, cvs. Inia 66, Opata 85, and Pavon 76. These evaluations took place in the field with a mixture of Mexican isolates of *F. graminearum*. Spikes were inoculated by placing a tiny tuft of cotton soaked with the inoculum in the middle spikelet close to the anthers just prior to anthesis. Glassine bags were placed over the inoculated spikes. These spikes were harvested 45 days after inoculation. Scab severity was determined by recording the number of infected and healthy spikelets. Analyses of variance showed significant differences in scab severities of the F_6 lines. The distribution of F_6 lines was continuous in each cross. The narrow-sense heritability estimates for the crosses evaluated during 1991 and 1993 were 0.66 and 0.93, respectively. Both quantitative and qualitative models were applied to estimate the number of segregating genes. The resistance of Frontana is controlled by the additive interaction of a minimum of three minor genes. Transgressive segregants were identified in each cross, indicating that the susceptible (or moderately susceptible) parents also carry one (or two) minor genes. The combinations of these genes with the genes in Frontana have given F_6 lines with significantly better scab resistance than that of Frontana.

Scab, or head blight, is a common wheat disease in humid wheat-growing regions. Losses are not only associated with reduced yield, but also with mycotoxins (5). Several species of the genus *Fusarium* are known to cause scab (13). *F. graminearum* Schwabe (perfect stage *Gibberella zeae* (Schw.) Petch) predominates in wheat growing areas of China and North and South America (4,8,15). Some bread wheat (*Triticum aestivum* L.) cultivars are known to carry scab resistance (4,8,11,15). Schroeder and Christensen (8) have determined that at least two components contribute scab resistance: *(1)* establishment of initial infection, and *(2)* rapidity of hyphal invasion of the host tissue. Therefore, two types of resistance, resistance to initial infection and resistance to hyphal spread, could be present in the host. They found that Frontana (a bread wheat cultivar released during 1943 in Brazil) possesses both types of resistance. Frontana is known to be resistant to scab in various parts of the world (5,11,15). Despite this, the inheritance of resistance to scab in Frontana is unknown.

The objective of our work was to study the genetic basis of resistance to scab in Frontana under controlled field inoculation.

MATERIALS AND METHODS

Random inbred F_6 lines from crosses of scab-resistant Frontana with two susceptible Mexican cultivars, Inia 66 and Opata 85, and one moderately susceptible cultivar, Pavon 76, were used. Parents and the F_6 lines were planted in a randomized complete block design with three replicates. Two planting dates at 12-day intervals were used to obtain tillers of a similar growth stage for a majority of the lines for same-day inoculation. The experimental site was CIMMYT's highland research station near Toluca (Mexico state), which is a representative location for cool, high-rainfall areas. Field plots, planted with approximately 60 seeds, consisted of two 1-m rows seeded 20 cm apart with 55 cm between plots.

The conidiospores of a mixture of *F. graminearum* isolates were multiplied in mungbean extract medium (1). The conidial suspension used for inoculation was adjusted to 5×10^4 spores per milliliter. Five spikes, close to anthesis, were chosen from each plot and were inoculated by placing a tiny tuft of cotton soaked with the inoculum in a floret of the middle spikelet. Glassine bags were placed over the inoculated spikes and left till harvesting.

Inoculated spikes were harvested 45 days after inoculation. Spikelets with shriveled grains and pink color mycelium were considered infected. Scab severity was recorded by counting the infected and uninfected spikelets; the percent severity was calculated from the above data. Severities of the five inoculated spikes/plot were averaged for the analysis.

Analyses of variance were conducted to determine the differences for scab severities among the F_6 lines using SAS (7). Narrow-sense heritability estimates were obtained using the formula $h^2 = \sigma^2 g / \sigma^2 p$; $\sigma^2 g$ and $\sigma^2 p$ were estimated from the ANOVA table according to the method of Singh and Chaudhary (9), $\sigma^2 g = (\sigma^2_L - \sigma^2_E)/r$, and $\sigma^2 p = \sigma^2 g + \sigma^2_E$; in this formula $h^2 =$ heritability estimate in the narrow sense, $\sigma^2 g =$ genetic variance, $\sigma^2_L =$ variance of the F_6 lines, $\sigma^2_E =$ error variance, and $r =$ number of replications. Although the genetic variance used in the formula to calculate heritability is the total genetic variance of the F_6 lines, the heritability estimate is considered to be narrow-sense because the dominance variance is negligible and the confounding effect of the additive-by-additive genetic variance can be included in the heritability estimate at the F_6 level of inbreeding.

The minimum number of genes controlling scab resistance was estimated using Wright's method (14). The modification for the level of inbreeding in the original formula is based on Cockerham (2). The modified formula is $n = (GR)^2 / 4.27\sigma^2 g$, in which $n =$ minimum number of effective genes, GR $=$ genotypic range, and $\sigma^2 g =$ genetic variance of the F_6 lines. GR was estimated by two different methods. In

Table 1. Wheat cultivars and their mean responses to scab during 1991 and 1993

Parent	Infected spikelets[a]		Percent[b]	
	1991	1993	1991	1993
Frontana	1.1 ± 0.2[c]	3.7 ± 0.3	5.0 ± 0.7	18.2 ± 1.1
Inia 66	...	13.6 ± 0.6	...	82.0 ± 2.9
Opata 85	8.6 ± 0.9	13.7 ± 0.5	46.6 ± 6.1	80.8 ± 2.4
Pavon 76	3.5 ± 0.1	10.3 ± 0.4	19.1 ± 0.8	59.3 ± 2.8

[a] Infected spikelets is on a per spike basis, and is the average for 5 spikes per plot in 3 replicates.
[b] Percent severity is the percentage of spikelets infected with scab, and is the average for 5 spikes per plot in 3 replicates.
[c] Standard error.

Table 2. Range, mean responses, and LSD of F_6 lines to scab and narrow-sense heritability estimates for crosses of cultivars

| Cross | Year tested | No. of F_6 lines tested | Response to scab | | | | | | Heritability |
| | | | Infected spikelets | | | Percent | | | |
			Range	Mean	LSD	Range	Mean	LSD	
Frontana/Inia 66	1993	107	1.4–17.5	6.3 ± 0.6[a]	1.7	8.8–100	37.8 ± 3.5[a]	9.9	0.93
Frontana/Opata 85	1991	57	1.3–10.5	4.7 ± 0.7	2.0	6.7–57.6	25.1 ± 2.9	10.9	0.66
Frontana/Opata 85	1993	56	3.3–19.0	9.5 ± 0.7	2.0	17.8–100	50.1 ± 3.7	10.4	0.93
Frontana/Pavon 76	1991	62	0.8–4.9	2.3 ± 0.3	0.9	4.4–30.6	13.2 ± 2.0	5.7	0.66
Frontana/Pavon 76	1993	55	1.3–15.5	6.3 ± 0.6	1.8	8.0–91.0	35.8 ± 3.6	10.3	0.92

[a] Standard error.
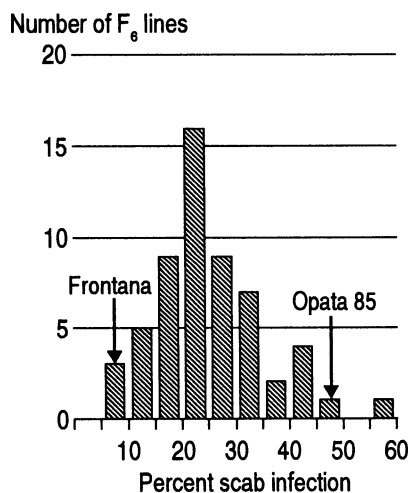
Fig. 1. Frequency distribution for percent scab severity of F_6 lines in the cross Frontana/Opata 85 tested during 1991.
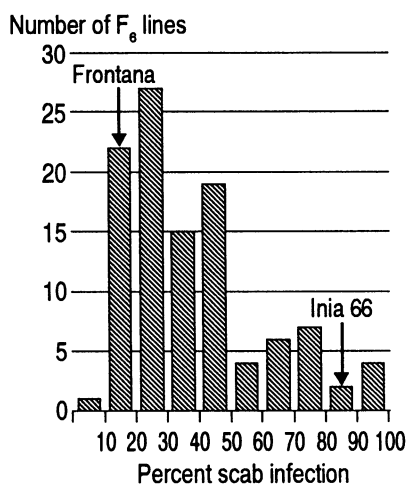

Fig. 3. Frequency distribution for percent scab severity of F_6 lines in the cross Frontana/Inia 66 tested during 1993.
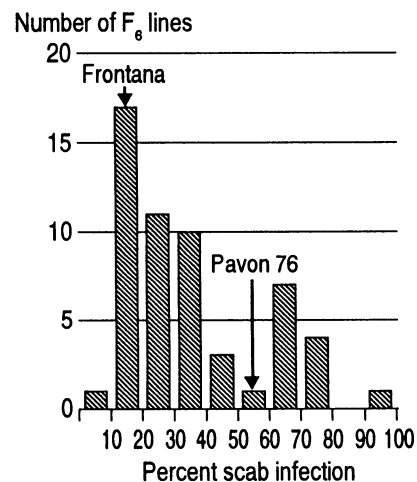

Fig. 5. Frequency distribution for percent scab severity of F_6 lines in the cross Frontana/Pavon 76 tested during 1993.
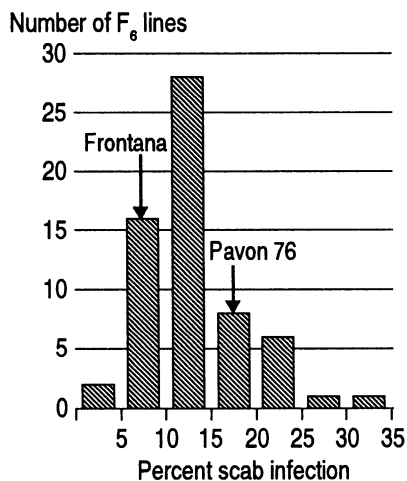

Fig. 2. Frequency distribution for percent scab severity of F_6 lines in the cross Frontana/Pavon 76 tested during 1991.
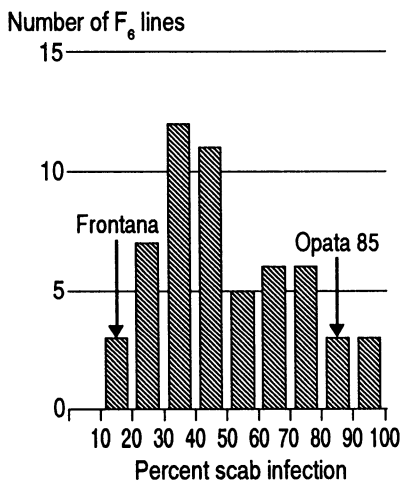

Fig. 4. Frequency distribution for percent scab severity of F_6 lines in the cross Frontana/Opata 85 tested during 1993.

method I, GR was the range of F_6 line means; in method II, GR was the range of F_6 line means multiplied by heritability, h^2, as calculated above. The correction in the second method tends to eliminate the environmental influence on the expression of the trait (6). The assumptions of the formula are: no linkage, no epistasis, no dominance, and all loci have equal effects.

The minimum number of effective genes that segregated for resistance to scab for crosses tested during 1993 was also calculated by grouping the F_6 lines in two categories: resistant-intermediate, and susceptible. The F_6 lines were considered susceptible when their responses were equal to the mean of the susceptible parent ±SE of the F_6 lines, or higher. Chi-square analyses were carried out to test the goodness of fit of the observed distribution with that expected for segregations of three, four, or five independent resistance genes.

RESULTS AND DISCUSSION

Mean responses of the cultivars to scab, expressed as infected spikelets and as percent infected spikelets, are given in Table 1. During 1991, disease was less severe than in 1993. Frontana was the most resistant cultivar during both years. Pavon 76 had an intermediate response, whereas Opata 85 was most susceptible. The susceptibility of Inia 66, tested only in 1993, was similar to that of Opata 85.

The range of scab severity recorded for the F_6 lines, population mean, and the LSD for each cross are given in Table 2. Frequency distributions for percent scab severity of the F_6 lines for each cross tested during 1991 and 1993 are shown in Figs. 1–5. The distribution of F_6 line means was continuous in both years for each cross. Transgressive segregants (based on LSD values at $P = 0.05$) for resistance or susceptibility were observed in the crosses of Frontana with Inia 66 and Pavon 76. However, the transgressive segregants in the cross Frontana/Opata 85 belonged to the higher susceptibility group only. During both years, the mean scab severity of the F_6 lines (Table 2) for the crosses of Frontana with Opata 85 and Pavon 76 was close to the midparental value (parental values are given in Table 1). However, the mean percent scab for F_6 lines from Frontana/Inia 66 was somewhat lower than the mid parental value.

Table 3. Estimates of minimum number of effective genes segregating for scab resistance in crosses of cultivars by using Wright's (1968) formula modified for F_6 generation

Cross	Year	Number of genes segregating for scab severity			
		Infected spikelets		Percent	
		Method I[a]	Method II[b]	Method I[a]	Method II[b]
Frontana/Inia 66	1993	4.5	3.8	4.2	3.6
Frontana/Opata 85	1991	6.4	2.9	6.8	3.0
Frontana/Opata 85	1993	3.4	2.8	3.4	2.9
Frontana/Pavon 76	1991	5.7	2.5	6.6	2.9
Frontana/Pavon 76	1993	3.5	2.9	3.6	3.0

[a] Genotypic range used in the formula = phenotypic range of the F_6 lines.
[b] Genotypic range used in the formula = phenotypic range of the F_6 lines × heritability.

Table 4. Frequencies and χ^2 analysis for F_6 lines tested during 1993

Cross	Number of F_6 lines[a]		χ^2 and P values[b]		
	Resistant-intermediate	Susceptible	3 genes	4 genes	5 genes
Frontana/Inia 66	98	9	0.41, $P > 0.5$	3.05, $P > 0.05$	17.8, $P < 0.01$
Frontana/Opata 85	50	6	0.01, $P > 0.9$	4.29, $P > 0.01$	...
Frontana/Pavon 76	52	3	1.40, $P > 0.1$	0.05, $P > 0.75$	2.44, $P > 0.1$

[a] F_6 lines with 78.5% scab in Frontana/Inia 66, and 77.1% scab in Frontana/Opata 85 and Frontana/Pavon 76, were considered to be susceptible. Remaining lines were grouped in the resistant-intermediate category.
[b] Ratios used for χ^2 analyses are 0.897:0.103, 0.952:0.048, and 0.977:0.023, which are expected for independent segregations of 3, 4, and 5 genes, respectively.

The narrow-sense heritability estimate for the two crosses tested during 1991 was 0.66 (Table 2). The heritabilities for all three crosses tested during 1993 were high (Table 2).

The minimum number of effective genes conferring resistance to scab in each cross, calculated from the modified formula of Wright (14), is given in Table 3. Data on actual infected spikelets or percent infected spikelets gave similar estimates of the gene number. However, the estimates were influenced by the measure of the genotypic range used in the formula. When the phenotypic range of the F_6 lines was used, the gene estimates varied between years. However, the correction of the phenotypic range of the F_6 lines by multiplying with the narrow-sense heritability (method II) gave similar estimates during both years. The overall results on gene estimation indicated that at least three additive genes confer resistance to scab.

The number of resistance genes was also estimated by classifying the F_6 lines in two categories: resistant-intermediate and susceptible (Table 4). Only 1993 data were used because of the high heritability estimates for the crosses. Although observed frequencies of F_6 lines in the cross Frontana/Inia 66 conformed with both the frequencies expected for segregations of three and four resistance genes, the distribution was closer to that expected for three-gene segregation. The observed frequencies of the F_6 lines for the cross Frontana/Opata 85 agreed with the ratio expected for segregation of three genes. In the cross Frontana/Pavon 76, the frequencies of the F_6 lines were in agreement with the frequencies expected for segregations of three, four, or five independent genes; however, a four-gene segregation model had the highest

probability.

Based on the two kinds of genetic analyses, i.e., quantitative and qualitative, it appears most likely that at least three additive genes are involved in Frontana's resistance to scab. Pavon 76, which was more resistant than Inia 66 and Opata 85, but less resistant than Frontana, should carry at least one or two minor genes. Because transgressive segregants with 100% scab were observed during 1993 tests, it can be inferred that Inia 66 and Opata 85 may also carry some resistance, most likely controlled by one minor gene. Segregants with significantly higher resistance than in either parent were recovered in crosses with Inia 66 and Pavon 76. The number of resistance genes reported to confer scab resistance in other wheat cultivars varies from one to six (3,10,12).

Scab inoculation, using a small tuft of cotton soaked with the inoculum, is a labor-intensive but precise method. Other inoculation methods result in uneven infection and disease spread, and therefore may complicate the genetic interpretation of the results.

Because only a few (three or four) additive genes are needed to achieve a good level of scab resistance, mild selection could be applied in the F_2 generation. Strong selection pressure should be applied in higher generations when high homozygosity has been achieved. Cultivars of diverse origin, reported to have resistance to scab, should be used in crossing. If the genes for resistance in the parents are different then the possibility of recovering transgressive segregants with improved resistance is increased.

LITERATURE CITED

1. Bekele, G. T. 1985. Head scab screening methods used at CIMMYT. Pages 169-173 in: Wheats for More Tropical Environments, a Proceedings of the International Symposium. CIMMYT, Mexico City.
2. Cockerham, C. C. 1983. Covariances of relatives from self-fertilization. Crop Sci. 23:1177-1180.
3. Gu, J. 1983. A study on the genetics of resistance to wheat scab. Sci. Agric. Sin. 6:61-64.
4. Luzzardi, G. C. 1985. Wheat breeding for scab resistance. Pages 158-168 in: Wheats for More Tropical Environments, a Proceedings of the International Symposium. CIMMYT, Mexico City.
5. Miller, J. D., and Arnison, P. G. 1986. Degradation of deoxynivalenol by suspension cultures of the fusarium head blight resistant wheat cultivar Frontana. Can. J. Plant Pathol. 8:147-150.
6. Mulitze, D. K., and Baker, R. J. 1985. Genotype assay and method of moments analysis of quantitative traits in a spring wheat cross. Crop Sci. 25:162-167.
7. SAS Institute. 1985. SAS user's guide: statistics. SAS Instute., Cary, NC.
8. Schroeder, H. W., and Christensen, J. J. 1963. Factors affecting resistance to wheat scab caused by *Gibberella zeae*. Phytopathology 53:831-838.
9. Singh, R. K., and Chaudhary, B. D. 1977. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India.
10. Snijders, C. H. A. 1990. The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:11-18.
11. Snijders, C. H. A. 1990. Genetic variation for resistance to fusarium head blight in bread wheat. Euphytica 50:171-179.
12. Yu, Y. J. 1982. Monosomic analysis for scab resistance and yield components in the wheat cultivar soo-moo-3. Cereal Res. Commun. 10:185-190.
13. Wiese, M. V., ed. 1977. Compendium of Wheat Diseases. American Phytopathological Society, St. Paul, MN.
14. Wright, S. 1968. Evolution and Genetics of Populations. Vol. I. Genetic and Biometric Foundations. University of Chicago Press, Chicago.
15. Zhong, W. Y., and Miller, J. D. 1988. Screening techniques and sources of resistance to fusarium head blight. Pages 239-250 in: Wheat Production Constraints in Tropical Environments. A.R. Klatt, ed. CIMMYT, Mexico City.