Detection and Manipulation of Resistance to Septoria nodorum in Wheat

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Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture; the Department of Plant Pathology, Montana State University; and the Montana Agricultural Experiment Station.

Journal Series Paper No. 791 of the Montana Agricultural Experiment Station.

Accepted for publication 25 July 1977.

ABSTRACT

SCHAREN, A. L., and J. M. KRUPINSKY. 1978. Detection and manipulation of resistance to Septoria nodorum in wheat. Phytopathology 68:245-248.

A method is described for obtaining resistance to *Septoria nodorum* in wheat. Seedling plants in each self-fertilized, but segregating generation (F_2, F_3, F_4, F_5) were subjected to a heavy inoculation with a mixture of isolates of *S. nodorum*. The most resistant plants, as measured by the degree of chlorosis and necrosis, as well as the number and size of lesions in seedling leaves from each generation, were grown to maturity and bulk-harvested to provide seed for the next generation. The F_6 progeny of all crosses were more resistant than their parents. The F_7 progeny were significantly better than the F_2 generation and in most cases, significantly better than their parents on the basis of number of lesions and percentage necrosis per square centimeter. The method proved to be effective for detecting and accumulating resistance to *S. nodorum* in wheat seedlings.

Additional key words: transgressive segregation.

The glume blotch disease of wheat, *Triticum aestivum* L., caused by *Septoria nodorum* (Berk.) Berk. *(Leptosphaeria nodorum* Müller) has become a major problem in recent years especially in Europe and South America (1, 12). The disease can cause severe reductions in yield (4, 7, 12, 14). Although sources of resistance have been sought (9, 10, 13, 14) and some have been found, one of the problems of utilizing known resistance is the apparent lack of single genes which alone give easily detectable levels of resistance (14). Brönnimann has described and developed tolerance to *S. nodorum* in wheat (2, 3).

Recessive, dominant, and partially dominant gene action has been implicated in previous studies of resistance to *S. nodorum* (5, 11). Plant breeding with emphasis on selection of transgressive types has been proposed as a method of extending the present limits and intensities of desired characters (6). The purpose of this paper is to describe a method used to increase levels of resistance in a segregating wheat population and to describe the development of wheat lines which are more resistant to *S. nodorum* than their parents.

MATERIALS AND METHODS

The parents, selected from among winter wheats that exhibited a range of resistance to *S. nodorum* (10), were: 0-6, a selection of Gasta (C.I. 11398); Redhart (C.I. 8898); Red Chief (C.I. 12109); Anderson (C.I. 12536); KS 43 B337 (C.I. 12752); Turkey selection (C.I. 11984); Hadden (C.I. 13488); and Candeal De Arevalo (P.I. 191037). A total of five crosses were used in this study. These included: 1 (0-6/12536); 2 (8898/12109); 4 (12109/8898); and 9 (12752/191037) made in 1971, and 18 (13488/11984) made in 1972. The spring wheat cultivar Fortuna (C.I. 13596) was used as a susceptible check.

After the initial cross, each subsequent, selfed generation (F_2, F_3, F_4, F_5) was space-planted in flats (32 × 21 × 5 cm) of sterile sand (125 seeds/flat). The plants, average 250 per generation, were grown in a greenhouse under natural light at 20 ± 3 C. The plants were allowed to grow to the third-leaf stage (10-14 days) before being subjected to a heavy inoculation with *S. nodorum*.

Isolates of *S. nodorum*, which were representative of a wide geographic area, were maintained on yeast-malt extract agar at 19-20 C under continuous illumination of a 15-w cool white fluorescent tube (5×10^3 ergs/cm^2/sec) in an incubator (8, 13), and were composited during preparation of inoculum. Pycnidiospores and mycelial fragments were obtained by scraping the fungus from agar plates into a blender, macerating, filtering through cheesecloth, and adding a surfactant (Ivory dish detergent, 0.1 ml/liter). The inoculum (15-30 × 10^6 spores/ml) was atomized onto the leaves until runoff occurred. After inoculation, plants were maintained in a saturated atmosphere provided by a plastic chamber and two cool-vapor humidifiers. Early generation material (F_2, F_3) was maintained in the chamber for 48 hr, but the time was increased to 72 hr when later generation material (F_4, F_5) was tested because of the increased levels of

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resistance (5). After the required moist period, the flats were returned to a greenhouse bench. Seven days after inoculation, lesions, chlorosis, and necrosis were recorded. The most resistant plants (5-10%) from each generation were selected, transplanted, vernalized, and grown to maturity in the greenhouse for seed production. The seed from each selected population was harvested for the next cycle of screening.

The first experiment, which was designed to compare parents with selected progeny, was conducted on one F_5 and four F_6 bulk populations. The parents and the advanced generation material were grown in 6-cm-diameter pots placed in a random design and the plants were inoculated with an inoculum suspension containing $1.7\text{-}1.9 \times 10^7$ spores/ml. Seven days after inoculation, individual plants were rated for lesions, chlorosis, and necrosis. Leaf sections were collected and stained according to the procedures of Shipton and Brown (15). After 1 mo exposure to the stain, six random leaf sections from each cross were mounted on microscope slides and photographed through a dissecting microscope. Photographs (20×25 cm) were used to count lesions and measure the area covered by them.

A second experiment was conducted on the F_2 and F_7 bulked populations of crosses 1 (0-6/12536) and 9 (12752/191037), and the parents used in making these crosses. Seven days after inoculation, the first leaf of each plant was collected and photographed. Using this method all the leaves could be collected and preserved for detailed measurements of lesions and necrosis at a later date. The areas of the leaves were computed; all lesions were counted and percent necrosis was calculated. Comparisons of numbers of lesions/cm^2 and percent necrosis cm^2 were made for Parent 1 (30 leaves), Parent 2 (30 leaves), F_2 (120 leaves), and F_7 (120 leaves). Data collected as percentages were transformed to angles for statistical analyses.

RESULTS

Fewer lesions and less necrosis usually developed in the F_5 and F_6 selected populations than in their parents (Table 1). Also an increased number of plants of the F_6 generation were symptomless. Leaf chlorosis in the advanced generations was less than either parent in cross 4 (12109/8898) and less than one parent in crosses 1 (0-6/12536) and 2 (8898/12109). A comparison of cross 4 (12109/8898) with 3 (8898/12109) yielded evidence that maternal or cytoplasmic factors may be involved. In crosses 9 (12752/191037) and 18 (13488/11984), there was an increase in the percentage of individuals having chlorosis in the F_5 and F_6 generations. This was

TABLE 1. Categorical classification of symptoms on wheat leaves of parental and F_6 selected progeny lines, and indices of leaf-tissue damage caused by inoculation with *Septoria nodorum*

	Plants with:[a]					
	No symptoms (%)	Typical lesions (%)	Chlorosis w/o lesions (%)	General necrosis (%)	Damage index[b]	Index as[b] percentage of check (%)
Cross 1						
0-6	35	12	45	9	863	58
C.I. 12536	0	20	3	76	592	40
F_6	89	5	5	1	63	4
Cross 2[c]						
C.I. 8898	37	29	27	4	586	39
C.I. 12109	87	5	8	0	452	30
F_6	72	6	17	4	368	25
Cross 4[c]						
C.I. 12109	87	5	8	0	452	30
C.I. 8898	37	29	27	4	586	39
F_6	94	5	1	0	441	30
Cross 9						
C.I. 12852	8	33	51	8	573	39
P.I. 191037	0	100	14	86	1,122	76
F_6	10	0	85	6	159	11
Cross 18						
C.I. 13488	1	9	61	28	1,035	69
C.I. 11984	0	0	67	33	522	35
F_5	2	0	97	1	254	17
Fortuna (Susceptible check)	0	100	0	100	1,487	100

[a]Percentages of total number examined.
[b]Lesion length × number = index. Also given as percentages of the check cultivar, Fortuna.
[c]Crosses 2 and 4 are reciprocal, the first listed parent being male, and the second female.

considered to be an improvement in the level of resistance because of the high level of necrosis in the parents, and the apparent shifting of advanced generations away from the more severe necrosis to less damaging chlorosis.

The F_5 and F_6 populations were also compared with their parents for average number of lesions and the average length of lesion, using stained leaves. A disease index, calculated by multiplying the average number of lesions by the average length of lesion, was used to compare the relative amounts of tissue damaged by *S. nodorum* (Table 1). Using the disease index, all results were reported as percentages of the susceptible check, Fortuna. All selected progeny lines exhibited fewer symptoms of *S. nodorum* than did their parents.

In a second series of experiments, the selected F_7 bulk progeny of cross 1 (0-6/12536) had significantly fewer lesions and less necrosis than either the F_2 or the parents (Table 2). The F_7 progeny exhibited 61% fewer lesions/cm^2 and 71% less necrosis/cm^2 than the best parent. Comparing the F_7 with the F_2 for lesion number/cm^2, there were twice as many plants with less than two lesions/cm^2 in the F_7 than in the F_2 (Table 2). Also, there were 1.7 times as many plants in the F_7 with less than 5% necrosis/cm^2 than in the F_2 (Table 2). The selected F_7 bulk progeny from cross 9 (12752/191037) had significantly fewer lesions and less necrosis than the F_2 and one parent, P.I. 191037 (Table 3). The F_7 bulk progeny were not significantly different from the parent, C.I. 12752. Ninety percent of the plants in the F_7 had less than two lesions/cm^2 in contrast to 1% of the F_2 plants in this category. Eighty-nine percent of the F_7 plants had less than 5% necrosis/cm^2 in contrast to 5% of the F_2 plants in this category.

TABLE 2. Comparison of numbers of lesions and amount of necrosis on seedling wheat leaves of parents, F_2 progeny, and F_7 progeny of a cross between lines 0-6 and C.I. 12536 inoculated with *Septoria nodorum*

Wheat lines	Leaves (no.)	Lesions/cm^2 (mean no.)	Necrosis/cm^2 (mean %)
0-6	30	8.26 a[x]	26.05 a
C.I. 12536	30	15.78 b	66.12 b
F_2	120	12.90 b[y]	38.35 a[z]
F_7	120	3.19 c	7.56 c

[x]Numbers followed by different letters are significantly different ($P = 0.05$).

[y]Number of leaves in each category of lesions/cm^2 were: for 0-1.9 lesions, $F_2 = 45$ and $F_7 = 94$; for 2-9 lesions, $F_2 = 26$ and $F_7 = 18$; for 10-19 lesions, $F_2 = 14$ and $F_7 = 7$; for 20-29 lesions, $F_2 = 17$ and $F_7 = 1$; for 30-39 lesions, $F_2 = 13$ and $F_7 = 0$; for 40-49 lesions, $F_2 = 4$ and $F_7 = 0$; and for 50-59 lesions, $F_2 = 1$ and $F_7 = 0$.

[z]Number of leaves in each category of percent necrosis/cm^2 were: for 0-4.9%, $F_2 = 42$ and $F_7 = 77$; for 5-9%, $F_2 = 10$ and $F_7 = 8$; for 10-19%, $F_2 = 9$ and $F_7 = 19$; for 20-29%, $F_2 = 7$ and $F_7 = 9$; for 30-39%, $F_2 = 4$ and $F_7 = 2$; for 40-49%, $F_2 = 4$ and $F_7 = 3$; for 50-59%, $F_2 = 2$ and $F_7 = 2$; for 60-69%, $F_2 = 3$ and $F_7 = 0$; for 70-79%, $F_2 = 6$ and $F_7 = 0$; for 80-89%, $F_2 = 6$ and $F_7 = 0$; for 90-99%, $F_2 = 11$ and $F_7 = 0$; and for 100%, $F_2 = 16$ and $F_7 = 0$.

TABLE 3. Comparison of numbers of lesions and amount of necrosis on seedling wheat leaves of parents, F_2 progeny and F_7 progeny of a cross between C.I. 12752 and P.I. 191037 inoculated with *Septoria nodorum*

Wheat lines	Leaves (no.)	Lesions/cm^2 (mean no.)	Necrosis/cm^2 (mean %)
C.I. 12752	30	5.43 a[x]	3.62 a
P.I. 191037	30	23.22 b	32.97 b
F_2	100	30.32 b[y]	51.80 c[z]
F_7	100	0.97 a	2.07 a

[x]Numbers followed by different letters are significantly different ($P = 0.05$).

[y]Number of leaves in each category of lesions/cm^2 were: for 0-1.9 lesions, $F_2 = 1$ and $F_7 = 90$; for 2-9 lesions, $F_2 = 15$ and $F_7 = 8$; for 10-19 lesions, $F_2 = 18$ and $F_7 = 1$; for 20-29 lesions, $F_2 = 14$ and $F_7 = 1$; for 30-39 lesions, $F_2 = 25$ and $F_7 = 0$; for 40-49 lesions, $F_2 = 15$, $F_7 = 0$; for 50-59 lesions, $F_2 = 4$ and $F_7 = 0$; for 60-69 lesions, $F_2 = 5$ and $F_7 = 0$; and for 70-79 lesions, $F_2 = 3$ and $F_7 = 0$.

[z]Number of leaves in each category of percent necrosis/cm^2 were: for 0-4.9%, $F_2 = 5$ and $F_7 = 89$; for 5-9%, $F_2 = 7$ and $F_7 = 5$; for 10-19%, $F_2 = 10$ and $F_7 = 4$; for 20-29%, $F_2 = 6$ and $F_7 = 0$; for 30-39%, $F_2 = 6$ and $F_7 = 1$; for 40-49%, $F_2 = 10$ and $F_7 = 1$; for 50-59%, $F_2 = 12$ and $F_7 = 0$; for 60-69%, $F_2 = 14$ and $F_7 = 0$; for 70-79%, $F_2 = 10$ and $F_7 = 0$; for 80-89%, $F_2 = 11$ and $F_7 = 0$; for 90-99%, $F_2 = 2$, $F_7 = 0$; and for 100%, $F_2 = 7$ and $F_7 = 0$.

DISCUSSION

Selection of the best plants from self-fertilized, but segregating, progeny of wheat crosses for several generations has been an effective technique for obtaining a population resistant to *S. nodorum*. The selected progeny exhibit fewer symptoms of disease than either the parents or the F_2 generation when subjected to heavy inoculations with the pathogen. This was demonstrated with the F_5 and F_6 generations which had fewer lesions and less chlorosis and necrosis, as well as a lower index of disease obtained by multiplying the number of lesions by the length of lesions. Enhanced resistance of the progeny lines was demonstrated subsequently when F_7 populations were compared with F_2 populations and the two parents on the basis of number of lesions/cm^2 and percent necrosis/cm^2. The method permits selection of transgressive types, thus increasing the resistance of wheat seedlings to *S. nodorum*. The enhanced resistance of progeny lines is probably due to additive gene action and/or dominance. Although it seems likely that the more resistant progeny are homozygous, it is possible that heterozygous plants are the most resistant.

At present, this method is being used to develop germplasm with higher levels of resistance than the wheat lines currently available. In the future, modern cultivars should be used as parents, and higher levels of resistance should be incorporated into modern cultivars. Indications are that factors for resistance to disease that can be accumulated are present in most lines and cultivars.

Preliminary data indicate that grain yields harvested from these derived lines after our selection procedure is completed are at least equal to yields obtained from the parents. Yields measured were from selected plants grown under controlled environments for seed increase. These had been inoculated as seedlings, and selected for low levels or absence of symptoms. Under continued disease pressure for a greater portion of the growing season, the resistant progeny could be expected to yield more than the parents.

LITERATURE CITED

1. ANONYMOUS. 1976. Multilines: safety in numbers. CIMMYT Today, No. 4, 1976. 12 pages. Published by Centro International de Mejoramiento de Maiz y Trigo, Apartado Postal 6-641, Mexico 6, D.F., Mexico.
2. BRÖNNIMANN, A. 1975. Beitrag zur Genetik der Toleranz auf Septoria nodorum Berk. bei Weizen (Triticum aestivum). Z. Pflanzenzeucht. 75:138-160.
3. BRŐNNIMANN, A. 1970. Zur Vererbung der Toleranz des Weizens gegenuber Befall durch Septoria nodorum Berk. Z. Pflanzenzeucht. 63:330-340.
4. BRŐNNIMANN, A., B. K. SALLY, and E. L. SHARP. 1972. Investigations on Septoria nodorum in spring wheat in Montana. Plant Dis. Rep. 56:188-191.
5. EYAL, Z., J. M. KRUPINSKY, and A. L. SCHAREN. 1976. Inheritance of symptom responses to inoculation with Septoria nodorum Berk. in wheat seedlings. Proc. Am. Phytopathol. Soc. 3:1 (Abstr.).
6. HEYNE, E. G., and G. S. SMITH. 1967. Wheat breeding. Pages 269-303 *in* K. S. Quisenberry and L. P. Reitz, eds. Wheat and wheat improvement. Am. Soc. Agronomy, Madison, Wisconsin. 560 p.
7. JENKINS, J. E. E., and W. MORGAN. 1969. The effect of Septoria diseases on the yield of winter wheat. Plant Pathol. 18:152-156.
8. KRUPINSKY, J. M. 1976. Techniques for screening wheat for Septoria resistance. Pages 28-33 *in* Septoria diseases. Proc. Septoria diseases of Wheat Workshop, Ga. Agric. Exp. Stn. Special Pub. No. 4. 69 p.
9. KRUPINSKY, J. M., J. C. CRADDOCK, and A. L. SCHAREN. 1977. Septoria resistance in wheat. Plant Dis. Rep. 61:632-636.
10. KRUPINSKY, J. M., J. A. SCHILLINGER, and A. L. SCHAREN. 1972. Resistance in wheats to Septoria nodorum. Crop Sci. 12:528-530.
11. LAUBSCHER, F. X., B. VON WECHMAR, and D. VAN SCHALKWYK. 1966. Heritable resistance of wheat varieties to glume blotch (Septoria nodorum Berk.). Phytopathol. Z. 56:260-264.
12. SAARI, E. E., and R. D. WILCOXSON. 1974. Plant disease situation of high-yielding dwarf wheats in Asia and Africa. Annu. Rev. Phytopathol. 12:49-68.
13. SCHAREN, A. L., and J. M. KRUPINSKY. 1970. Cultural and inoculation studies of Septoria nodorum, cause of glume blotch of wheat. Phytopathology 60:1480-1485.
14. SHIPTON, W. A., W. J. R. BOYD, A. A. ROSIELLE, and B. I. SHEARER. 1971. The common Septoria diseases of wheat. Bot. Rev. 37:231-262.
15. SHIPTON, W. A., and J. F. BROWN. 1962. A whole-leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology 52:1313.