Reciprocal Translocation Testcross Analysis of Genes for Anthracnose Stalk Rot Resistance in a Corn Inbred Line

M. L. Carson and A. L. Hooker

Graduate research assistant and professor, Department of Plant Pathology, University of Illinois, Urbana 61801.
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Present title and address of senior author: assistant professor of Plant Science, Department of Plant Science, South Dakota State University, Brookings 57007.
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ABSTRACT

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Nineteen reciprocal translocation testcross populations of corn of the form: (translocation stock × A556) × C123, were evaluated in the field for reaction to anthracnose stalk rot (ASR), which is caused by *Colletotrichum graminicola*. The translocation stocks involved 15 of the 20 chromosome arms. Partial sterility in translocation heterozygotes was found to reduce ASR severity when compared with fully fertile segregates (homozygous for "normal" chromosomes) in testcross populations. The long arms of chromosomes 1, 4, and 8 and both arms of chromosome 6 were found to carry genes for ASR resistance in A556. The relatively small number of genes or blocks of genes involved indicates that progress in selecting for ASR resistance should be rapid and effective.

Additional key words: maize, *Zea mays*.

Stalk rots are one of several major disease problems of corn in the United States (3). Yield losses from stalk rot can be caused by the premature death of plants, by stalk lodging, which results in unharvestable ears, and by ear rots that develop on lodged plants. Historically, *Diplodia maydis* (Schw.) Lev., *Gibberella zeae* (Schw.) Petch, and *Fusarium* spp. have been considered the primary causal agents of corn stalk rots in the U.S. Corn Belt (3). Evidence accumulated within the last two decades indicates that anthracnose stalk rot (ASR), which is caused by *Colletotrichum graminicola* (Ces.) Wils., has become a major stalk rot problem of corn in the warmer, more humid areas of the Corn Belt (5,9,14, 25). Williams and Willis (25) reported that *C. graminicola* was isolated from 50% of the infected stalks in plots at the Ohio Agricultural Experiment Station in 1961. Dale (5) reported in 1963 that corn anthracnose was common in Arkansas. Anthracnose diseases were the most destructive diseases of corn in North Carolina in 1972 and 1973 (14). In 1975, *C. graminicola* was associated with rotted stalks from 78% of fields examined in Illinois (9).

The potential for ASR to cause yield losses in dent corn has been demonstrated. Dale (5) reported no yield decreases or increased stalk lodging due to ASR when inoculated and uninoculated plots of two hybrids were compared. Perkins and Hooker (18) reported losses up to 17.2% when infected and uninfected plants of certain hybrids were compared under conditions of natural infection. Losses were attributed to premature plant death before the grain filling period was complete. Plot yield losses up to 40% have recently been demonstrated when plants of a susceptible hybrid artificially inoculated at anthesis were compared to uninoculated ones (24). The authors also reported that some hybrids, while not showing losses from premature killing by ASR, can suffer losses at harvest due to increased stalk lodging.

Genetic resistance is presently the only economical method available for the control of corn stalk rots. Although variation for reaction to ASR exists among corn inbred lines and hybrids, genetic information as it relates to resistance to ASR is limited. A diallel analysis of resistance to *C. graminicola* among 10 corn inbred lines indicated that resistance to ASR was polygenic, mostly additive in inheritance, and that resistant hybrids could be produced by crossing two resistant inbred lines (15). Other studies involving F_2 and backcross generations derived from crosses of resistant × susceptible inbred lines indicate resistance is controlled by several factors, acting in a mostly additive manner (*unpublished*).

Reciprocal chromosomal translocation stocks are a valuable tool in the study of inheritance of both qualitative and quantitative traits in corn. Translocation stocks have been used to identify chromosome arms involved in resistance in corn to *Helminthosporium carbonum* (23), *Helminthosporium turcicum* (11,12), *Ustilago maydis* (2, 19), *Diplodia maydis* (7), maize dwarf mosaic virus (8,20,21), and the European corn borer (*Ostrinia nubilalis*) (10). Chromosomal translocation studies can provide useful information about numbers of genes or blocks of genes involved in controlling the trait, the relative magnitude of the effects of these genes, possible linkage relationships with other genes, and corroborate or confirm data from other genetic studies of the trait.

The purpose of this study was to locate to chromosome arms genes or blocks of genes conditioning resistance to ASR in the highly resistant Corn Belt inbred A556 by means of reciprocal translocations and to provide additional information about the inheritance of resistance to ASR.

MATERIALS AND METHODS

A total of 19 reciprocal chromosomal translocation stocks, which involved 15 chromosome arms, were used to determine which chromosome arms in the highly resistant inbred A556 carry genes conditioning resistance to ASR. Five of the translocation stocks were in a W23 corn inbred line genetic background, while the remaining 14 stocks were in an M14 background. All the stocks had been backcrossed at least four times to the recurrent parent and were considered homozygous for the loci under study. Both inbred lines W23 and M14 are highly susceptible to ASR.

Since the inbred A556 confers some resistance in hybrid combination with ASR susceptible inbred lines, the type of testcross population that would segregate for both ASR reaction and semisterility would be the testcross: (translocation stock × A556) × susceptible tester (1). The tester used in this experiment

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was the highly susceptible inbred line, C123.

The 19 translocation testcross populations were grown in the summer of 1979 on the Agronomy South Farm, Urbana. Populations were planted in rows 76 cm apart and 419 cm long, with two kernels planted per hill in hills 38 cm apart. Plots were later thinned to 18 plants per row where feasible. Actual numbers of plants evaluated in each testcross population ranged from 132 to 181, the differences due to numbers of rows per population and loss of stand from poor seed germination or improper thinning.

Plants were inoculated, rated for ASR reaction, and the data analyzed statistically. Plants were inoculated approximately 2 wk after midsilk by injecting 2 ml of a 2×10^5 conidia per milliliter suspension in water into the first elongated internode above the top set of brace roots with a 50 cm^3 Vaco Pistol-Grip Rubber Plunger Syringe (Ideal Instruments, Inc., Chicago, IL 60612) fitted with a special stainless steel needle similar to that described by Koehler (13). Inoculum was prepared by washing conidia from the surface of oatmeal agar cultures of *C. graminicola* and adjusting the suspension to final concentration. Individual plants were classified for semisterile or normal fertility and for ASR reaction 4–5 wk after inoculation. Plants were classified as being semisterile or fertile based upon a visual determination of seed set. Plants classified as semisterile were tagged at the base for later identification. Individual plants were evaluated for ASR reactions by removing the upper portion of the plant above the ear, splitting the lower stalk longitudinally, and rating it for the total number of internodes with discoloration and the number of those internodes showing 75% or greater rotting. Data were later converted to a 12-class disease severity scale based upon the amount and spread of tissue discoloration in the inoculated internode and the five internodes above it. Disease scores of individual plants equalled the number of these six internodes with discoloration plus the number of these internodes >75% rotted.

Means and variances of ASR ratings of fertile and semisterile plants in each testcross were calculated separately and statistical tests performed. Since fertile plants consistently showed greater variation in ASR reaction than did semisteriles, a one-tailed *t*-test (4) designed for unpaired observations with unequal variances was used to test whether the mean difference in ASR reaction between normal and semisterile plants in a testcross was significantly less than the estimated effect of normal fertility on ASR.

RESULTS AND DISCUSSION

Mean differences in ASR reactions of fertile and semisterile plants in each testcross are shown in Table 1. Normal plants averaged 1.84 greater disease severity (12-class scale) than did semisteriles, averaged across all testcrosses. The overall variance of individual plant ASR reactions was 10.5611 among fertile plants compared to 6.5329 among semisteriles.

The usual procedure for determining which chromosome arms are carrying genes of interest is to test for a significantly greater expression of the trait among normal plants than among semisteriles within a testcross population (1). A significantly greater expression of the trait in normal plants indicates that one or both of the chromosome arms involved in the reciprocal translocation carry the gene or genes of interest. In none of the testcross populations in this study were the normal plants significantly more resistant (had a lower score) than semisteriles. Therefore, it was assumed in this study that full fertility was a factor that increased ASR severity and that when chromosome arms involved in the reciprocal translocation stock also carry genes for ASR resistance from A556, the mean ASR reaction of fertile plants will approach that of the semisteriles. In our initial analysis, the translocation was judged to involve a chromosome arm carrying a gene(s) for resistance when the mean difference in ASR reactions between normal and semisterile plants in testcross populations was significantly ($P \leq 0.05$) less than the difference between normals and semisteriles averaged over all testcrosses (1.84). This overall difference was considered an estimate of the effect of normal fertility on ASR reactions when no genes for resistance were involved. Because the overall difference was estimated with data from some testcrosses where genes for ASR resistance were apparently involved, it was considered an underestimate of the effect of normal fertility upon ASR, making our initial analysis too conservative (excessive Type I error rate). Testcrosses judged to involve ASR resistance genes in the initial analysis were omitted from the estimate of the effect of normal fertility (2.59) in the subsequent analysis.

Using reciprocal translocations to locate chromosome arms carrying genes for ASR resistance in A556 has several limitations. The set of translocation stocks used does not adequately cover the entire corn genome. Chromosome arms not represented in the set of 19 stocks are the short arms of chromosomes 3, 4, 5, and 8; the long arm of chromosome 10, and regions of chromosome 7 greater than 50 map units from the centromere. Several other chromosome arms are not represented in their entirety. Several chromosome arms are represented by two or more breakpoints in the set. The long arms of chromosomes 1,6, and 8 have two breakpoints apiece in the set of translocation stocks, while the long arm of chromosome 5 is represented by three breakpoints. Chromosome 9 is more than

TABLE 1. Mean differences in anthracnose stalk rot (ASR) reactions of fertile and semisterile corn plants in (translocation × A556) × C123 testcross populations following artificial inoculation with *Colletotrichum graminicola* in the field in 1979

Translocation	Background and No. of backcrosses	Number of plants evaluated	Breakpoints[a]		Mean difference in ASR reaction (fertiles-semisteriles)
1-9c	M14 (5)	137	1S.48	9L.22	2.06
1-9L	W23 (5)	157	1S.48	9L.22	2.56
$1\text{-}9_{4995}$	M14 (8)	173	1L.19	9S.20	0.05**[b]
$1\text{-}9_{8389}$	W23 (5)	132	1L.74	9L.13	1.06**
2-9b	M14 (8)	164	2S.18	9L.22	2.15
2-9d	M14 (4)	154	2L.83	9L.27	2.78
3-9c	M14 (7)	175	3L.09	9L.12	3.56
4-9b	M14 (8)	134	4L.90	9L.29	0.48**
$4\text{-}9_{5657}$	M14 (8)	175	4L.33	9S.25	2.92
5-9a	M14 (7)	156	5L.86	9S.40	1.96
5-9d	M14 (5)	156	5L.14	9L.10	2.42
$5\text{-}9_{4817}$	M14 (9)	180	5L.06	9L.07	2.89
6-9a	M14 (5)	170	6S.79	9L.10	−0.43**
6-9b	M14 (8)	181	6L.10	9S.37	0.02**
$6\text{-}9_{4505}$	M14 (8)	171	6L.13	9 cent.	−0.51**
$7\text{-}9_{4363}$	M14 (5)	169	7 cent.	9 cent.	3.57
8-9d	M14 (8)	164	8L.09	9L.16	1.02**
$8\text{-}9_{6673}$	M14 (7)	173	8L.35	9S.31	2.37
9-10b	W23 (5)	173	9S.13	10S.40	3.42

[a] Breakpoint locations are indicated as a proportion of the total distance of the chromosome arm from the centromere to the distal end.

[b] ** Indicates that the difference is significantly less ($P \leq 0.01$) than 2.594, the estimate of the effect of normal fertility on ASR reaction when no genes for resistance are involved in the translocation.

adequately represented in the set, since all the translocations involve chromosome 9. Another possible limitation is that not all the translocation stocks had been backcrossed an equal number of times to the recurrent parent. The reduction in chiasmata near the translocation breakpoint, however, would make the development of "isogenic" translocation stocks impossible.

Three types of genes can be detected by the reciprocal translocation technique (7): type 1—genes whose effect is small and that are close to the breakpoint; type 2—genes with a major effect and that are less than 50 map units from the breakpoint; and type 3—genes with major effects that are close to the breakpoint. While detection of the latter type of genes should be easy, the sensitivity of the method to detect genes of types 1 and 2 is dependent on the numbers of plants sampled, the variability of individual plant reactions, and the closeness of the genes to the breakpoint. The technique used will not detect either genes with small effects and that are distant from the breakpoint, or recessive genes. Therefore, estimates of gene numbers involved in ASR resistance are minimal estimates.

These data indicate that at least five chromosome arms in A556 carry genes conditioning ASR resistance. Genes with major effects on ASR reaction in A556 are carried on the long arms of chromosomes 1 and 4, both arms of chromosome 6, and the long arm of chromosome 8. The data suggests that the gene or genes on the long arm of chromosome 1 is closer to the breakpoint at 0.19 of the total distance from the centromere to the distal end than to the breakpoint at 0.74 of the distance, since the magnitude of the *t*-value for the mean difference in the $1\text{-}9_{4995}$ testcross was twice that of the $1\text{-}9_{8389}$ testcross. The gene or genes on the long arm of chromosome 4 appear to be closer to the breakpoint at 0.90 distance from the centromere than to the breakpoint at 0.33 of the distance, since the *t*-value for the 4-9b testcross was highly significant, but not the $4\text{-}9_{5657}$ testcross. The significant mean difference in the 8-9d testcross indicates that a gene or genes lie near the centromere on the long arm of chromosome 8 (Table 1). The effect of this gene was not detected in the $8\text{-}9_{6673}$ testcross where the break point on the long arm of chromosome 8 was more distal.

In a previous study using chromosomal translocations to locate genes for resistance to *Diplodia maydis* in corn it was assumed that partial seed set due to semisterility did not affect stalk rot development (7). These data cannot support this assumption in the case of ASR. The only feasible explanation for the observation that normal plants were never significantly more resistant (had lower scores) to ASR than semisteriles is that partial seed set in those translocation heterozygotes significantly reduced the severity of ASR. This explanation is supported by several studies which have shown that barreness, removal of the ear three weeks or earlier after anthesis, or the bagging of unfertilized ear shoots all reduce or prevent corn stalk rot (16,17,22). Others have suggested that when all other environmental factors are equal, plants with greater seed set will have more severe stalk rot than plants with lesser seed set (6). These studies did not, however, involve ASR.

Breeding for increased resistance to ASR in corn should be straightforward if, as the data suggests, the high level of resistance in A556 may be largely controlled by as few as five factors or genes. The transfer of such a relatively small number of genes into an agronomically suitable genotype by a backcrossing technique should be successful provided that population sizes are adequately large and extraneous variation can be held to a minimum.

Selection could be enhanced by selfing in backcross generations, selecting resistant plants at harvest, then backcrossing and selecting among and within S_1 families. Backcrossing may not result in an inbred with as high a level of resistance as A556, since minor genes, modifiers, and recessive genes would probably be lost in the process. If the genetic situation found in this study were extrapolated to random-mating corn populations, recurrent selection for ASR resistance in these populations would result in large gains for the first two or three cycles, with gains decreasing in subsequent cycles as genes with major effects became fixed early in the process.

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