

Inheritance of Resistance to *Sporisorium sorghi* in Grain Sorghum

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Introduction

Covered kernel smut, caused by *Sporisorium sorghi* (Ehrenberg ex Link [= *Sphacelotheca sorghi* (Link) G. P. Clinton] is an important disease of sorghum (*Sorghum bicolor* (L.) Moench). Grain losses may exceed those caused by other sorghum diseases (Casady 1961, Frowd 1980) particularly in lesser-developed countries where seed treatments are expensive, unavailable, or rarely used (Frowd 1980). Previous studies have shown that resistance to covered kernel smut exists in several sorghum genotypes and that cultivars resistant to various races of *S. sorghi* (Melchers et al. 1932) are also resistant to loose smut (*Sphacelotheca cruenta* [Kuhn] Potter) and head smut (*Sporisorium reiliana* [Kuhn] Langdon and Fullerton) (Reed 1923, Melchers et al. 1933, Casady et al. 1962, Frowd 1980). Most studies have shown that resistance to covered kernel smut is controlled by single genes (Casady 1961, Marcy 1937a,b, Reed 1928, Swanson and Parker 1931). Whether resistance or susceptibility is dominant depends on the parent used as the source of resistance (Reed 1928).

Reed (1928) concluded that susceptibility was dominant in crosses between reportedly resistant Feterita and susceptible varieties, and that resistance was dominant in crosses between resistant Standard White Milo and Blackhull Kafir. Swanson and Parker (1931) inoculated 284 F₃ lines of Red Amber x Feterita with race 1 of *S. sorghi* and reported a 3:1 segregation of smutted to healthy lines, indicating a single recessive gene as the mode of inheritance.

The reaction of various sorghum accessions to *S. sorghi*, *S. cruenta*, and *S. reiliana* was tested. Spur Feterita was reported (Reed 1923, Reed and Melchers 1925) to be highly resistant to covered kernel, loose and head smut diseases of sorghum. Five physiological races of *S. sorghi* were determined through the use of differential sorghum varieties (Melchers et al. 1932) and one selection from Spur Feterita (K.B. 2540) was immune to all races. Resistance to race 1 of *S. sorghi* was attributed to a single pair of genes with resistance being dominant to susceptibility. The 'blasting' reaction of sorghum to *S. sorghi* race 1 was eliminated by a single pair of genes considered to be epistatic to the gene for resistance (Casady 1963).

The blasting reaction occurred only under environmental conditions highly favorable for heavy infection by race 1 of *S. sorghi*.

Casady (1961) corroborated the reports (Marcy 1937a,b) of resistance to *S. sorghi* race 1, except for finding resistance to be incompletely dominant. Resistance to *S. sorghi* races 2 and 3 was controlled by 2 separate gene pairs with incompletely dominant resistance in both cases. Ss 1, Ss 2, and Ss3 were suggested as symbols to denote the genes for resistance to races 1, 2, and 3, respectively. The genes were linked. In studying the progeny of Spur Feterita x Pink Kafir, Casady et al. (1962) found that Spur Feterita carried both the gene (*Ss1*) for resistance and the gene for blasting to race 1. Blasting was expressed only in the presence of the recessive allele (*ss1*). Therefore the gene for blasting was not phenotypically expressed in Spur Feterita or K.B. 2450.

During the late 1950s and early 1960s, agronomically acceptable sorghum lines resistant to covered kernel smut were developed by Kansas State University and the United States Department of Agriculture (USDA) with a series of backcrosses using Spur Feterita as the source of resistance (Casady et al. 1962). These lines did not possess the same high level of resistance as Spur Feterita, and low levels of infection were observed under favorable environmental conditions.

Since the late 1960s, breeding work on covered kernel smut has not been reported. The All Disease and Insect Nursery (ADIN) from Texas A & M University was screened for genetic variability to *S. sorghi* for over 10 years at Kansas State University and B35-6, SC 414, and Sureno were identified as immune to *S. sorghi* under field conditions (Claflin and Ramundo 1996). The objective of this research was to investigate the inheritance of resistance to *S. sorghi* in B35-6, SC 414 and Sureno.

Materials and Methods

Seeds of true breeding lines and crosses used in this study were supplied by PJ Bramel (ICRISAT) and DT Rosenow (Texas A & M University, Lubbock). Immune accessions were B35-6 (IS 12555 derivative), SC414 (IS 2508 derivative), and Sureno ((SC423 x CS 3541) x E 35-1), recently released germplasm possessing acceptable agronomic characteristics. In all crosses, the susceptible parent was BTx623 (BTx3197 x SC 170-6). Crosses were made between resistant and susceptible accessions to determine inheritance. Crosses between resistant accessions were used to determine if they possessed the same genes. Crosses between resistant lines were SC 414 x Sureno, SC 414 x B35-6, and Sureno x B35-6. Crosses between resistant and susceptible lines were B35-6 x BTx623, SC 414 x BTx623, and Sureno x BTx623.

Greenhouse experiments. F₁ seed was sown and advanced by selfing to produce the F₂ generations. To produce the F₃ generation of crosses between resistant and susceptible parental lines, F₂ populations were grown and selfed in the greenhouse in 1995. The F₃ seed was harvested in May 1995 and stored in paper bags until sown in the field in June.

Eight seeds of each line were sown in pots (28 x 46 cm) containing a sterile soil mix consisting of Baccto potting soil (Michigan Peat Co., Houston, TX 77098)-sand-perlite (2:1:1). Plants were supplied with fertilizer (20-30-20; Schultz Company, St. Louis, MO 63043) according to Schultz Co. recommendations. To study inheritance of resistance, F₂ seed of all crosses were grown in pots in the greenhouse during the winter of 1995. The F₂ seed of the crosses between resistant and susceptible parents were divided into two equal portions and sown in different greenhouses (1 and 2). Potted plants in greenhouse 1 were placed on the cement floor and those in greenhouse 2 were placed on benches. Both greenhouses were maintained at 27°C day, 21°C night, with a 12 h photoperiod. Plants were thinned at the 3-4 leaf stage of growth and numbers of plants per pot varied from 3 to 5 depending on the pot size.

Field experiments. F₃ lines were machine sown in 3-m rows spaced 76 cm apart at the Rocky Ford Experimental Farm, Kansas State University, Manhattan, Kansas, in June, 1995. Ammonium nitrate fertilizer (34:0:0) was applied presowing at 94.2 kg ha⁻¹. Weeds were controlled

by applying Ramrod/Atrazine (Propachlor, (2-chloro-N-isopropylacetanilide, 48%); Atrazine, [2-chloro-4-(ethylamino)-6-(isopropylamino-s-tirazine, 15.5%]} pre-emergence at 12.6 L ha⁻¹ followed by hand weeding as needed. B35-6 x BTx623 provided 33 F₃ lines, Sureno x BTx623 provided 38 F₃ lines, and 41 F₃ lines were obtained from SC414 x BTx623.

Seed sterilization. Seeds were immersed in a mixture of formalin and water (1:300 v/v) for 1 h. Seeds were then washed in running tap water for 30 min, air-dried for 24 h and stored in paper envelopes (Dhingra and Sinclair 1985).

Inoculum preparation. Inoculum consisted of a mixture of *S. sorghi* teliospores from infected panicles of sorghum cultivars collected from the covered kernel smut nursery at the Rocky Ford Experiment Farm, Manhattan, Kansas. Smutted panicles were threshed by hand in plastic bags. The smut mass was sieved through 100 and 400 mesh screens to eliminate debris. Teliospores were stored at 4°C prior to use.

Seeds infested with teliospores. Dry teliospores (0.6% w/w) were added to F₃ seeds in paper envelopes. The envelopes were thoroughly shaken to ensure uniform distribution of spores on the seed coat. Infested seeds were sown under field conditions within 2 h of treatment.

Partial vacuum infestation. F₁ and F₂ seeds were mixed with teliospores (0.2% w/w), and a sufficient amount of water to wet the seed was added. The mixture was then placed under partial vacuum (180-200 mm Hg) for 1 h, and the vacuum was released at 15 min intervals. Seeds were dried at room temperature, and the procedure was repeated the following day. After the second application had dried, teliospores (0.2% by weight) were added to the F₁ and F₂ seeds in paper envelopes, shaken vigorously (Selvaraj 1980) and sown in the greenhouse within 2 h.

Disease incidence. Disease incidence (%) was determined by dividing the sum of smutted plants after all plants had reached physiological maturity by the total number of plants. Data were analyzed by Chi-square tests (Gomez and Gomez 1984).

Results

Smut reaction of parents. Resistant parents remained immune to *S. sorghi* under both field and greenhouse conditions (Table 1). The incidence (76%) of smutted panicles of BTx623 grown under greenhouse conditions was substantially higher than that in BTx623 plants grown in the field (2.3%).

The reactions of F₁ progenies of the crosses B35-6 x Sureno and Surefio x BTx623 suggest incomplete

Table 1. Number and percentage of covered kernel smutted plants in the F₁, F₂, and parental varieties.

Generation	Total plants	Smutted plants	Diseased (%)
Field sowing			
B35-6	329	0	0.0
BTx623	349	x	2.3
SC414	298	0	0.0
Sureno	455	0	0.0
Greenhouse			
B35-6	79	0	0.0
BTx623	71	54	76.1
SC414	80	0	0.0
Surefio	85	0	0.0
F ₁			
B35-6 x Surefio	19	0	0.0
Surefio x BTx623	18	4	22.2
F ₂			
B35-6 x Surefio	135	0	0.0
Surefio x SC414	122	32	26.2
B35-6 x SC414	47	2	4.3
SC414 x BTx623	147	31	21.1
Surefio x BTx623	165	56	33.9

dominance of resistance. The resistance of B35-6 is either dominant or it has the same gene(s) for resistance as Sureno (B35-6 x Sureno, Table 1). Reaction of other F₁ progenies to covered kernel smut could not be determined due to the limited number of seeds.

Smut reaction of R x R F₂ progenies. There was variability in smut reactions among crosses of resistant (R) by resistant (R) germplasm (Table 1). F₂ progenies of B35-6 x Sureno were free from smut and only 2 plants out of a total of 47 plants in the F₂ progenies of SC 414 x B 35-6 were smutted. Although unanticipated, 26% of the F₂ progenies of Sureno x SC 414 were smutted.

Smut reaction of R x S F₂ progenies. Covered kernel smut incidence varied among crosses of resistant (R) and susceptible (S) parents, depending on the greenhouse in which each cross was grown. Greenhouse 1 was located on a north exposure in the complex and temperature gradients were more severe in the winter than those in greenhouse 2, which was located on the south exposure. Both greenhouses contained the same heating and cooling equipment and were constructed in the same year, but pots in greenhouse 2 were placed on raised benches while those in greenhouse 1 were placed on the floor-which may have resulted in lower soil temperatures and lower light intensities for the plants grown in greenhouse 1. Plants in greenhouse 2 matured earlier than those in greenhouse 1, with a maturity difference of 2-4 weeks, although the time of sowing was identical in both greenhouses. Because of genotype x environmental interaction, a test for independence was performed. Contingency Chi-square values of 0.28, $P = 0.70-0.50$ for the cross SC414 x BTx623, and 0.022, $P = 0.90-0.70$ for Sureno x BTx623, indicated no significant differences in disease reaction between greenhouses. Therefore, the Chi-square test was calculated using the pooled data from both greenhouses.

Reaction of F₃ lines to smut. Conditions for smut infection were not conducive under field conditions as shown by the low incidence (2.3%) of infection in the susceptible parent (Table 1). F₃ lines of the crosses

exhibited a numerically higher incidence of covered kernel smut than the susceptible parent (BTx623, Table 1), with a mean covered kernel smut incidence of 6.2% in 38 F₃ lines of Sureno x BTx623, 3.8% in 41 F₃ lines of SC414 x BTx623, and 3.7% in 33 F₃ lines of B35-6 x BTx623 (data not shown).

The smutted F₃ lines were merely classed as susceptible, because it was impossible to separate segregating and homozygous susceptible lines. Therefore, a 1:3 ratio for homozygous resistant to segregating and homozygous susceptible classes was assumed, and the Chi-square values were calculated on this basis. The Chi-square and probability values indicated a close fit for the 1:3 ratio of the F₃ families of SC414 x BTx623 and Sureno x BTx623 (Table 2). These results are in good agreement with those of the greenhouse screens of F₂ families of each respective F₃ family, thus confirming the operation of a single dominant gene conferring resistance. These results are similar to those obtained by others (Casady 1961, Marcy 1937a, b, Swanson and Parker 1931).

Discussion

F₁ progenies of Sureno x BTx623 showed an incomplete dominance of resistance. Similar results were reported (Casady 1961) in F₁ plants of Combine Kafir 60 (S) x Spur Feterita (R) and Pink Kafir (S) x Spur Feterita (R). There was a very limited amount of F₁ seed from the other resistant x susceptible crosses and therefore no conclusion could be reached about the reactions of their progenies to *S. sorghi*.

A 3:1 ratio of resistant to susceptible plants was obtained in the F₂ population of SC414 x BTx623 (Table 1). The calculated Chi-square value of 1, $P = 0.50-0.10$ suggests the segregation of a single gene pair under the prevailing environmental conditions with the isolate of *S. sorghi* used in this experiment. A poor fit of the 3:1 ratio of resistant to susceptible plants was obtained in the F₂ population of Sureno x BTx623 (Table 1), suggesting incomplete dominance. Higher smut incidence in the F₁ progeny of Sureno x BTx623 was a further indication of the incomplete dominance of resistance from Sureno.

Table 2. Segregation for covered kernel smut among F₃ sorghum lines from three crosses grown under field conditions, Manhattan, KS, USA, 1995.

Cross	(N) ¹	(S) ²	Expected ratio	χ ²	P
B35-6 x BTx623	19	14	1:3	15.36	<0.001
SC414 x BTx623	12	29	1:3	0.20	0.5-0.3
Sureno x BTx623	11	27	1:3	0.14	0.7-0.5

1. N = Non-segregating.

2. S = Segregating or susceptible.

Data from the F₂ plants of B35-6 x SC414, B35-6 x Sureno, and Sureno x SC414 (Table 1) reveals that the lack of segregation in Sureno and B35-6 is attributable to 1) a number of lines that possess the same gene for resistance, 2) a number of different alleles at the same locus, or 3) that the parental lines have two tightly linked loci and are different from that in the line SC414. Therefore, in breeding sorghum for resistance to *S. sorghi*, it is immaterial whether Sureno or B35-6 is used as the resistant parent, although given data from this experiment, a breeder might choose to use B35-6, especially if producing F₁ hybrids. Because of pathogen variation, it is very useful to identify other sources of resistance such as that found in SC414. However, the two resistance genes from SC414 and B35-6/Surcno can be pyramided into one line or hybrid. It still remains to be determined whether the genes controlling resistance in B35-6 and SC414 occur in dominant or recessive form although the F₂ data in R x S crosses suggests that resistance is dominant.

Differences in covered kernel smut incidence in the susceptible parent (BTx623) in both greenhouse and field experiments were attributable to environmental differences as inoculation protocols utilized in this research are not significantly different (Nzioki et al. 2000). Disease escapes occur and the results are in agreement with long-term evaluations of the All Disease and Insect Nursery (Clafin and Ramundo 1996) where susceptible accessions seldom exhibit >50% smutted panicles.

The simple mode of inheritance to *S. sorghi* suggests that resistance can be easily transferred to susceptible sorghum cultivars with desirable agronomic characteristics. Due to the diversity of grain sorghum germplasm and possible occurrence of physiologic races of *S. sorghi* in less developed countries, additional research should be of high priority, particularly since such a program would be cost effective and productive in incorporating resistance genes in either development of new cultivars or in existing germplasm.

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