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Genetics of nonsenescence and charcoal rot resistance in sorghum

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Summary. Nonsenescence is a delayed leaf and plant death resistance mechanism in sorghum that circumvents the detrimental effects of reduced soil moisture combined with high temperatures during post-anthesis growth. This drought-tolerance mechanism is often equated with charcoal rot resistance, a widespread root and stalk disease of great destructive potential. Therefore, the inheritance of charcoal rot resistance was investigated directly, by exposure of sorghum to *Macrophomina phaseolina*, the causal organism, and indirectly, by determination of the inheritance of nonsenescence. Sorghum families derived from diallel crosses between two nonsenescent, resistant inbreds (B35, SC599-*11E*) and two senescent, susceptible inbreds (BTx378, BTx623) were evaluated in 1989 at College Station and at Lubbock, Texas, under controlled and field conditions. We determined that nonsenescence was regulated by dominant and recessive epistatic interactions between two nonsenescence-inducing loci and a third locus with modifying effects. The same conclusion was reached for charcoal rot resistance. The presence of different genetic mechanisms within SC599-*11E* for nonsenescence and charcoal rot resistance verifies that these two forms of resistance are not different manifestations of a single trait, i.e., they are not to be equated with each other. We conclude that nonsenescence alone cannot account for, and should not be used as the sole breeding criterion for, resistance to charcoal rot in sorghum.

Key words: *Sorghum bicolor* – Post-anthesis drought tolerance – Charcoal rot resistance – Breeding

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Introduction

Charcoal rot of sorghum [*Sorghum bicolor* (L.) Moench] is a root and stalk disease caused by *Macrophomina phaseolina* (Tassi) Goid. This pathogen has a wide host range and has been reported from nearly all sorghum growing areas of the world (Mihail et al. 1988). *M. phaseolina* has great destructive potential (Mughogho and Pande 1984; Bowen and Schapaugh). However, actual yield losses caused by charcoal rot per se are difficult to assess due to the association of the disease with post-anthesis soil moisture stress and high temperature (Edmunds 1964; Jordan et al. 1984; Seetharama et al. 1987) and secondary invading fungi (Mughogho and Pande 1984). Charcoal rot can be controlled through soil and water management techniques aimed at increasing soil water storage, especially during post-anthesis growth (Jordan et al. 1984; Seetharama et al. 1987). However, this approach is impracticable for many drought-prone, low input agricultural areas in the world (ICRISAT 1984), which leaves host plant resistance as the most practical type of control for charcoal rot under non-irrigated conditions.

The biology of *M. phaseolina* and the epidemiology of charcoal rot are well documented (Odvody and Dunkle 1979; Mughogho and Pande 1984; Mihail et al. 1988), but little is known about the mechanisms of host-pathogen interactions that prevent or allow fungal ingress to proceed. Studies on the reaction of sorghum to charcoal rot suggested that both dominant and recessive genes were involved in active resistance to the rotting organism (Rosenow 1984; Bramel-Cox et al. 1988; Bramel-Cox and Claflin 1989). It was concluded that resistance may be controlled by a multiple-locus complex with distinct heterotic patterns (Bramel-Cox et al. 1988). Whether these genes interact or operate as independent polygenes was

not discussed and the number of loci involved was not determined.

The reaction of sorghum plants to charcoal rot may be primarily a post-flowering drought response trait since cultivars that resist post-anthesis drought stress also resist charcoal rot (Rosenow 1984). The precise cellular and molecular mechanisms associated with drought tolerance are unknown. The differential speed and extent of leaf tip and margin firing, especially during post-anthesis growth, is the most noticeable response to soil moisture deficit. While drought-susceptible cultivars suffer extensive firing, many drought-tolerant cultivars maintain significant amounts of green leaf area (Rosenow et al. 1983). This characteristic of drought-tolerant cultivars has been termed reduced progressive senescence (McBee 1984) or nonsenescence (Duncan 1977), and has been used as the main criterion in breeding for drought tolerance and, indirectly, charcoal rot resistance (Duncan et al. 1981; Duncan 1984; Rosenow 1984). The genetic control of nonsenescence has been examined and both dominant and recessive inheritances have been reported for this trait (Duncan 1984; Rosenow 1984). Whether drought tolerance and resistance to *M. phaseolina* in sorghum are under pleiotropic gene control has not been determined.

Several sources of charcoal rot resistance that are also nonsenescent have been routinely used in breeding programs in Texas. These materials provide a basis for further understanding of the genetic mechanisms underlying the nonsenescence and charcoal rot resistance complex. Elucidation of these mechanisms is required to increase the efficiency of nonsenescence and of resistance incorporation into desired sorghum cultivars. To understand the genetics of these characters we evaluated the reaction of progenies derived from two resistant inbreds and two susceptible inbreds.

The objectives of this study were: (1) to determine the mode of inheritance of nonsenescence and resistance to *M. phaseolina* in sorghum, and (2) to estimate the number of loci involved.

Materials and methods

Plant materials and inoculation procedures

Four cultivars of sorghum, B35, SC599-*11E*, BTx378, and BTx623, were used in this study. B35 and SC599-*11E* are known sources of nonsenescence (N) and resistance (R) to charcoal rot, whereas BTx378 and BTx623 are senescent (S) and susceptible (S) (Duncan 1984; Rosenow 1984). Twelve F_1 hybrids were produced from these cultivars during the summer of 1988 following a full diallel mating scheme. The F_1s were self-pollinated and backcrossed to both parents during the spring of 1989, and 12 F_2 and 24 BC_1F_1 families were obtained.

M. phaseolina was isolated from NaOCl surface-disinfected sorghum roots or stems and grown on potato-dextrose agar (PDA) in 9-mm Petri plates at 25 °C in the dark. The inoculum was transferred periodically until a pure culture was obtained. Fungal growth was rapid and sclerotia were formed within 5 days, with little production of mycelia due to the high surface area to volume ratio of the medium (Odvody and Dunkle 1979). The pure culture was then allowed to colonize sterilized round wooden tooth picks which had been initially alkali (KOH)-cooked to eliminate tannins and soaked in potato-dextrose broth. The toothpicks were covered with black sclerotia after incubation for 4 weeks at 25 °C in the dark. Sclerotia-bearing toothpicks were used to inoculate sorghum plants as previously described (Frederiksen and Rosenow 1980).

Table 1. Green leaf retention and charcoal rot reaction of sorghum inbred cultivars B35, SC599-*11E*, BTx378, and BTx623, and their F_1 progeny

Cultivars	Green leaf retention				Lesion size			
	Percentage			Class[a]	In millimetres			Class[b]
	Min	Max	Mean ± SE		Min	Max	Mean ± SE	
Inbred parents								
B35	45	85	71.3 ± 0.78	N	9	48	24.1 ± 1.51	R
SC599-*11E*	45	85	67.7 ± 1.04	N	5	41	18.0 ± 1.17	R
BTx378	0	40	19.8 ± 1.24	S	54	250	122.0 ± 6.99	S
BTx623	0	40	20.7 ± 1.29	S	52	334	131.0 ± 7.62	S
F_1 progeny								
B35 × SC599-*11E*	60	80	72.0 ± 1.19	N	5	23	14.1 ± 1.45	R
B35 × BTx378	45	80	63.7 ± 1.84	N	22	46	28.8 ± 2.23	R
B35 × BTx623	45	80	64.0 ± 1.67	N	11	48	28.3 ± 3.05	R
SC599-*11E* × BTx378	0	40	28.2 ± 1.79	S	13	44	26.5 ± 2.87	R
SC599-*11E* × BTx623	45	75	57.5 ± 1.64	N	10	48	26.8 ± 3.40	R
BTx378 × BTx623	0	40	18.1 ± 2.69	S	59	362	168.1 ± 25.39	S

[a] N, nonsenescent; S, senescent
[b] R, resistant; S, susceptible

Screening plants for nonsenescence and resistance

Parents and progenies were evaluated for resistance to charcoal rot during the summer of 1989. Experiments were grown at College Station in the greenhouse (GH), and at Lubbock in the field (LB) and the rainshelter (RS). Sowing dates were 19 May 1989 for the greenhouse, 31 May 1989 for the field, and 27 June 1989 for the rainshelter. Nonsenescence studies were conducted in the Lubbock field experiment and in another field experiment sown near College Station (CS) on 7 April 1989. Due to the lack of seed, backcross progenies were not included in the CS, GH and RS experiments, and reciprocal cross progenies were exluded from the LB experiment.

The soil type at College Station is Ships Clay (very fine, mixed thermic Udic Chromusterts) intergrading toward a Norwood clay loam (fine-silty, mixed thermic Typic Udifluvent). The soil type at Lubbock is Olton loam (fine, mixed, thermic Aridic Paleustolls). Field plots were fertilized with a preplant application of N, P, and K at the rate of 29, 12.7, and 24 kg ha^{-1}, respectively. Additional N from NH_4NO_3 (50 kg ha^{-1}) was applied as a side-dressing approximately 6 weeks after planting. Irrigation was provided for all experiments until anthesis when all irrigation was withheld for 3 weeks for the GH and RS experiments. Natural shortage of rainfall produced the desired post-anthesis drought stress for the field experiments.

Three replications of an unbalanced randomized block design were used for each experiment. Five plants of each parent, five plants of each F_1, and 20 plants of each F_2 were grown in the greenhouse and in the rainshelter, for each replication. Field experiments were grown with three 6-m-rows (1 m spacing) for each F_2 and one row for each parent, F_1, and backcross. The population density was kept at approximately 110,000 plants ha^{-1}. All GH and RS plants and alternate field plants were inoculated at anthesis. The plants were grown to maturity, and charcoal-rot development was assessed by splitting the basal stem (culm) lengthwise and recording the extent of pith degradation (Rosenow 1984; Bramel-Cox et al. 1988). This was expressed as lesion length. The distributions of lesion length in populations of genetically uniform structure (resistant or susceptible) did not overlap (Table 1). Plants with lesions ≤ 50 mm were designated as resistant since plants of the resistant inbreds had lesions ≤ 50 mm. Nonsenescence was determined on all field-grown plants by subjective estimation of green leaf retention (GLR) at maturity. Previous studies have shown that visual estimates correlated well ($r = 0.93^{**}$) with percent green leaf area obtained by actual measurements of leaf area (Wanous et al. 1991). Nonsenescent inbreds and F_1s had a GLR $\geq 45\%$ whereas plants of the senescent inbreds or F_1 had less than 45% green leaf area (Table 1). Therefore, plants with 45% or more GLR were classified as nonsenescent and plants with GLR $< 45\%$ were considered senescent.

Results

No statistically significant difference occurred between the means of reciprocal F_1 progenies produced from the same parental pair for either green leaf retention or for reaction to charcoal rot. This indicated the absence of maternal cytoplasmic effects, and progenies from reciprocal crosses were pooled for genetic analyses.

Crosses between the nonsenescent, charcoal-rot-resistant (NR) cultivars B35 and SC599-*11E* produced only NR plants in the F_1, F_2, and backcross to SC599-*11E*.

Table 2. Chi-square analysis of F_2 populations of the crosses B35 × SC599-*11E*, B35 × BTx378, B35 × BTx623, SC599-*11E* × BTx378, SC599-*11E* × BTx623, and BTx378 × BTx623 of sorghum segregating for green leaf retention and resistance to *Macrophomina phaseolina*

Crosses	Green leaf retention [a]						Resistance to *M. phaseolina* [b]								
	College Station			Lubbock			Lubbock			Greenhouse			Rainshelter		
	Obs [c]	Exp	χ^2	Obs	Exp	χ^2	Obs	Exp	χ^2	Obs	Exp	χ^2	Obs	Exp	χ^2
B35 × SC599-*11E*	– [d]	All N	–	147:0	All N	na [e]	72:0	All R	na	36:0	All R	na	23:0	All R	na
B35 × BTx378	122:20	13N:3S	2.03	114:30	13N:3S	0.41	57:17	13R:3S	0.87	29:6	13R:3S	0.07	13:3	13R:3S	0.00
B35 × BTx623	116:30	3N:1S	1.54	119:31	3N:1S	1.50	52:20	3R:1S	0.30	20:3	3R:1S	1.75	20:3	3R:1S	1.75
SC599-*11E* × BTx378	–	1N:3S	–	41:109	1N:3S	0.44	55:19	13R:3S	2.33	37:4	13R:3S	2.18	23:3	13R:3S	0.89
SC599-*11E* × BTx623	141:39	3N:1S	1.07	111:39	3N:1S	0.08	54:19	3R:1S	0.04	31:7	3R:1S	0.88	19:4	3R:1S	0.71
BTx378 × BTx623	–	3N:1S	–	20:130	3N:1S	2.89	20:54	3R:1S	3.33	3:21	3R:1S	0.62	0:13	3R:1S	3.00

[a] Numbers indicate nonsenescent (N): senescent (S) ratios, where nonsenescent denotes green leaf retention (GLR) ≥45% and senescent means GLR < 45%

[b] Numbers indicate resistant (R): susceptible (S) ratios, where resistant designates lesion ≤ 50 mm and susceptible means lesion > 50 mm

[c] Obs, observed segregation; Exp, expected ratio

[d] Data not available

[e] na, not applicable

The backcross to B35 was not evaluated due to the lack of seed. Crosses between the senescent, charcoal-rot-susceptible (SS) cultivars BTx378 and BTx623 produced only SS progeny in the F_1 and backcross to BTx378, but segregated 3NR:13SS in the F_2, and 1NR:3SS in the backcross to BTx623. Thus nonsenescent, resistant plants were recovered from crosses between the two senescent, susceptible parents (Tables 2 and 3).

The F_1 progenies from the crosses B35(NR) × BTx378 (SS) and B35(NR) × BTx623 (SS) were nonsenescent and resistant. The F_2 offspring of these crosses segregated 13NR:3SS and 3NR:1SS, respectively (Table 2). Backcrosses to the NR parent were all NR whereas backcrosses to SS parents produced equal numbers of NR and SS progenies (Table 3).

When SC599-*11E* (NR) was crossed to BTx623 (SS), the F_1 was NR, the F_2 segregated 3NR:1SS, the backcross to SC599-*11E* was NR, and the backcross to BTx623 segregated 1NR:1SS. In contrast, the cross SC599-*11E*(NR) × BTx378 (SS) produced senescent but resistant (SR) offspring in the F_1 generation. The F_2 of this cross segregated 1N:3S for nonsenescence and 13R:3S for charcoal rot resistance. The backcross (SC599-*11E* × BTx378) × SC599-*11E* segregated 1:1 for nonsenescence but was all resistant. The backcross (SC599-*11E* × BTx378) × BTx378 was all senescent but segregated 1:1 for resistance. Thus not all nonsenescent individuals were charcoal-rot-resistant and not all nonsenescent plants were susceptible to the disease (Tables 2 and 3).

Discussion

The presence of a major dominant allele for nonsenescence and charcoal rot resistance in B35 was revealed by the NR phenotype of its F_1 progenies and the 3NR:1SS segregation in the F_2 of the cross B35 × BTx623. Involvement of a recessive allele for both traits was evident from the 13NR:3SS segregation of the cross B35 × BTx378 (F_2), which is typical of dominant and recessive epistatic interactions between two loci, one with dominant effect and one with recessive effect. Recovery of NR plants from crosses between SS parents (F_2 and backcross to BTx623) further indicated the presence of silenced genes in one of the senescent parents, BTx623, barring the possibility that NR progeny could merely be escapes. We propose that silencing could result from the action of a modifying factor that is completely epistatic to nonsenescence- and resistance-determinants.

Under the modifier hypothesis, cultivars with genes for nonsenescence and charcoal rot resistance, but lacking the favorable allele of the modifier (type 1), would be senescent and susceptible. However, when such cultivars are crossed to SS cultivars that have the favorable allele of the modifier, but lack the genes for nonsenescence and charcoal rot resistance (type 2), some of the progeny will be NR. Similarly, SS inbreds of the type-2 genotype should produce senescent progeny when crossed to an inbred with recessive nonsenescence. This would account for the apparent differences observed for nonsenescence when SC599-*11E* was crossed to BTx378 (presumably

Table 3. Chi-square analysis of backcross populations of the crosses B35 × SC599-*11E*, B35 × BTx378, B35 × BTx623, SC599-*11E* × BTx378, SC599-*11E* × BTx623, and BTx378 × BTx623 of sorghum segregating for green leaf retention and resistance to *Macrophomia phaseolina*

Crosses	Green leaf retention[a]			Resistance to *M. phaseolina*[b]		
	Obs[c]	Exp	χ^2	Obs	Exp	χ^2
(B35 × SC599-*11E*) × B35	–[d]	All N	–	–	All R	–
(B35 × SC599-*11E*) × SC599-*11E*	35:0	All N	na[e]	19:0	All R	na
(B35 × BTx378) × B35	59:0	All N	na	30:0	All R	na
(B35 × BTx378) × BTx378	44:43	1N:1S	0.01	22:18	1R:1S	0.22
(B35 × BTx623) × B35	86:0	All N	na	43:0	All R	na
(B35 × BTx623) × BTx623	53:43	1N:1S	1.04	26:21	1R:1S	0.53
(SC599-*11E* × BTx378) × SC599-*11E*	27:27	1N:1S	0.00	25:0	All R	na
(SC599-*11E* × BTx378) × BTx378	0:77	All S	na	19:22	1R:1S	0.22
(SC599-*11E* × BTx623) × SC599-*11E*	44:0	All N	na	24:0	All R	na
(SC599-*11E* × BTx623) × BTx623	35:31	1N:1S	0.24	20:13	1R:1S	1.49
(BTx378 × BTx623) × BTx378	0:16	All S	na	0:8	All S	na
(BTx378 × BTx623) × BTx623	18:52	1N:3S	0.02	13:25	1R:3S	1.72

[a] Numbers indicate nonsenescent (N): senescent (S) ratios, where nonsenescent denotes green leaf retention (GLR) ≥45% and senescent means GLR <45%

[b] Numbers indicate resistant (R): susceptible (S) ratios, where resistant designates lesion ≤50 mm and susceptible means lesion >50 mm

[c] Obs and Exp refer to observed and expected segregation, respectively

[d] The backcross to B35 was not tested due to lack of seed

[e] na, not applicable

type 2) or BTx623 (presumably type 1). This study agreed with earlier reports on recessive inheritance of nonsenescence in SC599-*11E* (Rosenow 1984). Dominance reversal was not observed for charcoal rot resistance, which does not support claims for the exclusively recessive inheritance of resistance in SC599-*11E* (Rosenow 1984).

The cross SC599-*11E* × BTx378 produced senescent but charcoal-rot-resistant F_1 progeny and segregated 3 resistant : 1 susceptible and 1 nonsenescent : 3 senescent in the F_2 generation. Such dissimilar segregation patterns of nonsenescence and resistance in the F_2, and the occurrence of senescent but resistant offspring in backcrosses to either parent, argue against equating charcoal rot resistance to nonsenescence. Consequently, nonsenescence alone cannot account for, and should not be used as the sole breeding criterion for, resistance to charcoal rot in sorghum.

Mendelian analysis was adequate for our data despite reports of the quantitative inheritance of nonsenescence (Mughogho and Pande 1984) and resistance to charcoal rot (Bramel-Cox et al. 1988; Bramel-Cox and Claflin 1989). Although heterogeneous populations did not segregate into discrete classes, the range of disease reaction in uniformly resistant entries did not overlap with the range of values obtained for uniformly susceptible entries. Similarly, green leaf retention in uniformly nonsenescent entries was distinct in range from green leaf retention in uniformly senescent entries. Thus, the breakdown of the variation into discrete classes was not artificial and had a genetical significance. Those circumstances conformed to the principle of Mather and Jinks (1971) for Mendelian analysis of apparently continuous data.

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