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Deanna L. Funnell-Harris
University of Nebraska-Lincoln, Deanna.Funnell-Harris@ars.usda.gov

Scott E. Sattler
University of Nebraska-Lincoln, Scott.Sattler@ars.usda.gov

Patrick M. O'Neill
University of Nebraska-Lincoln

Kent M. Eskridge
University of Nebraska-Lincoln, keskridge1@unl.edu

Jeffrey F. Pedersen
University of Nebraska-Lincoln, jpedersen1@unl.edu

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First and third authors: Grain, Forage and Bioenergy Research Unit (GFBRU), U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), and Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0937; second and fifth authors: GFBRU, USDA-ARS, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0937; and fourth author: Department of Statistics, University of Nebraska, Lincoln, NE 68583-0937.

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ABSTRACT

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Loss of function mutations in *waxy*, encoding granule bound starch synthase (GBSS) that synthesizes amylose, results in starch granules containing mostly amylopectin. Low amylose grain with altered starch properties has increased usability for feed, food, and grain-based ethanol. In sorghum, two classes of *waxy* (*wx*) alleles had been characterized for absence or presence of GBSS: wx^a ($GBSS^-$) and wx^b ($GBSS^+$, with reduced activity). Field-grown grain of wild-type; *waxy*, $GBSS^-$; and *waxy*, $GBSS^+$ plant introduction accessions were screened for fungal infection. Overall, results showed that *waxy* grains were not more susceptible than wild-type. $GBSS^-$ and wild-type grain had similar infection levels. However, height was a factor with *waxy*, $GBSS^+$ lines: short accessions (wx^b allele) were more susceptible than tall accessions (undescribed allele). In greenhouse experiments, grain from accessions and near-isogenic wx^a, wx^b, and wild-type lines were inoculated with *Alternaria* sp., *Fusarium thapsinum*, and *Curvularia sorghina* to analyze germination and seedling fitness. As a group, *waxy* lines were not more susceptible to these pathogens than wild-type, supporting field evaluations. After *C. sorghina* and *F. thapsinum* inoculations most *waxy* and wild-type lines had reduced emergence, survival, and seedling weights. These results are valuable for developing *waxy* hybrids with resistance to grain-infecting fungi.

Numerous *waxy* mutants, identified in sorghum (*Sorghum bicolor* (L.) Moench), millets, barley (*Hordeum vulgare* L.), wheat (*Triticum* spp.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.), reduce the amylose content of the starch (11,12,19,20,65,72). In wild-type plants, amylose makes up approximately one-fourth of starch and amylopectin encompasses the remaining portion. Having greatly reduced to undetectable amounts of amylose, *waxy* can increase usability of the grain for animal feeds and some food products (10,11,34,46,64). The *waxy* trait results from loss of function mutations in the *waxy* gene(s) responsible for granule bound starch synthase (GBSS), critical for amylose production (56). Recently, *waxy* sorghum and corn have gained attention for possible use in ethanol production in the bioenergy industry (26,73). Greater fermentation efficiencies have been reported with *waxy* grains because amylose forms lipid complexes that are resistant to enzymatic hydrolysis, and greater swelling and fragmentation of starch granules during heating has been reported with *waxy* grain (56,73,76). *Waxy* starch also has lower pasting temperature, reducing energy demands during ethanol production (76).

In sorghum, wild-type grain contains 22 to 24% amylose and *waxy* grain contains 2% or less amylose (49,53). A previous study identified two types of *waxy* mutants, based on the presence or absence of the GBSS protein, designated wx^a and wx^b, respectively (49,56). Further characterization revealed that wx^b had a missense mutation that affected a highly conserved amino acid in GBSS, resulting in only partial function (56). Further characterization of wx^a showed that the gene contained a large insertion, which likely prevented the accumulation of GBSS protein. At least two more *wx* alleles were identified by screening diverse sorghum germplasm (24,38), using DNA markers developed for the wx^a and wx^b alleles (56).

Despite interest in *waxy* grain, published reports specifically examining effects of *waxy* on response to grain pathogens are limited. In a study of aflatoxin contamination (correlated with *Aspergillus flavus* Link. infection [7]) in kernels of maize hybrids with differing endosperm characteristics, a *waxy* hybrid had significantly greater mean aflatoxin levels than its wild-type counterpart (35). Other modifications of the endosperm may also indirectly affect amylose content of grains. For example, the *Opaque-2* (*O2*) gene encodes a transcriptional activator, specifically expressed in maize endosperm. *o2* mutants have greatly reduced amounts of the endosperm protein zein (60) and somewhat reduced amylose content (28 to 29% compared with 32 to 34% in wild-type) (21); *o2* lines are known to be susceptible to pathogens (37). Due to the pleotropic effects of the mutation, including increased softness, also associated with disease susceptibility (22,70), it is difficult to ascertain the factors involved in these observations. These few studies illustrate the necessity of a thorough investigation to determine whether *waxy* sorghum is more susceptible than wild-type to grain pathogens.

There is evidence that impaired GBSS activity alters the structure of the grain. Scanning electron microscopy (SEM) revealed differences in endosperm appearance between wild-type and *waxy* grain (56). Wild-type grains had a floury-appearing endosperm in the center of the grain, surrounded by the vitreous (slightly translucent) region, while *waxy* grains had a smaller floury endosperm region surrounding the embryo while the vitreous region was found in the middle of the grain (56). The vitreous region of *waxy* grains was more loosely packed than that of wild-type grains (56). Physicochemical properties of the starch from wild-type and *waxy* grains are also different. GBSS, as the name implies, is primarily associated with starch granules; the granules in *waxy* grains are made up almost entirely of amylopectin, and wx^a

Corresponding author: D. Funnell-Harris;
E-mail address: Deanna.Funnell-Harris@ars.usda.gov

*The *e-Xtra* logo stands for "electronic extra" and indicates that five supplementary tables are published online.

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sorghum genotypes, do not have GBSS (49). *wx*^{b} genotypes do include the low activity GBSS bound in the starch granules (49). Other proteins in sorghum endosperm include the major storage proteins, kafirins, similar to maize zeins (72). Kafirins form protein bodies, which are held together by other proteins, making a matrix that surrounds starch granules. In endosperm of a wild-type line, the protein bodies were shown to be more closely associated with starch granules, as compared with that of a closely related *waxy* line (72). Other differences between *waxy* and wild-type grains include starch thermal properties, mean gelatinization temperatures being significantly higher in *waxy* lines than wild-type (50), and increased digestibility of proteins in *waxy* grains (72).

In addition to grain proteins just described, there are those implicated in resistance to grain fungal infections. Although little is known about these antifungal proteins in *waxy* grain, much research has been conducted with wild-type grain. Several proteins (sormatin, a thaumatin-like protein; a β-1,3-glucanase; chitinases; and a ribosomal-inactivating protein) from sorghum grain that strongly inhibit in vitro fungal growth have been found (62). These proteins are present in the grain throughout development (29,51,52,61), including when the immature grain is most vulnerable to fungal attack (39,44). Additionally, sormatin and chitinase have been shown to be inducible or retained under high grain mold pressure (51,52).Thus, changes to endosperm structure, including how the protein matrix interacts with starch granules, the composition of starch granules, and increased protein digestibility observed in *waxy* grain (23,56,72), may alter its interactions with fungi relative to wild-type grains.

Grain mold is a destructive disease of sorghum, reducing yield and quality (44,69). A complex of fungi infecting flowers at anthesis, or developing grain, causes this disease. Because of the complexity of the disease, resistance is a polygenic trait, influenced strongly by environment (2,3,63). In sorghum, numerous species have been implicated in grain mold but most commonly detected are species of *Fusarium, Cochliobolus*, and *Alternaria* (5,39,71). Some *Fusarium* and *Alternaria* species produce mycotoxigenic compounds that cause serious diseases in both humans and livestock (33,36,58). These fungi cause grain deterioration in the field or during storage, and therefore, represent a threat for sorghum produced for feed, food, and fuel (9,57).

In the present study, field grown grain from photoperiod insensitive plant introductions (PIs), were screened for the presence of grain mold fungi. Additionally, grain from a subset of the PIs and near-isogenic *wx*^{a} and *wx*^{b} lines were inoculated with grain mold fungi. In this way, the following hypothesis was tested: *waxy* grain is not more susceptible to the grain mold pathogens *Alternaria*, *Fusarium*, and *Cochliobolus* spp. than wild-type grain.

MATERIALS AND METHODS

Fungi and media. Fungi utilized in this study were *Alternaria* sp. isolate H02-781S-3b, *Gibberella thapsina* Klittich, J.F. Leslie, P.E. Nelson & Marasas isolate H03-11S-9, and *Curvularia sorghina* R.G. Shivas & Sivan. 1987 isolate H05-531S-2 (13,14,16). The three were isolated from sorghum grain grown at the University of Nebraska Havelock field in Lincoln. For ease and familiarity to the reader, *G. thapsina* will be referred to as *Fusarium thapsinum*. Working cultures of the fungi were maintained on one-half strength potato dextrose agar (PDA), prepared using potato dextrose broth (Becton, Dickinson and Co. [BD], Sparks, MD), with 100 µm ampicillin (Sigma-Aldrich [SA], St. Louis). For sporulation cultures, *F. thapinum* cultures were transferred to 80 mM potassium chloride/1.5% agar (KCl), as previously described (16) and grown at room temperature for 10 days. For sporulation of *Alternaria* sp., transfers were made to 2% water agar with sterile filter paper (1 cm^2) aseptically placed over the inoculation site and then grown at room temperature for 10 days. Sporulation of *C. sorghina* was also accomplished using water agar, except cultures were incubated at 22°C with 8 h light for 10 days.

For isolation of fungi from grain, the semiselective media used were dichloran rose bengal chloramphenicol (DRBC) agar, dichloran chloramphenicol peptone agar (DCPA) and pentchloronitrobenzene (PCNB) agar (1,27,43). DRBC is a general purpose fungal medium that contains rose bengal, a stain that suppresses bacterial growth and slows growth of rapidly-growing fungi. DCPA is semiselective for *Fusarium* spp., and *Alternaria* spp. and other dark-spored ascomycetes. PCNB is semiselective for *Fusarium* spp. Dichloran, rose bengal and chloramphenicol were from SA, pentachloronitrobenzene, formulated as Terrachlor, was from Uniroyal Co. (Middlebury, CT), and agar and peptone, under the name Bacto, were from BD.

Plant lines. Information about the sorghum PIs, the known *waxy* line, 'Ellis', and nine wild-type lines, 'Atlas', 'Brawley', 'Dale', E35-1, IS2261, 'Kansas Collier', N98, 'Rox Orange', and 'Wray', is provided in Table 1. The PIs were obtained from the USDA-ARS Southern Regional Plant Introduction Station, Griffin, GA. There are no known previous reports of responses to grain mold fungi for any of these lines (http://www.ars-grin.gov/npgs/). *Waxy* phenotypes and presence (+) or absence (–) of GBSS were previously determined (49,50). All lines listed in Table 1 were utilized in the field study, and those lines marked with asterisks were chosen for use in a greenhouse seed inoculation study. In separate greenhouse studies, grain from the S7 generation of 11 pairs of near-isogenic *waxy* lines and corresponding wild-type lines, released May 2014 (75), an unreleased pair ([F2 BTx2752 ms3 × B 94C274])/B94C274 BC1), along with wild-type lines RTx430 and Wheatland, were inoculated (Table 2). Grain from the S6 generation of three *waxy* near-isogenic pairs containing either *wx*^{a} (GBSS$^-$) or *wx*^{b} (GBSS$^+$) allele, (BTxAGR1 × [BTx630 × BTxAGR1] BC1), the parental lines BTx630 (*wx*^{a}) and BTxAGR1 (*wx*^{b}), and the wild-type lines BWheatland and RTx430, were also inoculated. For greenhouse studies, grain produced in the greenhouse was utilized. Grain for PIs was grown in 2004, 2006, 2010, and 2012, grain for the near-isogenic *wx*^{a} and *wx*^{b} and corresponding wild-type lines was grown in 2009, and grain for *wx*^{a} and *wx*^{b} near-isogenic lines was grown in 2008 and 2009. Our previous studies have shown that greenhouse-grown sorghum grain has very low levels of endophytic fungi (less than 0.2%) (15).

DNA marker analyses and iodine staining of GBSS$^+$ and GBSS$^-$ *waxy* PIs. To determine whether lines with GBSS$^-$ or GBSS$^+$ *waxy* phenotypes, previously characterized (49), had *wx*^{a} or *wx*^{b} alleles, respectively, markers for these alleles were utilized (56). DNA from *waxy* PIs and the control lines BWheatland (wild-type), RTx2901 (*wx*^{a}) and BTxAGR1 (*wx*^{b}) were screened for the *wx*^{a} or *wx*^{b} allele as previously described (56). DNA was extracted using the "paint shaker" method (55), modified as follows. Metal beads with 40 µl of 10 mM Tris, pH 8.0, 1 mM EDTA (TE) were used to disrupt tissue and then, after shaking, the mixtures were briefly centrifuged at room temperature. Extraction buffer (60 µl of a solution of 2% cetyl trimethylammonium bromide, 1.42 M sodium chloride, 20 mM EDTA, 100 mM Tris, pH 8.0, 2% polyvinylpolypyrrolidone, and 5 mM ascorbic acid) and 1 µl of RNase A were added and mixtures were shaken by hand, following room temperature incubation for 15 min and then briefly centrifuged. Chloroform/isoamyl alcohol (24:1) (75 µl) was added and the mixtures were mixed by hand and then centrifuged at room temperature for 15 min. The supernatant was collected and DNA from six plants of each cultivar was pooled. DNA was precipitated with isopropanol (×0.7) and incubated for at least 1 h at 4°C and then centrifuged at maximum speed, 4°C, for 15 min. The resulting pellet was washed, dried, and resuspended in 50 µl of TE. PIs previously listed as *wx*^{a} were screened with the allele specific multiplex primer set and those previously listed as *wx*^{b} were screened using a cleavage amplified polymorphic sequence (CAPS) marker that distinguishes *Wx* from *wx*^{b} (56). DNA from PI 23231, PI 217897, and PI 548008 were tested with both markers. The polymerase used was AmpliTaq Gold (Life Technologies, Grand Island, NY) and HF *Nco*I was

purchased from New England Biolabs, Ipswich, MA. Grain from PI 23231, PI 217897, and PI 548008, along with BWheatland (wild-type) and 94C289 (*waxy*), was stained with iodine to determine presence or absence (*waxy* phenotype) of amylose, as previously described (48,50). Chemicals were purchased from Sigma Chemical Co., St. Louis, and Fisher Scientific, Waltham, MA.

Field study. The PIs, Ellis (*waxy*), and nine wild-type lines were planted in a randomized complete block (RBC) design with four replications each year, during 2004 and 2005, at the University of Nebraska, Agricultural Research and Development Center near Mead, NE. In a previously published study (17), planted near the present study in the same field, disease pressure for grain infections was significantly less in 2004 than in 2005 (P = 0.01). Plots consisted of single 7.6-m rows spaced 76 cm apart. Grain was sown on 21 May 2004 and on 23 May 2005. Details of field management for 2004 were previously described (50). In 2005, nitrogen fertilizer was applied at 157 kg ha^{-1} before planting. Atrazine was applied at 2.2 kg ha^{-1} immediately after planting, followed by an application

TABLE 1. Accession number, local common name, country of origin, height, days to anthesis, grain phenotype, plant genotype, and presence or absence of the granule bound starch synthase protein (GBSS) in starch granules, of plant introductions (PIs) and other lines used in this study

Accession[v]	Common name	Country	Height (cm)[w]	Anthesis (days)[x]	Phenotype	Genotype[y]	GBSS[z]
*PI 23231	Brown Kaoliang	China	265.6	77.9 ± 3.9	*waxy*	Unknown	+
PI 55123	Hemaise	Sudan	281.9	75.9 ± 3.6	*waxy*	*wx^a*	–
*PI 76407	North West Gold Kaoliang	China	269.4	69.8 ± 3.9	*waxy*	*wx^a*	–
*PI 82340	Kaoliang-Wx	Korea	287.5	76.0 ± 3.6	*waxy*	*wx^a*	–
*PI 87355	Bomususu	Korea	304.4	79.5 ± 3.7	*waxy*	*wx^a*	–
PI 88004	Susu Zairai Shu	Korea	288.8	71.8 ± 3.6	*waxy*	*wx^a*	–
*PI 173971	Jawar	India	289.4	76.9 ± 4.1	Wild-type	*Wx*	+
PI 175316	Mal Giunra	India	330.6	78.1 ± 3.6	Wild-type	*Wx*	+
PI 192876	Katengu	Indonesia	216.3	72.6 ± 3.9	*waxy*	*wx^a*	–
*PI 217897	305	Indonesia	245.6	79.4 ± 3.7	*waxy*	Unknown	+
PI 220636	Nai-shaker	Afghanistan	287.5	77.9 ± 3.7	*waxy*	*wx^a*	–
PI 234456	Unknown	Japan	250.0	74.0 ± 3.7	*waxy*	*wx^a*	–
PI 246699	IS 1024	India	262.5	70.3 ± 3.8	Wild-type	*Wx*	+
PI 250230	MN 4116	Pakistan	211.3	66.4 ± 3.8	Wild-type	*Wx*	+
*PI 455543	ETS 3634	Ethiopia	221.9	75.1 ± 3.8	*waxy*	*wx^a*	–
*PI 547915	Bai Ruan Gao Liang	China	260.0	67.6 ± 3.7	Wild-type	*Wx*	+
PI 548008	Huang Ke Jiao	China	251.9	65.9 ± 3.7	*waxy*	*wx^a*	–
*PI 562758	Basuto Red Q2-1-29	USA	294.4	90.1 ± 4.3	*waxy*	Unknown	–
*PI 563015	Kaura Mai Faran Kona	Nigeria	274.4	78.8 ± 4.0	*waxy*	*wx^a*	–
*PI 563068	IS 8303	USA	167.5	81.5 ± 3.8	*waxy*	*wx^a*	–
*PI 563402	IS 10497	USA	127.5	72.0 ± 3.8	*waxy*	*wx^a*	–
*PI 563576	LV 129	China	292.5	77.9 ± 4.5	*waxy*	*wx^a*	–
PI 563611	LR 390	China	148.8	85.3 ± 3.7	Wild-type	*Wx*	+
PI 563612	LR 395	China	148.1	81.6 ± 3.7	Wild-type	*Wx*	+
*PI 563670	L 1999B-17	China	129.4	82.0 ± 3.7	*waxy*	*wx^b*	+
*PI 563671	L 1999B-18	China	125.6	76.8 ± 3.7	*waxy*	*wx^b*	+
*PI 563672	LR 2409	China	130.0	82.5 ± 3.7	Wild-type	*Wx*	+
*PI 565116	SDS 1412	Zimbabwe	123.8	71.8 ± 3.6	Wild-type	*Wx*	+
PI 567796	Pyungchang local	South Korea	295.0	77.1 ± 3.6	*waxy*	*wx^a*	–
PI 567803	Yungju local	South Korea	301.9	77.9 ± 3.7	*waxy*	*wx^a*	–
PI 567809	Unknown	South Korea	305.0	77.3 ± 3.7	*waxy*	*wx^a*	–
*PI 567811	Unknown	South Korea	280.6	73.9 ± 3.7	*waxy*	*wx^a*	–
*PI 567910	Bai Liu Ai (Fu Yang)	China	293.8	74.0 ± 3.9	Wild-type	*Wx*	+
PI 567913	Bai She Yan (Sui Zhong)	China	249.4	72.8 ± 3.9	Wild-type	*Wx*	+
*PI 567931	Da Shan Dong (Wen Shui)	China	310.0	75.6 ± 3.6	Wild-type	*Wx*	+
PI 567939	Gao Liang	China	265.6	75.9 ± 3.8	Wild-type	*Wx*	+
PI 568012	Niu Xin	China	270.0	71.5 ± 3.6	Wild-type	*Wx*	+
PI 586448	Cody	Hungary	141.5	75.4 ± 3.7	*waxy*	*wx^a*	–
PI 586454	Leoti	Hungary	252.5	75.5 ± 3.7	*waxy*	*wx^a*	–
*PI 586524	IS 27929	China	273.8	70.5 ± 3.7	*waxy*	*wx^a*	–
PI 586526	IS 27931	China	261.3	71.4 ± 3.6	*waxy*	*wx^a*	–
PI 586529	IS 27935	China	275.0	73.0 ± 3.6	*waxy*	*wx^a*	–
Known *waxy* line							
*PI 667647	Ellis	USA	250.0	76.4 ± 3.7	*waxy*	*wx^a*	–
Known wild-type lines							
*PI 586537	Atlas	Australia	271.9	79.9 ± 3.6	Wild-type	*Wx*	+
*NSL 4346	Brawley	USA	262.5	79.5 ± 3.9	Wild-type	*Wx*	+
*PI 651495	Dale	USA	311.3	93.0 ± 3.7	Wild-type	*Wx*	+
*Grif 652	E35-1	Ethiopia	226.3	96.9 ± 4.9	Wild-type	*Wx*	+
*PI 217672	IS 2261	Sudan	301.9	98.4 ± 4.3	Wild-type	*Wx*	+
*PI 586540	Kansas Collier	Australia	236.9	81.4 ± 4.0	Wild-type	*Wx*	+
*PI 535783	N98	USA	253.8	76.3 ± 3.8	Wild-type	*Wx*	+
*PI 641836	Rox orange	USA	243.8	74.3 ± 3.8	Wild-type	*Wx*	+
*PI 653616	Wray	USA	280.0	83.0 ± 4.1	Wild-type	*Wx*	+

[v] PIs and other lines listed were utilized in field study; PIs and lines with asterisks were used in grain inoculation study. NSL 4346 is maintained at National Center for Genetic Resources Preservation, Fort Collins, CO, while all others are maintained at the Plant Genetic Resources Conservation Unit, Griffin, GA.

[w] Average plant height was estimated when the plants in a plot reached anthesis (defined below). Plot measurements were rounded to the nearest 5 cm.

[x] Days to anthesis was determined when the anthers from half the plants in a plot were exerted.

[y] *Wx* is the wild-type allele. *wx^a* and *wx^b* alleles were determined in the current study using allele-specific primers (*wx^a*) or cleavage amplified polymorphic sequence marker (*wx^b*) (56). Those indicated with unknown had the wild-type-sized band for both markers.

[z] *Waxy* phenotype and presence of protein in the starch were determined in previous studies (49,50). For GBSS$^+$ lines, starch granules in wild-type lines contain functional GBSS and *waxy* lines have partially active GBSS. GBSS$^-$ lines lack the protein in starch granules.

of alachlor (2-chloro-2',6'-diethyl-*N*-[methoxymethyl] acetanilide) at 4.75 liters ha^{-1} and atrazine at 1.1 kg ha^{-1}, approximately 14 days postemergence. Supplemental irrigation (3.8 cm) was applied on 25 May, 28 June, 28 July, 4 August, and 11 August.

In both years, five grain heads were randomly chosen from each plot and the threshed grain from all five heads was pooled, dried at room temperature, and stored in a cold room (4°C, 40% humidity). To prepare grain for screening, 30 grains from each plot were placed in a 15-ml conical tube and washed with 95% ethanol for 2 min, followed by 1% sodium hypochlorite (NaClO) with 0.01% Tween 20 (Sigma) for 10 min, using continuous gentle rocking, and then rinsed three times in sterile purified water (Labconco, Kansas City, MO). The grain samples were dried in a laminar-flow hood. Grain was aseptically applied to DRBC, DCPA, and PCNB media at five grains per medium. Grains with fungal growth were enumerated for each medium.

Individual fungal colonies growing from each grain onto the medium were transferred to PDA. Colony morphology on PDA, and conidiophore structures, conidial types, and conidial morphologies on appropriate sporulation media were used to identify *Fusarium*, *Alternaria*, and *Cochliobolus* spp. (6,32,45,66). Fungal infection of each grain was enumerated for the following categories: presence of fungal growth, *Alternaria* spp., *Fusarium* in the *Gibberella fujikuroi* species complex (SC), *Fusarium* spp. not in the *G. fujikuroi* ('other *Fusarium* spp.'), and members of the *Cochliobolus* SC.

Because several *Fusarium* spp. infect sorghum grain (14,17), many of which are difficult to distinguish due to lack of morphological characteristics and variability in culture (18), molecular identification using sequences from the translation elongation factor gene (*TEF*) was conducted. A subset of representative isolates from grain of each plot was randomly chosen for molecular identification. Isolates were single-spored and DNA was extracted from ground, lyophilized mycelium (31). The 5′ region of *TEF* was polymerase chain reaction (PCR)-amplified using primers EF-1 and EF-2 (18). Amplification products were sequenced and sequences were assembled using Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, MI). Assembled sequences were compared with those in the publically available FUSARIUM-ID database (http://isolate.fusariumdb.org/index.php) (18). Sequences were submitted to GenBank as accession numbers KM462914 through KM463008.

Percentage of grain infected by fungi within a category were analyzed to determine effects of *waxy* phenotype (wild-type or *waxy*) and presence or absence of GBSS in *waxy* grains. Results obtained from each medium were analyzed separately using PROC MIXED of SAS/STAT software (54). Years (2004 and 2005), lines (23 wild-type; 25 *waxy*, $GBSS^-$; and 4 *waxy*, $GBSS^+$), and replications (four per year) were considered random variables. Grain phenotype was the only fixed variable. In the MODEL statement, the DDFM = KR option was specified to calculate denominator degrees of freedom using the Kenward-Rogers method (25). Levene's homogeneity of variance tests were conducted for each class variable, which indicated heterogeneity in covariance structure of some class variables. On the basis of these results, the REPEATED/GROUP option of PROC MIXED (54) was used to specify those structures, as appropriate. For each medium, the percent of grain infected in each of the five categories was the dependent variable. In the text and tables, means are expressed as least squares means followed by the standard error. Pearson correlations were generated for the response variables days to anthesis, height at maturity, and percentage of grain with internal fungal infection as determined on DCPA, PCNB, and DRBC using SAS Proc Corr software (45). Correlations were also generated for the same response variables and peak starch gelatinization temperatures (previously reported) for the two replications that this study overlaps with the previous study (50). Wild-type; *waxy*, $GBSS^-$; and *waxy*, $GBSS^+$ were conducted separately.

Sorghum has four loci, *Dw1* through *Dw4*, that control plant height; forage types tend to have recessive alleles at one or two loci while grain types have recessive alleles at three loci (67). Since previous research suggested that plant height was a factor in determining susceptibility to grain mold (67), a preliminary correlation analysis comparing plant height with grain infection levels was conducted. This analysis indicated that plant height could not be discounted as a factor in the results. To determine if the distribution of plant height at maturity for each grain phenotype was a covariate of the mean percentage of grain infected with fungi, partial correlations were made with SAS/STAT software (54) using the MANOVA/PRINTE statement in PROC GLM. Based on these results, percent of grain with internal fungal infection for PI 23231, PI 217897, PI 535783, PI 563015, PI 563402, PI 563670, PI 563671, PI 563672, PI 565116, PI 567939, PI 586448, and PI 667647 were compared with phenotype and height class (Table 1) using PROC MIXED of SAS/STAT software (54) as described above.

Grain inoculations. Grain inoculations were performed on lines described in Tables 1 and 2, using previous protocols with modifications (4,13). Experiment 1 used PIs indicated with asterisks in Table 1, experiment 2 used near-isogenic wx^a or wx^b lines and their corresponding wild-type lines, listed in Table 2, and experiment 3 used wx^a and wx^b lines, also listed in Table 2. For each repetition, five sets of 50 seeds were washed as described above, except wash times were extended by 3 and 5 min, respectively. After rinsing in sterile purified water and drying, four sets of 50 seeds from each line were placed into individual polystyrene petri dishes

TABLE 2. Sorghum near-isogenic line pairs, *Wx* and wx^a, *Wx* and wx^b, and wx^a and wx^b, utilized in this study

Line[v]	Pair[w]	Phenotype[x]	Genotype[y]
Wx and wx^a or wx^b			
N619	1	*waxy*	wx^b
N620	1	Wild-type	*Wx*
N621	2	*waxy*	wx^a
N622	2	Wild-type	*Wx*
N623	3	*waxy*	wx^a
N624	3	Wild-type	*Wx*
N625	4	*waxy*	wx^b
N626	4	Wild-type	*Wx*
N627	5	*waxy*	wx^b
N628	5	Wild-type	*Wx*
N629	6	*waxy*	wx^a
N630	6	Wild-type	*Wx*
N631	7	*waxy*	wx^b
N632	7	Wild-type	*Wx*
N633	8	*waxy*	wx^b
N634	8	Wild-type	*Wx*
N635	9	*waxy*	wx^b
N636	9	Wild-type	*Wx*
N637	10	*waxy*	wx^a
N638	10	Wild-type	*Wx*
N639	11	*waxy*	wx^a
N640	11	Wild-type	*Wx*
U[z]	12	*waxy*	wx^a
U	12	Wild-type	*Wx*
wx^a and wx^b			
U	1	*waxy*	wx^a
U	1	*waxy*	wx^b
U	2	*waxy*	wx^a
U	2	*waxy*	wx^b
U	3	*waxy*	wx^a
U	3	*waxy*	wx^b

[v] N followed by a number refers to Agricultural Research Division, Institute of Agriculture and Natural Resources (75).

[w] Lines with the same pair number are near-isogenic. Refer to Materials and Methods for details.

[x] *Waxy* indicates starch with low (0 to 2%) amylose while wild-type indicates normal (22 to 24%) amylose starch.

[y] The wild-type *Waxy* gene (*Wx*) encodes for the protein granule bound starch synthase (GBSS), associated with starch granules ($GBSS^+$). wx^a lines have starch granules lacking GBSS ($GBSS^-$); wx^b lines have a partially active GBSS protein associated with the starch granule ($GBSS^+$).

[z] U indicates the line is unreleased. See Materials and Methods for genetic background information.

(60 × 15 mm) (Falcon, BD Labware, Franklin Lakes, NJ). Conidial suspensions were prepared in sterile purified water, quantified using a hemocytometer, and diluted to 1×10^3 conidia ml^{-1}. For each line, 10 ml of each conidial suspension was added to each of three petri dishes and 10 ml of sterile purified water (control) was added to the fourth petri dish. The petri dishes were incubated in the dark at 25°C for 2 days and then in the dark at 4°C for 2 days. Two days after the start of the experiment, for the fifth set of 50 grains, a standard germination protocol was conducted by placing the grain into a previously prepared, sterilized glass petri dish (100 mm) containing paper towels saturated with purified water, and then incubating for 2 days at 25°C (null treatment) (13). Grain germination for all treatments was scored based on growth of the radicle and shoot past the length of each seed.

For each treatment (three fungal inoculations, water or null), seven germinated seeds were planted, using surface-disinfested forceps, into soil mix (one part sand, one part coarse vermiculite, one part top soil and two parts shredded peat moss) previously moistened with either purified water (PIs, Ellis, and wild-type lines) or sterile purified water (near-isogenic lines, RTx430 and Wheatland), and then covered with vermiculite. A single germinated seed was planted in a Ray Leach "Cone-tainer" (Stuewe & Sons, Inc., Corvalis, OR), 3.8 cm diameter at the top by 21 cm long, which was then placed in a rectangular rack that holds 98 Cone-tainers in a 7 × 14 grid. Reverse osmosis (RO) water was purified using a four filter (one carbon and three deionizing) general chemistry polishing system with a hollow fiber final filter with 0.2 µm pore size to remove bacteria (Labconco). Water was dispensed to Cone-tainers with sterilized apparatuses constructed from 50-ml conical tubes. Immediately after planting, germinated seeds were fertilized with 10 ml of sterilized one-third strength Hoagland's solution lacking micronutrients prepared with purified water. Cone-tainers were watered with 10 ml of water, three times per week.

Three weeks after planting, seedling emergence and survival were recoded. Aboveground fresh weight of emerged plants was determined; if weight was indicated as 0.00 on the balance, "0.004 g" was recorded (just below the level of detection).

The experimental design for PIs (experiment 1) was a randomized incomplete block (RIB) with three replications (10 entries in blocks 1 and 2 and 11 in block 3), planted every two weeks, for each of 31 lines and five treatments (*Alternaria* sp., *C. sorghina*, or *F. thapsinum* conidia, or water or null controls) per line. The experimental design for the near-isogenic wx^a and wx^b lines, and their wild-type counterparts (experiment 2), was also a RIB (blocks 1 and 2 each had six entries and blocks 3 and 4 each had seven) with three replications per line with 22 lines and five treatments per line. For experiments 1 and 2, lines were randomly assigned to a block within each replication. The experimental design for wx^a and wx^b near-isogenic lines (experiment 3) was a RCB with three replications with 10 lines and five treatments per line. For all three experiments, each line was planted in five adjacent rows of Cone-tainers (whole plot) and treatments were randomly assigned to a row of seven Cone-tainers (split plot). Percent germination, and percent emergence and survival following planting, and fresh weights of emergent plants were analyzed separately using PROC MIXED of SAS/STAT software (54). Replication was considered a random effect. For PIs, grain type (wild-type and *waxy*), phenotype ($GBSS^{+/-}$), and treatment (*Alternaria* sp., *F. thapsinum*, *C. sorghina*) were considered fixed effects. For near-isogenic lines, genotype and pair (Table 2), as well as treatment, were considered fixed effects. The KENWARD-ROGER option was specified for estimating degrees of freedom. Least squares means, and differences among least squares means were compared using the DIFF option. Data from PI inoculations were analyzed by comparing grain type (*waxy* versus wild-type) and by comparing the three phenotypes (wild-type; *waxy*, $GBSS^+$; and *waxy*, $GBSS^-$). The measurements percent germination, emergence or survival, were analyzed separately. Analysis of aboveground weights of individual plants was confounded because certain treatments had high numbers of Cone-tainers with no emergent plants, resulting in missing data (e.g., amount of missing data were treatment-dependent). Therefore, mean weight of emergent plants per row was estimated and these values ($n = 3$) were used in analyses.

TABLE 3. Percentage of field-grown wild-type or *waxy* grain, with the presence (+) or absence (–) of granule bound starch synthase (GBSS) in starch granules, infected by total fungi, *Alternaria* spp., members of the *Cochliobolus* species complex (SC), members of the *Gibberella fujikuroi* SC, and other *Fusarium* spp., in plant introductions (PI) and other lines, as determined on three semiselective media[v]

Medium Fungal group	Phenotype, $GBSS^{+/-}$ (percent) Wild-type, $GBSS^+$	 *waxy*, $GBSS^-$	 *waxy* $GBSS^+$
DCPA[w]			
Total fungi	10.0b[x] ± 5.8	11.3b ± 5.7	26.9a ± 7.9
Alternaria spp.	3.3b ± 1.9	7.0ab ± 1.9	16.3a ± 5.1
Cochliobolus SC	0.0b ± 0.1	0.1b ± 0.1	0.6a ± 0.3
Gibberella fujikuroi SC	4.4b ± 4.4	4.5b ± 4.4	5.7a ± 4.5
Other *Fusarium* spp.	0.8a ± 0.3	0.6a ± 0.4	0.6a ± 0.7
PCNB[y]			
Total fungi	4.5b ± 4.1	4.4b ± 4.1	6.9a ± 4.1
Gibberella fujikuroi SC	4.0a ± 2.7	1.5a ± 2.7	8.1a ± 4.3
Other *Fusarium* spp.	0.4a ± 0.4	0.3a ± 0.4	0.6a ± 0.8
DRBC[z]			
Total fungi	10.1b ± 5.9	10.7b ± 5.9	16.9a ± 6.4
Alternaria spp.	4.4a ± 1.9	6.1a ± 1.9	10.6a ± 4.0
Cochliobolus SC	0.0a ± 0.1	0.1a ± 0.1	0.0a ± 0.2
Gibberella fujikuroi SC	4.5a ± 4.3	4.2a ± 4.3	4.3a ± 4.4
Other *Fusarium* spp.	0.4a ± 0.2	0.2a ± 0.2	0.6a ± 0.5

[v] Grain was surface-disinfested, dried, and plated onto three semiselective media. Each grain with fungal growth was counted to determine percent infected by total fungi. Fungal colonies growing onto the medium were transferred and identified using morphological characteristics to determine percentage of grain infected by those in each of the fungal groups.

[w] Dicholoran chloramphenicol peptone agar (DCPA) is semiselective for *Fusarium* spp., and *Alternaria* spp. and other dark-spored ascomycetes.

[x] Data were analyzed using PROC MIXED procedure of SAS/STAT software (54) by comparing percentage of grain with colonies within a fungal group for a plant phenotype, as detected on a given medium. Least squares means and standard errors are shown. Different letters (across rows, comparing plant phenotypes) indicate that comparisons are significant at $P \leq 0.05$.

[y] Pentachloronitrobenzene (PCNB) agar is semiselective for *Fusarium* spp.; thus, only percent infection by *G. fujuikuroi* SC members and other *Fusarium* spp. are shown.

[z] Dichloran rose bengal chloramphenicol (DRBC) agar is a general purpose fungal medium.

RESULTS

Marker and iodine analyses of $GBSS^-$ and $GBSS^+$ *waxy* PIs. To determine whether the $GBSS^-$ lines contained the wx^a allele and the $GBSS^+$ lines contained wx^b, marker analyses were performed. Multiplex PCR of DNA from $GBSS^-$ *waxy* PIs revealed that all but one PI had the PCR product size indicative of the wx^a allele; PI 562758 produced a product size similar to that of wild-type lines, even though it possesses *waxy* starch (Table 1). CAPS marker analysis of DNA from $GBSS^+$ *waxy* PIs revealed that two (PI 563670 and PI563671) had the restriction pattern similar to known wx^b cultivars and two (PI 23231 and PI 217897) were not digested by *Nco*I and had the wild-type sized band. Iodine staining was repeated (from a previous study) on grain from PI 23231, PI 217897 and PI 548008, along with controls BWheatland (wild-type) and 94C289 (*waxy*). The three PIs all resulted in a purple iodine response, while BWheatland scored royal blue and 94C289 also scored purple; these results are similar to what was previously observed (50).

Field study. When grain were screened and fungi selected on DCPA media, $GBSS^+$ *waxy* grain had significantly more infection by *Alternaria* spp., and members of the *Cochliobolus* and

G. fujikuroi SC than wild-type grain ($P = 0.02$; Table 3). GBSS$^-$ *waxy* grain had similar levels of infection to wild-type grain in these three fungal groups ($P \geq 0.09$). For other *Fusarium* spp. detected on DCPA, for *G. fujikuroi* SC isolates and other *Fusarium* spp. isolates detected on PCNB, and for all four fungal groups detected on DRBC, there were no significant differences between the three grain phenotypes for percentage of fungal infection (Table 3).

Alternaria spp. were identified to species using morphological techniques and *Fusarium* spp. were also identified using morphological techniques, which were confirmed using molecular identification. The most commonly isolated *Alternaria* spp. was by far *A. alternata* (Fr.) Keissl. 1912, but also detected were *A. arborescens* E.G. Simmons 1999, *A. dumosa* E.G. Simmons 1999, *A. gaisen* Nagano 1920, and *A. longipes* (Ellis & Everh.) E.W. Mason 1928. The *G. fujikuroi* SC species found in grain were *F. thapsinum*, which was most prevalent. *F. proliferatum* (Matsush.) Nirenberg, *F. andiyazi* Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie 2001, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas 1983 (Supplementary Table 1), The only other Fusarium spp. detected was *F. incarnatum–F. equiseti* species complex (FIESC) genotype 25-a, b, c.

Because of the variety of sorghum lines utilized in this study, correlation analyses were used to compare the physical parameters, plant height at maturity, and days to anthesis, when examining percent of grain with fungal infection (Table 4). Internal fungal infection was significantly negatively correlated with plant height for each of three phenotypic classes (wild-type; *waxy*, GBSS$^-$; and *waxy*, GBSS$^+$) when fungi were selected on DCPA ($r \leq -0.36$; $P < 0.01$) and DRBC ($r \leq -0.21$; $P < 0.01$) media; additionally, for wild-type and GBSS$^-$ *waxy* grain, height was significantly negatively correlated with plant height when fungi were selected on PCNB ($r \leq -0.25$; $P < 0.01$) but not for GBSS$^+$ *waxy* grain ($r = -0.22$; $P = 0.23$) (Table 4). Analyses of percentage of grain across all plant phenotypes with members of individual fungal genera or of the *G. fujikuroi* SC revealed significant negative correlation between height at anthesis and percentage of grain with *Alternaria* spp. (on DCPA, $r = -0.39$, $P < 0.01$; on DRBC, $r = -0.34$, $P < 0.01$), *G. fujikuroi* SC (on DCPA, $r = -0.25$, $P < 0.01$; on DRBC, $r = -0.19$, $P < 0.01$; on PCNB, $r = -0.19$, $P = 0.05$), and other *Fusarium* spp. (on DCPA, $r = -0.11$, $P = 0.02$; on PCNB, $r = -0.22$, $P < 0.01$; on DRBC, r not significant at $P = 0.08$). To examine the role of plant height in grain mold further, a subset of 12 lines, representing two significantly different height classes ($P < 0.01$; Table 1) were compared for fungal infection of grain on DCPA, PCNB, and DRBC (Table 5). For all three tall plant phenotypes, there were no significant differences for fungi infecting grain selected on all three media ($P \geq 0.20$). However, *waxy* GBSS$^+$ short plants had significantly greater percentages of grain with internal fungal growth than grain from short *waxy* GBSS$^-$ plants, when selected on DCPA or DRBC media ($P \leq 0.05$) (Table 5). On these two media, there were also significantly greater percentages of infected grain from short wild-type and *waxy* GBSS$^+$ plants than tall ones within the same *waxy* phenotype ($P \leq 0.02$).

Correlation analyses were also conducted utilizing two repetitions from 2004 that were previously analyzed for gelatinization temperatures (50). When peak gelatinization temperatures were compared with percent fungal infection found in grain of the three phenotypes (wild-type, *waxy* GBSS$^-$, and *waxy* GBSS$^+$) correlations were not significant ($r \leq 0.68$; $P \geq 0.06$).

TABLE 4. Pearson correlation coefficient (r) and probability (*P*) for the variables days to anthesis, height, and percent of grain with internal fungal infection, as determined on three media, for wild-type; *waxy*, granule bound starch synthase (GBSS)$^-$; and *waxy*, GBSS$^+$ plant introductions and other lines[x]

Variables	Height at anthesis[y]	Percent grain with fungi selected on DCPA[z]	PCNB[z]	DRBC[z]
Wild-type (n = 192)				
Days to anthesis	$r = 0.17$	$r = -0.16$	$r = 0.17$	$r = -0.25$
	$P = 0.02$	$P = 0.02$	$P = 0.02$	$P < 0.01$
Height		$r = -0.39$	$r = -0.41$	$r = -0.41$
		$P < 0.01$	$P < 0.01$	$P < 0.01$
DCPA			$r = 0.75$	$r = 0.77$
			$P < 0.01$	$P < 0.01$
PCNB				$r = 0.58$
				$P < 0.01$
waxy, GBSS$^-$ (n = 200)				
Days to anthesis	$r = 0.15$	$r = -0.09$	$r = -0.06$	$r = -0.09$
	$P = 0.04$	$P = 0.19$	$P = 0.36$	$P = 0.18$
Height		$r = -0.36$	$r = -0.25$	$r = -0.21$
		$P < 0.01$	$P < 0.01$	$P < 0/01$
DCPA			$r = 0.58$	$r = 0.71$
			$P < 0.01$	$P < 0.01$
PCNB				$r = 0.50$
				$P < 0.01$
waxy, GBSS$^+$ (n = 32)				
Days to anthesis	$r = -0.16$	$r = -0.18$	$r = -0.42$	$r = -0.22$
	$P = 0.37$	$P = 0.31$	$P = 0.02$	$P = 0.23$
Height		$r = -0.59$	$r = -0.22$	$r = -0.57$
		$P < 0.01$	$P = 0.23$	$P < 0.01$
DCPA			$r = 0.63$	$r = 0.66$
			$P < 0.01$	$P < 0.01$
PCNB				$r = 0.47$
				$P = 0.01$

[x] Pearson correlations were generated for the response variables days to anthesis, height at maturity, percentage of grain with internal fungal infection as determined on dicholoran chloramphenicol peptone agar (DCPA), pentachloronitrobenzene (PCNB) agar, and dichloran rose bengal chloramphenicol (DRBC) agar using SAS Proc Corr software (54).

[y] Average plant height in each plot was estimated when the plants in a plot reached anthesis (defined as half the plants having anthers exerted).

[z] To determine internal fungal infection, grain was surface-disinfested, dried, and plated onto DCPA, PCNB, and DRBC. DCPA is semiselective for *Fusarium* spp. and *Alternaria* spp. and other dark-spored ascomycetes. PCNB is semiselective for *Fusarium* spp. DRBC is a general purpose fungal medium. Each grain with fungal colonies growing onto the medium was counted.

TABLE 5. Mean percent grain with internal fungal infection of short or tall, wild-type with granule bound starch synthase present in grain (GBSS$^+$), *waxy* GBSS$^-$, or *waxy* GBSS$^+$ sorghum plants, as determined on three semiselective media[w]

Medium[x]	Height[y]	Percent infected grain: Wild-type, GBSS$^+$	*waxy*, GBSS$^-$	*waxy*, GBSS$^+$
DCPA	Short	25.0b*[z] ± 7.9	18.8b ± 7.9	48.8a* ± 7.9
DCPA	Tall	7.5a ± 6.1	8.8a ± 6.1	5.0a ± 6.1
PCNB	Short	7.9a ± 5.3	6.8a ± 5.1	11.0a ± 5.4
PCNB	Tall	4.4a ± 5.3	3.6a ± 5.1	4.6a ± 5.4
DRBC	Short	30.0ab* ± 7.4	15.0b ± 7.4	31.3a* ± 7.4
DRBC	Tall	8.8a ± 5.7	6.3a ± 5.7	2.5a ± 5.7

[w] Grain was surface-disinfested, dried and plated onto three semiselective media. Grains with fungal growth were counted. GBSS is the enzyme responsible for amylose production in wild-type plants. It is absent in starch granules of many *waxy* (no amylose) plants (GBSS$^-$), but is present (partially active) in starch granules of a few *waxy* lines (GBSS$^+$).

[x] Dichloran chloramphenicol peptone agar (DCPA) is semiselective for *Fusarium* spp. and *Alternaria* spp. and other dark-spored ascomycetes. Pentachloronitrobenzene (PCNB) agar is semiselective for *Fusarium* spp. Dichloran rose bengal chloramphenicol (DRBC) agar is a general purpose fungal medium.

[y] Two lines of each *waxy* (GBSS$^{+/-}$) phenotype and height class were chosen for analysis (Table 1). The lines were PI 23231, PI, 217897, PI 535783, PI 563015, PI 563402, PI 563670, PI 563671, PI 563672, PI 565116, PI 567939, PI 586448, and PI 667647. Difference in heights between short and tall plants was significant ($P < 0.01$).

[z] Data were analyzed using PROC MIXED procedure of SAS/STAT software (54). Comparisons were made of percent of grain with colonies for a height class, as detected on a given medium. Differing letters indicate that comparisons are significant at $P \leq 0.05$. Comparisons also were made between percent of grain with colonies from short and tall plants within a plant phenotype as detected on a given medium. An asterisk indicates the value is significantly greater than the comparable value at $P \leq 0.05$.

Grain inoculations. In experiment 1, fungal conidia inoculations or water treatments did not affect germination of grain from wild-type PIs ($P \geq 0.12$) (Table 6). Similar to wild-type, *waxy* GBSS$^-$ PIs mean percent germination was not significantly different than the water control ($P \geq 0.07$). Mean percent germination was reduced for grain of GBSS$^+$ *waxy* PIs by inoculation with *Alternaria* sp. and *C. sorghina* when compared with water controls ($P \leq 0.04$); subsequent measurements using the same inoculated seed were not significantly reduced ($P \geq 0.06$). However, emergence, survival, and mean plant weight of wild-type or GBSS$^-$ *waxy* grain inoculated with *C. sorghina* or *F. thapsinum* could be reduced compared with water controls (Table 6).

When response of *waxy* PIs was compared with that of wild-type PIs, inoculations of GBSS$^-$ grain did not have reduced germination ($P \geq 0.08$), but had significantly reduced mean percent emergence (*C. sorghina*) and survival (*Alternaria* sp. and *C. sorghina*) ($P = 0.04$) (Table 6). Inoculation of *waxy* GBSS$^+$ grain by *Alternaria* sp. and *C. sorghina* reduced germination compared with wild-type grain ($P \leq 0.05$), but mean percent emergence and survival, and mean fresh weight, were similar to wild-type ($P \geq 0.37$). Treatment of *waxy* GBSS$^+$ grain with *F. thapsinum* conidia did not significantly affect responses compared with wild-type ($P \geq 0.11$).

In experiment 2, 12 near-isogenic pairs, in different genetic backgrounds, were used to directly compare effects of either wx^a or wx^b to wild-type (75) (this work). There was no consistent effect indicating that *waxy* increased susceptibility to any of the three fungi relative to wild-type; e.g., seedling weights were not significantly affected (Table 7). wx^a grain was not significantly affected by inoculation when comparing wild-type grain with the same treatment, for any of the four measurements ($P \geq 0.52$). However, following inoculations with *C. sorghina* or *F. thapsinum*, mean percent emergence of wx^b grain was significantly less than wild-type grain (Table 7). Interestingly, mean seedling weights were significantly greater for *Alternaria*-treated wx^b grain, as well as for untreated grain compared with wild-type grain. Comparisons of individual *waxy* isogenic line and comparable wild-type line within individual pairs revealed that germination was significantly reduced in inoculated *waxy* grain from line pair 5 (*Alternaria* sp. and *F. thapsinum*), 6 (*C. sorghina* and *F. thapsinum*), 7 (*C. sorghina*), 9 (*C. sorghina* and *F. thapsinum*), and 12 (*Alternaria* sp.) ($P \leq 0.04$) (Table 2; Supplementary Table 2). Seedling emergence and survival were significantly reduced in the *waxy* line of pair 4 after inoculation with *F. thapsinum* ($P = 0.05$), and in the *waxy* line of pair 8 inoculated with *C. sorghina* ($P \leq 0.02$), compared with respective wild-type lines. The *waxy* lines from pairs 1 and 4 had significantly greater percent germination ($P = 0.01$) and mean plant weight ($P = 0.02$), following *Alternaria* sp. inoculation, and the *waxy* line from pair 9 had significantly greater mean seedling plant weight following inoculation with *F. thapsinum*, than their near-isogenic wild-type counterparts.

To directly compare the wx^a (GBSS$^-$) and wx^b (GBSS$^+$) alleles, near-isogenic pairs were developed (Table 2) (M. K. Yerka, J. J. Toy, D. L. Funnell-Harris, S. E. Sattler, and J. F. Pedersen, *unpublished data*). In experiment 3, grain from these three isogenic line pairs also were inoculated with *Alternaria* sp., *F. thapsinum*, and *C. sorghina* conidia. There were no significant differences between wx^a and wx^b for any of the treatments and the untreated control ($P \geq 0.11$) (Table 8). *C. sorghina* treatment significantly reduced emergence, survival, and plant weight for both *wx* genotypes compared with water controls, and likewise, *F. thapsinum* significantly reduced seedling survival and plant weight ($P \leq 0.01$) for both *wx* genotypes. Again, there was no case where a significant difference was observed between wx^a and wx^b alleles near-isogenic pairs following fungal conidia treatment ($P \geq 0.13$) (Table 2; Supplementary Table 3).

DISCUSSION

The results presented herein indicated that the use of the *waxy* trait to increase usability of sorghum grain for food products, feed, and grain-based bioenergy (34,53,73,74) will not result in increased susceptibility to grain mold pathogens. These results were obtained

TABLE 6. Grain from sorghum plant introductions that are wild-type, or *waxy* that either lack granule bound starch synthase (GBSS$^-$) or have the GBSS protein (GBSS$^+$) in starch granules were treated with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia, or water or were untreated[x]

	Conidial treatments			Controls	
Assessment	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
Wild-type grain					
Germination	71.8a[y] ± 4.4	73.7a ± 4.4	74.8a ± 4.4	72.8a ± 4.4	75.0a ± 4.4
Emergence	69.9bc ± 11.3	67.4bc ± 11.3	65.2c ± 11.1	74.1ab ± 11.2	82.3a ± 11.3
Survival	58.5bc ± 13.7	54.0c ± 13.7	50.8c ± 13.7	66.7ab ± 13.7	72.1a ± 13.7
Weight (g)	0.37b ± 0.11	0.28c ± 0.11	0.28c ± 0.11	0.40ab ± 0.11	0.45a ± 0.11
waxy grain					
Germination	69.6c ± 4.3	72.0bc ± 4.3	73.0bc ± 4.3	74.8ab ± 4.3	76.8a ± 4.3
Emergence	63.0bc ± 11.3	55.6c*[z] ± 11.3	57.7c ± 11.3	65.7b ± 11.1	75.8a ± 11.2
Survival	50.0c ± 13.7	44.1c* ± 13.7	43.5c ± 13.7	58.9b ± 13.7	67.0a ± 13.7
Weight (g)	0.31b ± 0.11	0.28bc ± 0.11	0.22c ± 0.11	0.35b ± 0.11	0.45a ± 0.11
waxy, GBSS$^-$ grain					
Germination	74.9b ± 4.7	77.8ab ± 4.7	77.2ab ± 4.7	78.9ab ± 4.7	80.9a ± 4.7
Emergence	62.6bc ± 12.1	55.4c* ± 12.1	59.0c ± 11.8	66.9b ± 11.8	76.8a ± 12.0
Survival	47.3b* ± 13.8	42.9b* ± 13.8	44.9b ± 13.8	60.4a ± 13.8	67.9a ± 13.8
Weight (g)	0.30bc ± 0.11	0.28bc ± 0.11	0.23c ± 0.11	0.36ab ± 0.11	0.44a ± 0.11
waxy, GBSS$^+$ grain					
Germination	53.7b* ± 7.9	54.6b* ± 7.9	60.4ab ± 7.9	62.5a ± 7.9	64.7a ±7.9
Emergence	64.1ab ± 12.4	55.7b ± 13.0	53.4b ± 13.1	61.7ab ± 12.5	72.4a ± 12.6
Survival	58.4ab ± 15.0	47.7bc ± 15.0	39.3bc ±15.0	54.8abc ± 15.0	64.3a ± 15.0
Weight (g)	0.35ab ± 0.13	0.28b ± 0.13	0.19b ± 0.13	0.33ab ± 0.13	0.45a ± 0.13

[x] Mean percent germination, emergence and survival, and mean fresh weight (g) were determined. Grain from *waxy* and wild-type sorghum plant introductions, a known *waxy* line, and known wild-type lines were incubated in suspensions of conidia or sterile water or germinated without treatment. Percentage of germinated seeds was determined. A subset of germinated seeds was grown for 3 weeks in soil at which time numbers of emerged plants and surviving plants were determined. Fresh weight of above-ground plant parts was determined and analyzed by total weights for a row and then divided by number of surviving plants to determine mean weight per plant.

[y] Least squares means and standard errors are shown. Within plant genotype and a given assessment, means with different letters are significantly different at $P \leq 0.05$.

[z] Within a treatment and a given assessment, asterisk indicates that the mean of the *waxy* lines is significantly less than that of the wild-type phenotype at $P \leq 0.05$.

from screening a diverse collection (PIs) of field-grown grain and in vitro inoculation of grain from PIs with grain mold fungi, and in vitro inoculations of near-isogenic *waxy* lines with grain mold fungi.

Field-grown grain of the PI collection were assessed for internal fungal infection by *Alternaria* spp., *Fusarium* spp., and *Cochliobolus* SC. Across all genotypes, the most commonly detected species, *A. alternata*, *F. thapsinum*, and *F. proliferatum*, are those known to infect sorghum grain (14,17,69). Greater colonization of members from *Alternaria* spp., *Cochliobolus* SC, and *G. fujikuroi* SC on *waxy* GBSS$^+$ indicated that these lines are more susceptible to infection (Table 3). Correlation analyses indicated that height was a factor in grain infection for all three phenotypes (wild-type, *waxy* GBSS$^-$, and *waxy* GBSS$^+$) (Table 4). Therefore, a subset of lines was compared within height classes (Table 5), which indicated that short lines tended to have more fungal infection of grain than tall lines, but short *waxy* GBSS$^+$ plants had a higher fungal infection percentage than short lines of the other two phenotypes. The four GBSS$^+$ *waxy* lines are all the known such lines from the extensive U.S. photoperiod insensitive collection (http://www.ars-grin.gov/npgs/); nonetheless, caution must be observed when making broad conclusions. What is known is that the two short *waxy* GBSS$^+$ plants are wx^b and likely related (Table 1), while the two tall cultivars have similar infection levels as the tall wild-type lines (Table 5). Although the two tall *waxy* lines were GBSS$^+$, they did not carry the wx^b allele, as indicated by previously developed markers (56). These new *wx* alleles, as represented by the tall GBSS$^+$ *waxy* lines, may provide valuable materials for breeding for new *waxy* grain production lines.

Plant height was a significant factor affecting fungal infection in this study (Tables 4 and 5). Nearly all the lines were tall and

TABLE 7. Comparison of wild-type (*Wx*) and *waxy* (wx^a or wx^b) grain in near-isogenic sorghum lines following seed inoculations with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia, or water and untreated controls as determined by mean percent germination, emergence and survival, and mean fresh weight (g)[v]

		Conidial treatments			Controls	
Assessment	Genotype	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
Combined lines with wx^a backgrounds[w]						
Germination	*Wx*	79.9a[x] ± 5.2	77.0a ± 5.2	79.5a ± 5.1	81.7a ± 5.2	72.3a ± 7.2
Emergence	*Wx*	81.6ab ± 7.2	65.0c ± 7.3	68.9bc ± 8.8	79.2b ± 7.1	91.9a ± 6.5
Survival	*Wx*	72.5b ± 8.0	51.0c ± 8.4	47.9c ± 9.6	70.9b ± 7.5	89.1a ± 5.5
Weight (g)	*Wx*	0.22bc ± 0.06	0.15cd ± 0.05	0.13d ± 0.05	0.24b ± 0.05	0.36a ± 0.05
Germination	wx^a	75.2b ± 5.3	70.9b ± 5.2	74.0b ± 5.1	81.1a ± 5.2	68.1b ± 7.2
Emergence	wx^a	69.2b ± 7.2	54.1c ± 7.4	53.3bc ± 8.8	69.2b ± 7.2	84.3a ± 6.5
Survival	wx^a	68.1b ± 7.0	50.7cd ± 8.0	36.4d ± 8.6	63.4bc ± 8.7	84.8a ± 6.8
Weight (g)	wx^a	0.26ab ± 0.06	0.13c ± 0.05	0.11c ± 0.05	0.24b ± 0.05	0.31a ± 0.05
Combined lines with wx^b backgrounds[y]						
Germination	*Wx*	81.4b ± 4.4	82.8ab ± 4.7	83.0b ± 4.1	87.2a ± 4.4	80.0ab ± 5.9
Emergence	*Wx*	71.2abc ± 7.2	59.3c ± 7.7	68.8bc ± 6.9	76.0ab ± 6.7	83.0a ± 5.8
Survival	*Wx*	64.4a ± 9.2	45.3b ± 9.2	44.5b ± 9.2	65.2a ± 9.2	77.7a ± 9.2
Weight (g)	*Wx*	0.15bc ± 0.05	0.10c ± 0.05	0.11c ± 0.05	0.18b ± 0.05	0.25a ± 0.05
Germination	wx^b	80.8a ± 4.5	78.4a ± 4.4	79.3a ± 4.8	81.4a ± 4.4	77.2a ± 4.5
Emergence	wx^b	67.6b ± 7.4	39.0c*[z] ± 7.9	50.9c* ± 7.1	69.2b ± 6.9	83.5a ± 6.0
Survival	wx^b	63.1b ± 9.4	33.8c ± 9.4	26.6c ± 9.4	60.0b ± 9.4	79.0a ± 9.4
Weight (g)	wx^b	0.20b* ± 0.05	0.09c ± 0.05	0.11c ±0.06	0.25b ± 0.05	0.37a* ± 0.06

[v] Grain from wild-type and near-isogenic wx^a or wx^b lines were incubated in suspensions of conidia or sterile water, or germinated without treatment. Number of germinated seeds was determined. A subset of germinated seeds was grown for 3 weeks in soil at which time numbers of emerged plants and surviving plants were determined. Fresh weights were determined and analyzed by total weights for a row and then divided by number of emergent plants to determine mean weight per plant.

[w] Data are from grain of six near-isogenic line pairs in backgrounds with two different sources of wx^a gene (75) (present work).

[x] Least squares means and standard errors are shown. Mean separations are for response to four treatments or untreated control, within a genotype and for a particular assessment. Means with different letters are significantly different at $P \leq 0.05$.

[y] Data are from grain of six near-isogenic line pairs in backgrounds with two different sources of wx^b gene (75).

[z] Asterisk indicates that mean for *waxy* genotype for the particular assessment and treatment, or untreated control, is significantly different than corresponding wild-type mean ($P \leq 0.05$).

TABLE 8. Comparison of *waxy* sorghum grain of wx^a (absence (–) of the granule bound starch synthase protein in starch granules [GBSS]) or wx^b (GBSS$^+$) alleles, in near-isogenic lines, following seed inoculations with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia, or sterile water and untreated as determined by mean percent germination, emergence and survival, and mean fresh weight (g)[y]

		Conidial treatments			Controls	
Assessment	Genotype	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
Near-isogenic line pairs						
Germination	wx^a	88.7a[z] ± 2.6	88.4a ± 2.6	92.3a ± 2.6	89.4a ± 2.6	89.1a ± 2.6
Emergence	wx^a	74.6ab ± 11.6	57.1b ± 13.4	69.8ab ± 11.6	85.7a ± 8.3	84.1a ± 8.5
Survival	wx^a	66.7ab ± 13.6	52.4b ± 13.8	55.6b ± 11.8	84.1a ± 11.4	79.4a ± 11.0
Weight (g)	wx^a	0.30b ± 0.03	0.17c ± 0.03	0.20c ± 0.03	0.37ab ± 0.03	0.41a ± 0.03
Germination	wx^b	91.7a ± 1.7	91.8a ± 1.7	90.3a ± 1.7	90.7a ± 1.7	92.1a ± 1.7
Emergence	wx^b	84.1ab ± 11.6	58.7c ± 13.4	65.1bc ± 11.6	90.5a ± 8.3	92.1a ± 8.5
Survival	wx^b	73.0ab ± 13.6	47.6b ± 13.9	49.2b ± 11.8	81.0a ± 11.4	90.5a ± 11.0
Weight (g)	wx^b	0.29c ± 0.03	0.14d ± 0.03	0.19d ± 0.03	0.32bc ± 0.03	0.42a ± 0.03

[y] Grain from near-isogenic wx^a and wx^b lines were incubated in suspensions of conidia, sterile water, or were germinated without treatment. Number of germinated grains was determined. A subset of germinated grains was grown for three more weeks in soil at which time numbers of emerged plants and surviving plants were determined. Fresh weights were also determined and analyzed by total weights for a row and then divided by number of emergent plants in the row to determine mean weight per plant.

[z] Data were analyzed using the PROC MIXED procedure of SAS/STAT software (54). Least squares means and standard errors are shown. Mean separations are for response to four treatments or untreated, within a genotype and for a particular assessment. Means with different letters are significantly different at $P \leq 0.05$.

comparable in stature to forage-type plants (50), but there were nine shorter, grain-height accessions. RCB design with single row plots was planted. A grain line was often planted beside taller plants, which could have affected light levels, day- and nighttime temperatures, air movement and relative humidity; changes in these conditions for infection, release and dispersal of airborne inoculum, and proximity to soil- or debris-borne inoculum, could have affected infection levels in shorter plants (8,68). A previous study showed significant negative correlations between plant height and visual grain mold rating, demonstrating using QTL analyses that there were two genomic regions that affected both grain mold incidence and plant height or peduncle length (28). In the present study, there were no visible symptoms of grain mold, so grain was screened for internal, asymptomatic infection, yet a negative correlation between plant height and internal infection was still apparent (Table 4). The results of this and the previous studies may be relevant for intercropping sorghum with other crops (59) or possibly sorghum monoculture planted on sloped terrains with low areas or depressions (30,40).

Grain from a subset of the accessions and wx^a and wx^b near-isogenic lines were challenged with conidia from an *Alternaria* isolate, and *C. sorghina* and *F. thapsinum*, previously isolated from grain sorghum (16,17). These inoculation studies, which measured germination and seedling traits, supported the field results. There were incidences where all three fungal treatments had greater effects on *waxy* grain than on wild-type grain (Tables 6 and 7). However, there was no consistent and prevalent response that indicated that *waxy* lines were more susceptible to these grain pathogens than wild-type lines. This included near-isogenic wx^b lines, indicating that through breeding at least some negative qualities associated with the wx^b PI lines have been ameliorated (75). A direct comparison between *waxy* alleles wx^a ($GBSS^-$) and wx^b ($GBSS^+$) near-isogenic lines showed again no evidence that one allele was superior to the other (Table 8). Within individual lines, it was clear that inoculation with *C. sorghina* or *F. thapsinum* conidia could significantly reduce percent emergence and survival and fresh weight of inoculated seedlings (Tables 6, 7, and 8). In previous studies, maize kernels were inoculated with conidia of *F. verticillioides* and grown to maturity: a systemic infection occurred throughout entire plants and even infected kernels were produced on these plants (41,42). In another study, kernels planted in *F. verticillioides*-infested soil were systemically infected as seedlings (47). In the present study, the results suggested that systemic fungal growth may be occurring, and this growth may be detrimental to survival or plant weight of sorghum seedlings. In a previous study, the grain produced from *F. thapsinum*-inoculated sorghum grain showed not to be infected with the pathogen (13). Thus, there is no evidence at this time that *F. thapsinum* continues to grow through the maturing plant, unlike the maize–*F. verticillioides* system (41). Even in this system where infected kernels were observed from plants systemically infected, in most cases fungal growth did not progress beyond plant crowns.

A beneficial consequence of this research was the identification of at least one new *waxy* allele among the *waxy* PIs. We wanted to ascertain that all the $GBSS^+$ lines had the wx^b allele and all $GBSS^-$ lines had wx^a. Therefore, DNA from these *waxy* lines was subjected to marker analyses, previously published: all but one of the *waxy* alleles of the $GBSS^-$ lines were identified as containing the wx^a allele, and two of the four $GBSS^+$ lines were identified as containing the wx^b allele (Table 1). Two other *wx* alleles had been recently identified in Asian germplasm collections, and based on the predicted amino acid sequences resulting from these mutations, both alleles produce the GBSS protein (24,38). Therefore, the present study has discovered at least one and possibly as many as three novel *waxy* alleles. We are currently analyzing these potentially new *waxy* alleles.

In summary, this study used both field conditions and growth chamber inoculations of a diverse set of *waxy* and wild-type sorghum materials to demonstrate that the *waxy* trait does not increase susceptibility to common grain pathogens. Another benefit of this study was the finding of additional *waxy* alleles within existing accessions. The field study provided evidence that shorter plants may be more susceptible to fungal infection of their grain even if there are no visible symptoms. This study presented valuable results that could be utilized to choose *waxy* lines with resistance to grain-infecting fungi for hybrid seed production (Supplementary Tables 4 and 5).

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Supplementary Table 1. *Fusarium* species isolated from sorghum grain grown on wild-type plants with granule bound starch synthase (GBSS) present in starch granules ($GBSS^{+}$), *waxy* $GBSS^{-}$ plants, or *waxy* $GBSS^{+}$ plants and as determined by comparing sequences from the translation elongation factor (*TEF*) gene with those in the FUSARIUM-ID database

	Phenotype, $GBSS^{+/-}$					
	Wild-type, $GBSS^{+}$		Waxy, $GBSS^{-}$		Waxy, $GBSS^{+}$	
Species[x]	% similarity	*n*	% similarity	*n*	% similarity	*n*
F. andiyazi	100	1	n/a[y]	0	100	1
F. proliferatum	99-100	5	100	2	100	8
F. subglutinans	n/a	0	100	2	n/a	0
F. thapsinum[z]	100	32	100	15	100	28
FIESC 25-a,b,c	100	1	n/a	0	100	1

[x]The 5' region of *TEF* was amplified and sequenced (2) and compared with sequences of type isolates in the database (http://isolate.fusariumdb.org/index.php). FIESC indicates genotype of the *Fusarium incarnatum-F. equiseti* species complex.

[y]"n/a" indicates "not applicable."

[z]Sequence from *Gibberella thapsina* (*Fusarium thapsinum*) type isolate M-3790 has 100% identity to sequence from isolate FD 01851 in FUSARIUM-ID database (1, 3). Sequences from isolates from this study with 100% similarity with sequence from isolate FD 01851 were thus identified as *F. thapsinum*.

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Supplementary Table 2. Comparison of wild-type (*Wx*) and waxy (*wx*^{a} or *wx*^{b}) grain within near-isogenic sorghum line pairs following seed inoculations with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia or sterile water and untreated controls as determined by mean percent germination, emergence and survival, and mean fresh weight (g).[t]

			Conidial treatments			Controls	
Line pair[u]	Assessment	Genotype	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
Lines with *wx*^{a} backgrounds[v]							
2	Germination	*Wx*	90.6a[w] ± 7.4	88.7a ± 7.1	90.5a ± 6.5	90.5a ± 7.4	81.4a ± 13.5
2	Emergence	*Wx*	80.6ab ± 13.4	23.5c ± 14.4	52.0bc ± 18.7	66.3ab ± 14.8	94.9a ± 10.4
2	Survival	*Wx*	68.1ab ± 16.3	20.4c ± 17.5	44.3bc ± 20.9	53.8bc ± 14.9	91.9a ± 7.8
2	Weight (g)	*Wx*	0.24ab ± 0.09	0.12b ± 0.09	0.21ab ± 0.08	0.14b ± 0.07	0.36a ± 0.08
2	Germination	*wx*^{a}	80.6a ± 7.2	76.9a ± 6.9	79.7a ± 6.3	86.7a ± 7.2	84.5a ± 13.4
2	Emergence	*wx*^{a}	82.0a ± 12.9	53.5a ± 14.0	58.2a ± 18.4	63.0a ± 14.3	82.0a ± 9.7
2	Survival	*wx*^{a}	73.9a ± 12.7	54.9a ± 15.6	54.9a ± 17.6	59.6a ± 17.9	83.4a ± 12.0
2	Weight (g)	*wx*^{a}	0.15ab ± 0.09	0.07ab ± 0.09	0.06b ± 0.08	0.12ab ± 0.08	0.24a ± 0.08
3	Germination	*Wx*	89.3a ± 7.4	81.1a ± 7.1	84.8a ± 6.5	81.6a ± 7.4	50.4b ± 13.5
3	Emergence	*Wx*	89.3a ± 13.3	79.8a ± 14.3	60.8a ± 18.6	79.8a ± 14.7	84.6a ± 10.2

3	Survival	*Wx*	73.2ab ± 16.3	35.1b ± 17.5	25.6b ± 20.9	68.4ab ± 14.9	77.9a ± 7.7
3	Weight (g)	*Wx*	0.18ab ± 0.09	0.08b ± 0.08	0.02b ± 0.07	0.24a ± 0.07	0.31a ± 0.08
3	Germination	*wx*[a]	78.5ab ± 7.2	79.0ab ± 6.9	83.3a ± 6.3	87.3a ± 7.2	53.1b ± 13.4
3	Emergence	*wx*[a]	81.4a ± 13.0	43.3b ± 14.0	62.3ab ± 18.4	76.6ab ± 14.4	95.7a ± 9.8
3	Survival	*wx*[a]	78.8ab ± 12.7	45.5b ± 15.6	40.7b ± 17.6	78.8ab ± 17.9	97.9a ± 12.0
3	Weight (g)	*wx*[a]	0.31ab ± 0.09	0.23bc ± 0.07	0.10c ± 0.07	0.33ab ± 0.07	0.44a ± 0.08
6	Germination	*Wx*	93.5a ± 7.4	90.8a ± 7.1	89.3a ± 6.5	90.1a ± 7.4	75.0a ± 13.5
6	Emergence	*Wx*	84.6 a± 13.3	65.5a ± 14.3	70.3a ± 18.6	70.3a ± 14.7	84.6a ± 10.2
6	Survival	*Wx*	68.4ab ± 16.3	44.6b ± 17.5	44.6ab ± 20.9	63.7ab ± 14.9	82.7a ± 7.7
6	Weight (g)	*Wx*	0.19ab ± 0.09	0.10b ± 0.08	0.08b ± 0.07	0.20ab ± 0.07	0.30a ± 0.08
6	Germination	*wx*[a]	92.0a ± 7.3	71.5b*[x] ± 7.0	69.3b* ± 6.4	77.3ab ± 7.3	84.2ab ± 13.4
6	Emergence	*wx*[a]	53.4ab ± 13.0	43.9ab ± 14.1	43.9ab ± 18.5	39.1b ± 14.4	72.5a ± 9.9
6	Survival	*wx*[a]	56.1ab ± 12.9	41.8ab ± 15.8	32.3ab ± 17.7	27.5b ± 18.0	70.4a ± 12.2
6	Weight (g)	*wx*[a]	0.34a ± 0.11	0.13a ± 0.09	0.14a ± 0.08	0.11a ± 0.08	0.18a ± 0.08
10	Germination	*Wx*	63.8a ± 7.2	60.0a ± 6.9	66.1a ± 6.3	74.1a ± 7.2	69.3a ± 13.3
10	Emergence	*Wx*	90.7a ± 12.8	71.7a ± 13.9	66.9a ± 18.3	81.2a ± 14.3	100.3a ± 9.6

10	Survival	*Wx*	87.7ab ± 15.9	68.7ab ± 17.1	49.6b ± 20.6	82.9ab ± 14.4	97.2a ± 6.8
10	Weight (g)	*Wx*	0.24abc ± 0.09	0.16bc ± 0.07	0.09c ± 0.07	0.28ab ± 0.07	0.34a ± 0.08
10	Germination	*wx*[a]	68.4a ± 7.2	68.2a ± 6.9	70.0a ± 6.3	74.1a ± 7.2	50.6a ± 13.4
10	Emergence	*wx*[a]	70.9a ± 12.9	75.6a ± 14.0	51.8a ± 18.4	75.6a ± 14.4	75.6a* ± 9.7
10	Survival	*wx*[a]	71.9a ± 12.8	71.9a ± 15.7	14.7b ± 17.6	76.6a ± 17.9	76.6a ± 12.1
10	Weight (g)	*wx*[a]	0.16ab ± 0.09	0.10b ± 0.07	0.12b ± 0.07	0.25a ± 0.07	0.23ab ± 0.08
11	Germination	*Wx*	60.8a ± 7.3	59.7a ± 7.0	66.0a ± 6.4	68.3a ± 7.3	75.1a ± 13.4
11	Emergence	*Wx*	57.4b ± 13.1	86.0ab ± 14.2	81.2ab ± 18.5	90.7ab ± 14.6	90.7a ± 10.0
11	Survival	*Wx*	59.0b ± 16.4	82.8ab ± 17.5	54.2ab ± 20.9	78.0ab ± 15.0	92.3a ± 7.9
11	Weight (g)	*Wx*	0.23b ± 0.09	0.19b ± 0.08	0.16b ± 0.07	0.25b ± 0.07	0.43a ± 0.08
11	Germination	*wx*[a]	62.8b ± 7.2	54.0b ± 6.9	59.8b ± 6.3	77.9a ± 7.2	73.5ab ± 13.4
11	Emergence	*wx*[a]	66.0a ± 12.8	70.8a ± 14.0	51.7a ± 18.3	80.3a ± 14.3	85.0a ± 9.6
11	Survival	*wx*[a]	67.6ab ± 12.7	58.1ab ± 15.6	29.5b ± 17.5	67.6ab ± 17.9	86.7a ± 12.0
11	Weight (g)	*wx*[a]	0.28ab ± 0.10	0.13b ± 0.07	0.09b ± 0.08	0.24ab ± 0.07	0.35a ± 0.08
12	Germination	*Wx*	85.1a ± 7.2	85.5a ± 6.9	83.9a ± 6.3	89.3a ± 7.2	86.0a ± 13.4
12	Emergence	*Wx*	81.9ab ± 12.9	58.1b ± 13.9	77.1ab ± 18.4	81.9ab ± 14.3	91.4a ± 9.7

12	Survival	*Wx*	78.5ab ± 15.9	54.6b ± 17.1	68.9ab ± 20.6	78.5ab ± 14.5	92.7a ± 6.9
12	Weight (g)	*Wx*	0.26ab ± 0.09	0.21b ± 0.07	0.21b ± 0.07	0.33ab ± 0.07	0.40a ± 0.08
12	Germination	*wx*[a]	69.5b* ± 7.2	76.8ab ± 6.9	83.1a ± 6.2	84.2ab ± 7.2	63.7ab ± 13.3
12	Emergence	*wx*[a]	57.5bc ± 12.8	33.7c ± 13.8	48.0bc ±18.3	76.6ab ± 14.2	90.8a ± 9.5
12	Survival	*wx*[a]	60.5b ± 12.7	31.9b ± 15.6	46.2b ± 17.6	70.0ab* ± 17.9	93.8a ± 12.0
12	Weight (g)	*wx*[a]	0.32ab ±0.09	0.09c ± 0.07	0.16bc ± 0.08	0.37a ± 0.07	0.41a ± 0.08
Lines with *wx*[b] backgrounds[y]							
1	Germination	*Wx*	70.2a ± 5.1	68.2a ± 5.6	73.9a ± 4.2	74.4a ± 5.8	68.0a ± 11.2
1	Emergence	*Wx*	78.0ab ± 14.3	54.2b ± 15.9	59.0b ± 13.7	78.0ab ± 13.1	87.5a ± 9.6
1	Survival	*Wx*	70.1ab ± 15.5	36.8bc ± 15.5	32.0c ± 15.5	70.1ab ± 15.5	79.6a ± 15.5
1	Weight (g)	*Wx*	0.20bc ± 0.06	0.12c ± 0.06	0.15c ± 0.06	0.25b ± 0.06	0.37a ± 0.07
1	Germination	*wx*[b]	89.5a* ± 5.9	85.5a* ± 6.0	80.9a ± 6.9	90.7a* ± 6.3	88.7a ± 5.9
1	Emergence	*wx*[b]	81.5ab ± 14.1	29.1c ± 15.8	57.7bc ± 13.5	81.5ab ±12.9	91.0a ± 9.3
1	Survival	*wx*[b]	74.1a ± 15.3	31.2b ± 15.3	26.4b ± 15.3	74.1a ± 15.3	93.1a ± 15.3
1	Weight (g)	*wx*[b]	0.23ab ± 0.06	0.11b ± 0.07	0.09bc ± 0.09	0.30ac ± 0.09	0.38a ± 0.10
4	Germination	*Wx*	74.1b ± 5.2	78.9ab ± 5.7	72.7b ± 4.2	89.5a ± 5.8	91.6ab ± 11.2

4	Emergence	*Wx*	71.9a ± 14.5	62.4a ± 16.2	57.6a ± 14.0	81.4a ± 13.3	76.7a ± 9.9
4	Survival	*Wx*	51.1a ± 15.8	55.9a ± 15.8	46.3a ± 15.8	60.6a ± 15.8	65.4a ± 15.8
4	Weight (g)	*Wx*	0.05b ± 0.06	0.06b ± 0.06	0.10b ± 0.06	0.13ab ± 0.06	0.24a ± 0.07
4	Germination	*wx*[b]	77.6a ± 6.0	72.1a ± 6.2	76.4a ± 7.0	76.7a ± 6.5	82.2a ± 6.1
4	Emergence	*wx*[b]	79.8a ± 14.4	70.3a ± 16.1	17.9b* ± 13.9	75.0a ± 13.2	94.1a ± 9.8
4	Survival	*wx*[b]	74.5a ± 15.6	65.0a ± 15.6	3.1b* ± 15.6	69.7a ± 15.6	93.5a ± 15.6
4	Weight (g)	*wx*[b]	0.21a* ± 0.07	0.06b ± 0.06	0.00b ± 0.14	0.21ab ± 0.09	0.30a ±0.10
5	Germination	*Wx*	92.6a ± 5.1	89.9a ± 5.6	94.8a ± 4.1	91.3a ± 5.8	59.3b ± 11.2
5	Emergence	*Wx*	77.1ab ± 14.2	58.1b ± 15.8	72.4ab ± 13.6	72.4ab ± 12.9	96.2a ± 9.4
5	Survival	*Wx*	81.8ab ± 15.3	58.0b ± 15.3	58.0b ± 15.3	77.1ab ± 15.3	100.9a ± 15.3
5	Weight (g)	*Wx*	0.26a ± 0.06	0.17ab ± 0.06	0.13b ± 0.06	0.31a ± 0.06	0.30a ± 0.07
5	Germination	*wx*[b]	73.5a* ± 5.9	79.0a ± 6.0	79.5a* ± 6.9	82.9a ± 6.3	69.7a ± 5.9
5	Emergence	*wx*[b]	67.9ab ± 14.1	58.4ab ± 15.8	48.9b ± 13.5	63.2ab ± 12.9	87.0a ± 9.3
5	Survival	*wx*[b]	69.3ab ± 15.3	50.3bc ± 15.3	21.7c ± 15.3	64.5ab ± 15.3	88.4a ± 15.3
5	Weight (g)	*wx*[b]	0.22bc ± 0.06	0.08d ± 0.06	0.09cd ± 0.09	0.40a ± 0.09	0.42ab ± 0.10
7	Germination	*Wx*	85.3a ± 5.1	85.5a ± 5.6	87.2a ± 4.1	91.9a ± 5.7	97.8a ± 11.2

7	Emergence	*Wx*	63.8ab ± 14.2	49.5b ± 15.8	78.1ab ± 13.6	68.6ab ± 12.9	92.4a ± 9.4
7	Survival	*Wx*	47.1b ± 15.3	28.1b ± 15.3	51.9b ± 15.3	47.1b ± 15.3	90.0a ± 15.3
7	Weight (g)	*Wx*	0.05b ± 0.06	0.09ab ± 0.06	0.10ab ± 0.06	0.09ab ± 0.06	0.20a ± 0.07
7	Germination	wx^b	74.8a ± 5.9	69.7a* ± 6.0	78.7a ± 6.9	75.3a* ± 6.3	70.5a* ± 5.9
7	Emergence	wx^b	41.6ab ± 14.2	17.8b ± 15.8	41.6ab ± 13.6	41.6ab ± 12.9	51.1a* ± 9.4
7	Survival	wx^b	30.2a ± 15.3	11.2a ± 15.3	15.9a ± 15.3	20.7a ± 15.3	30.2a* ± 15.3
7	Weight (g)	wx^b	0.14ab ± 0.06	0.08b ± 0.07	0.13ab ± 0.09	0.14ab ± 0.09	0.33a ±0.10
8	Germination	*Wx*	83.7a ± 5.1	85.2a ± 5.6	80.3a ± 4.1	84.8a ± 5.8	72.2a ± 11.2
8	Emergence	*Wx*	75.1a ± 14.3	75.1a ±15.9	79.9a ± 13.7	84.6a ± 13.0	60.0a ± 9.5
8	Survival	*Wx*	69.4ab ± 15.4	50.4ab ± 15.4	36.1b ± 15.4	78.9a ± 15.4	54.3ab ± 15.4
8	Weight (g)	*Wx*	0.19ab ± 0.06	0.09b ±0.06	0.12ab ± 0.06	0.16ab ± 0.06	0.23a ± 0.07
8	Germination	wx^b	86.1a ± 5.9	88.7a ± 6.0	79.9a ± 6.9	80.9a ± 6.3	87.2a ± 5.9
8	Emergence	wx^b	66.9a ± 14.1	9.7b* ± 15.8	62.1a ±13.5	71.6a ± 12.9	85.9a* ± 9.3
8	Survival	wx^b	61.0ab ± 15.3	3.9c* ± 15.3	37.2bc ± 15.3	61.0ab ± 15.3	80.1a ± 15.3
8	Weight (g)	wx^b	0.19ab ± 0.06	0.06b ± 0.07	0.11ab ± 0.09	0.19ab ± 0.09	0.29a ± 0.10
9	Germination	*Wx*	86.3a ± 5.2	92.9a ± 5.7	92.9a ± 4.2	95.1a ± 5.8	94.2a ± 11.2

9	Emergence	*Wx*	62.2a ± 14.5	57.4a ± 16.1	66.9a ± 13.9	71.7a ± 13.3	86.0a ± 9.9
9	Survival	*Wx*	59.8a ± 15.7	35.9a ± 15.7	35.9a ± 15.7	50.2a ± 15.7	69.3a ± 15.7
9	Weight (g)	*Wx*	0.14ab ± 0.06	0.10ab ± 0.06	0.06b ± 0.06	0.18a ± 0.06	0.18a ± 0.07
9	Germination	*wx*[b]	76.6a ± 5.9	68.5a* ± 6.1	73.4a* ± 6.9	74.7a* ± 6.4	57.9a* ± 5.9
9	Emergence	*wx*[b]	66.8ab ± 14.2	47.8b ± 15.9	76.3ab ± 13.6	81.1ab ± 13.0	90.6a ± 9.4
9	Survival	*wx*[b]	70.6ab ± 15.4	42.0b ± 15.4	56.3ab ± 15.4	70.6ab ± 15.4	89.6a ± 15.4
9	Weight (g)	*wx*[b]	0.23b ± 0.06	0.13b ± 0.06	0.35a* ± 0.09	0.28ab ± 0.09	0.49a* ± 0.10
Wild-type cultivars[z]							
RTx430	Germination	*Wx*	63.0a ± 6.6	63.2a ± 6.7	70.4a ± 6.0	60.8a ± 7.4	56.5a ± 11.5
RTx430	Emergence	*Wx*	85.4a ± 17.1	52.1a ± 17.1	61.6a ± 17.1	85.4a ± 17.1	80.7a ± 17.1
RTx430	Survival	*Wx*	83.2a ± 18.1	45.1a ± 18.1	35.5a ± 18.1	83.2a ± 18.1	73.6a ± 18.1
RTx430	Weight (g)	*Wx*	0.26a ± 0.06	0.13bc ± 0.07	0.08c ± 0.06	0.22ab ± 0.06	0.32a ± 0.06
Wheatland	Germination	*Wx*	92.1a ± 6.5	90.7a ± 6.6	90.7a ± 6.0	91.4a ± 7.5	84.4a ± 11.5
Wheatland	Emergence	*Wx*	86.1a ± 17.1	57.6a ± 17.1	52.8a ± 17.1	76.6a ± 17.1	90.9a ± 17.1
Wheatland	Survival	*Wx*	53.7a ± 18.8	53.7a ± 18.8	39.4a ± 18.8	53.7a ± 18.8	82.3a ± 18.8
Wheatland	Weight (g)	*Wx*	0.15b ± 0.07	0.15b ± 0.07	0.19b ± 0.08	0.15b ± 0.07	0.44a ± 0.07

[t]Grain from wild-type and near isogenic *wx*^{a} or *wx*^{b} lines were incubated in suspensions of conidia, sterile water, or were germinated without treatment. Number of germinated seeds was determined. A subset of germinated seeds were grown for three more weeks in soil at which time number of emerged plants and surviving plants were determined. Fresh weights were also determined and analyzed by total weights for row, then divided by number of surviving plants to determine mean weight per plant.

[u]Line pairs are from the same genetic background, segregating for the indicated *waxy* allele (See Table 2.).

[v]Data are from grain of six near-isogenic line pairs in backgrounds with two different sources of *wx*^{a} gene (1) (present work). Data were analyzed using the PROC MIXED procedure of SAS/STAT software ® (SAS 2002-2008).

[w]Least Squares Means and Standard Errors are shown. Mean separations are for response to four treatments or untreated control, within a genotype and for a particular assessment. Means with different letters are significantly different at $P \leq 0.05$.

[x]Asterisk indicates that mean for waxy genotype for the particular assessment and treatment, or untreated control, is significantly different than corresponding wild-type mean ($P \leq 0.05$).

[y]Data are from grain of six near-isogenic line pairs in backgrounds with two different sources of *wx*^{b} gene (1)

[z]Wild-type cultivars RTx430 and Wheatland were included in grain inoculation assay but analyzed separately. The means are presented here as further information for the reader.

1. Yerka , M. K., Toy, J. J., Funnell-Harris, D. L., Sattler, S. E., and Pedersen, J. F. 2015. Registration of N619 to N640 grain sorghum lines with waxy or wild-type endosperm. J. Plant Reg. 9:(in press).

Supplementary Table 3. Comparison of grain within near-isogenic waxy sorghum line pairs with *wx*^{a} [absence (-) of the granule bound starch synthase protein in starch granules (GBSS)] or *wx*^{b} (GBSS^{+}) alleles, the *wx*^{a} and *wx*^{b} parents, and lines with wild-type (*Wx*) grain, following seed inoculations with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia or sterile water and untreated controls as determined by mean percent germination, emergence and survival, and mean fresh weight (g).[w]

Line pair[x]/			Conidial treatments			Controls	
Cultivar	Assessment	Genotype	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
Waxy near-isogenic line pairs							
1	Germination	*wx*^{a}	93.8a[y] ± 2.6	93.7a ± 2.6	97.9a ± 2.6	92.3a ± 2.6	95.1a ± 2.6
1	Emergence	*wx*^{a}	76.2a ± 12.1	57.1a ± 23.3	85.7a ± 14.4	100.0a ± 10.9	90.5a ± 10.1
1	Survival	*wx*^{a}	61.9ab ± 19.2	57.1b ± 19.5	76.2ab ± 15.0	100.0a ± 14.0	85.7ab ± 13.1
1	Weight (g)	*wx*^{a}	0.27b ± 0.05	0.21b ± 0.05	0.27b ± 0.05	0.41ab ± 0.05	0.46a ± 0.05
1	Germination	*wx*^{b}	92.2a ± 1.9	94.4a ± 1.9	93.2a ± 1.9	96.4a ± 1.9	92.4a ± 1.9
1	Emergence	*wx*^{b}	85.7a ± 12.1	52.4a ± 23.3	76.2a ± 14.4	85.7a ± 10.9	85.7a ± 10.1
1	Survival	*wx*^{b}	76.2ab ± 19.2	47.6b ± 19.5	66.7ab ± 15.0	76.2ab ± 14.0	85.7a ± 13.1
1	Weight (g)	*wx*^{b}	0.27bc ± 0.05	0.16c ± 0.06	0.23bc ± 0.05	0.33ab ± 0.05	0.47a ± 0.05
2	Germination	*wx*^{a}	88.6a ± 2.3	88.4a ± 2.3	91.8a ± 2.3	91.0a ± 2.3	89.1a ± 2.3

2	Emergence	*wx*[a]	67.7a ± 23.4	47.6a ± 17.9	52.4a ± 22.0	66.7a ± 9.0	71.4a ± 10.3
2	Survival	*wx*[a]	61.9a ± 19.2	38.1ab ± 19.5	33.3b ± 15.0	66.7a ± 14.0	66.7a ± 13.1
2	Weight (g)	*wx*[a]	0.28a ± 0.06	0.08b ± 0.05	0.09b ± 0.05	0.28a ± 0.05	0.33a ± 0.05
2	Germination	*wx*[b]	87.6a ± 4.2	89.7a ± 4.2	88.8a ± 4.2	87.2a ± 4.2	90.6a ± 4.2
2	Emergence	*wx*[b]	71.4a ± 23.4	52.4a ± 17.9	57.1a ± 22.0	90.5a ± 9.1	90.5a ± 10.3
2	Survival	*wx*[b]	66.7ab ± 19.2	28.6b ± 19.5	33.3b ± 15.0	81.0a ± 14.0	90.5a ± 13.1
2	Weight (g)	*wx*[b]	0.29a ± 0.05	0.09b ± 0.05	0.16b ± 0.05	0.31a ± 0.05	0.35a ± 0.05
3	Germination	*wx*[a]	83.6a ± 7.0	83.0a ± 7.0	87.1a ± 7.0	85.0a ± 7.0	83.2a ± 7.0
3	Emergence	*wx*[a]	81.0a ± 13.5	66.7a ± 20.0	71.4a ± 13.6	90.5a ± 8.6	90.5a ± 9.8
3	Survival	*wx*[a]	76.2ab ± 19.2	61.9ab ± 19.4	57.1b ± 15.0	85.7ab ± 14.0	85.7a ± 13.1
3	Weight (g)	*wx*[a]	0.35ab ± 0.05	0.21b ± 0.05	0.25b ± 0.05	0.44a ± 0.05	0.43a ± 0.05
3	Germination	*wx*[b]	95.1a ± 2.0	91.3ab ± 2.0	88.8b ± 2.0	88.5b ± 2.0	93.2ab ± 2.0
3	Emergence	*wx*[b]	95.2ab ± 13.5	71.4ab ± 20.0	61.9b ± 13.6	95.2a ± 8.6	100.0a ± 9.8
3	Survival	*wx*[b]	76.2ab ± 19.2	66.7ab ± 19.5	47.6b ± 15.0	85.7a ± 14.0	95.2a ± 13.1
3	Weight (g)	*wx*[b]	0.32ab ± 0.05	0.18bc ± 0.05	0.17c ± 0.05	0.33a ± 0.05	0.44a ± 0.05

Waxy parent lines[z]

BTx630	Germination	*wx*[a]	89.3b ± 2.5	90.4ab ± 2.5	94.6ab ± 2.5	97.3a ± 2.5	93.2ab ± 2.5
BTx630	Emergence	*wx*[a]	48.4bc ± 17.4	57.9abc ± 17.8	43.7c ± 16.5	77.1ab ± 13.1	86.7a ± 11.9
BTx630	Survival	*wx*[a]	41.9bc ± 19.4	41.9bc ± 18.2	32.4c ± 15.1	70.5ab ± 16.8	80.2a ± 13.8
BTx630	Weight (g)	*wx*[a]	0.31ab ± 0.07	0.12c ± 0.04	0.23bc ± 0.06	0.33ab ± 0.05	0.45a ± 0.05
BTxAGR1	Germination	*wx*[b]	85.1ab ± 3.7	92.3a ± 3.7	95.8a ± 3.7	92.6a ± 3.7	80.9b ± 3.7
BTxAGR1	Emergence	*wx*[b]	62.5a ± 18.3	67.2a ± 18.6	67.2a ± 17.3	62.5a ± 14.1	76.7a ± 13.1
BTxAGR1	Survival	*wx*[b]	50.3a ± 20.5	54.9a ± 19.4	55.0a ± 16.4	50.3a ± 17.9	73.9a ± 15.4
BTxAGR1	Weight (g)	*wx*[b]	0.28abc ± 0.06	0.14c ± 0.05	0.23bc ± 0.06	0.31ab ± 0.05	0.39a ± 0.05
Wild-type lines[z]							
RTx430	Germination	*Wx*	60.4a ± 8.2	60.0a ± 8.2	64.9a ± 8.2	59.0a ± 8.2	55.9a ± 8.2
RTx430	Emergence	*Wx*	77.1ab ± 18.0	67.5b ± 18.4	77.3ab ± 17.1	86.7ab ± 13.8	101.0a ± 12.8
RTx430	Survival	*Wx*	60.3ab ± 20.2	31.7bc ± 19.1	17.3c ± 16.1	60.4ab ± 17.6	84.1a ± 15.1
RTx430	Weight (g)	*Wx*	0.21a ± 0.04	0.05b ± 0.04	0.01b ± 0.07	0.24a ± 0.07	0.32a ± 0.07
Wheatland	Germination	*Wx*	87.1a ± 3.3	85.5a ± 3.3	81.4a ± 3.3	90.4a ± 3.3	89.6a ± 3.3
Wheatland	Emergence	*Wx*	72.3a ± 17.5	57.9a ± 18.0	58.0a ± 16.6	72.3a ± 13.2	81.8a ± 12.1
Wheatland	Survival	*Wx*	56.1a ± 19.5	41.7a ± 18.4	46.5a ± 15.2	65.6a ± 16.9	79.8a ± 14.1

Wheatland	Weight (g)	*Wx*	0.25b ± 0.04	0.18b ± 0.04	0.31ab ± 0.07	0.41ab ± 0.07	0.49a ± 0.07

[w]Grain from near isogenic *wx*^{a} and *wx*^{b} lines, parents and wild-type lines were incubated in suspensions of conidia, sterile water, or were germinated without treatment. Number of germinated grains was determined. A subset of germinated grains was grown for three more weeks in soil at which time number of emerged plants and surviving plants were determined. Fresh weights were also determined and analyzed by total weights for row, then divided by number of surviving plants to determine mean weight per plant.

[x] Line pairs are from the same genetic background, segregating for the indicated *waxy* allele (see Materials and Methods).

[y]Data were analyzed using the PROC MIXED procedure of SAS/STAT software ® (SAS 2002-2008). Least Squares Means and Standard Errors are shown. Mean separations are for response to four treatments or untreated control, within a genotype and for a particular assessment. Means with different letters are significantly different at $P \leq 0.05$.

[z]*waxy* parents and wild-type lines were analyzed separately from near-isogenic line pairs. Means are presented as further information for the reader.

Supplementary Table 4. Waxy phenotype, genotype, presence or absence of Granule Bound Starch Synthase (GBSS$^{+/-}$), and percent internal fungal infection of grain from Plant Introductions and *waxy* and wild-type lines.

				Percent infection as determined on:[z]		
Accession[v]	Phenotype[w]	Genotype[x]	GBSS[y]	DCPA	PCNB	DRBC
*PI 23231	waxy	unk	+	8.0	4.2	5.4
PI 55123	waxy	*wx*^{a}	-	9.4	4.0	12.6
*PI 76407	waxy	*wx*^{a}	-	5.3	3.7	3.1
*PI 82340	waxy	*wx*^{a}	-	2.5	3.9	3.1
*PI 87355	waxy	*wx*^{a}	-	5.3	3.9	5.4
PI 88004	waxy	*wx*^{a}	-	6.1	3.7	4.3
*PI 173971	wild-type	*Wx*	+	8.9	3.7	10.4
PI 175316	wild-type	*Wx*	+	2.5	3.7	3.1
PI 192876	waxy	*wx*^{a}	-	11.6	3.7	15.4
*PI 217897	waxy	unk	+	2.5	3.9	3.1
PI 220636	waxy	*wx*^{a}	-	11.6	3.7	18.2
PI 234456	waxy	*wx*^{a}	-	11.6	3.9	8.7
PI 246699	wild-type	*Wx*	+	3.9	3.7	3.1
PI 250230	wild-type	*Wx*	+	23.5	5.2	26.5
*PI 455543	waxy	*wx*^{a}	-	5.3	3.9	8.7
*PI 547915	wild-type	*Wx*	+	2.5	3.7	3.1
*PI 548008	waxy	*wx*^{a}	-	7.5	3.7	9.3
*PI 562758	waxy	unk	-	15.8	8.7	23.2

*PI 563015	waxy	*wx*^{a}	-	3.9	3.7	7.6
*PI 563068	waxy	*wx*^{a}	-	51.3	5.4	26.5
*PI 563402	waxy	*wx*^{a}	-	31.6	9.2	21.5
*PI 563576	waxy	*wx*^{a}	-	5.3	3.7	5.4
PI 563611	wild-type	*Wx*	+	19.0	4.8	16.0
PI 563612	wild-type	*Wx*	+	40.4	5.7	24.3
*PI 563670	waxy	*wx*^{b}	+	41.6	4.6	26.0
*PI 563671	waxy	*wx*^{b}	+	57.5	14.8	33.2
*PI 563672	wild-type	*Wx*	+	12.1	4.8	17.6
*PI 565116	wild-type	*Wx*	+	31.6	10.4	48.7
PI 567796	waxy	*wx*^{a}	-	6.1	3.7	10.9
PI 567803	waxy	*wx*^{a}	-	2.5	3.7	3.1
PI 567809	waxy	*wx*^{a}	-	6.1	3.7	10.9
*PI 567811	waxy	*Wx*^{a}	-	12.1	4.0	12.1
*PI 567910	wild-type	*Wx*	+	3.9	3.7	3.1
PI 567913	wild-type	*Wx*	+	13.9	3.9	12.6
*PI 567931	wild-type	*Wx*	+	9.4	4.0	11.5
PI 567939	wild-type	*Wx*	+	3.9	3.7	4.3
PI 568012	wild-type	*Wx*	+	2.5	3.7	4.3
*PI 586448	waxy	*wx*^{a}	-	7.5	3.7	9.3
PI 586454	waxy	*wx*^{a}	-	24.7	8.5	16.5
*PI 586524	waxy	*wx*^{a}	-	7.5	3.7	5.4
PI 586526	waxy	*wx*^{a}	-	8.9	3.9	5.4

PI 586529	waxy	*wx*[a]	-	16.6	3.7	11.0
Known *waxy* line						
*PI667647	waxy	*wx*[a]	-	11.6	4.0	10.4
Known wild-type lines						
*PI 586537	wild-type	*Wx*	+	8.9	8.5	12.0
*NSL 4346	wild-type	*Wx*	+	2.5	3.7	3.1
*PI 651495	wild-type	*Wx*	+	3.9	4.2	3.1
*Grif 652	wild-type	*Wx*	+	3.9	3.9	3.1
*PI 217672	wild-type	*Wx*	+	3.9	3.7	3.1
*PI 586540	wild-type	*Wx*	+	2.5	3.7	3.1
*PI 535783	wild-type	*Wx*	+	10.8	4.0	14.3
*PI 641836	wild-type	*Wx*	+	2.5	3.9	5.4
*PI 653616	wild-type	*Wx*	+	6.1	3.7	4.3

[v]PIs and other lines listed were utilized in a two year field study, with four replicated plots per year, at Mead, NE. NSL 4346 is maintained at National Center for Genetic Resources Preservation, Fort Collins, CO, while all others are maintained at Plant Genetic Resources Conservation Unit, Griffin, GA. Lines with asterisks were used in grain inoculation study (Supplementary Table 5).

[w]Grain phenotype was determined in previous studies using an iodine staining method.

[x] *Wx* is the wild-type allele. *wx*[a] and *wx*[b] alleles were determined in the current study using allele specific primers (*wx*[a]) or Cleavage Amplified Polymorphic Sequence marker (*wx*[b]). Those indicated with "unk," for "unknown," had the wild-type-sized band for both markers.

[y]Presence of the GBSS protein in starch granules was determined in previous studies. For $GBSS^+$ lines, starch granules in wild-type lines contain functional GBSS and *waxy* lines have partially active GBSS. $GBSS^-$ lines lack the protein in starch granules.

[z]Field-grown grain was surface disinfested, dried and plated onto three semi-selective media. Dicholoran chloramphenicol peptone agar (DCPA) is semi-selective for *Fusarium* spp., and *Alternaria* spp. and other dark-spored ascomycetes. Pentachloronitrobenzene (PCNB) agar is semi-selective for *Fusarium* spp. Dichloran rose bengal chloramphenicol (DRBC) agar is a general purpose fungal medium. Each grain with fungal growth was counted to determine percent infection. Least squares means are shown. Standard errors for DCPA are 7.5, for PCNB, 4.4 and for DRBC, 7.8.

Supplementary Table 5. Response of sorghum grain from *waxy* and wild-type plant introductions (PI) following seed inoculations with *Alternaria* sp., *Curvularia sorghina* or *Fusarium thapsinum* conidia or sterile water and untreated controls as determined by mean percent germination, emergence and survival, and mean fresh weight (g).[y]

			Conidial treatments			Controls	
Line[z]	Assessment	Phenotype	*Alternaria* sp.	*C. sorghina*	*F. thapsinum*	Water	Untreated
PI 23231	Germination	waxy	60.5	53.6	57.6	61.3	61.3
PI 23231	Emergence	waxy	71.4	61.8	52.3	61.8	85.7
PI 23231	Survival	waxy	69.5	59.9	45.6	59.9	83.7
PI 23231	Weight (g)	waxy	0.50	0.46	0.25	0.33	0.49
PI 76407	Germination	waxy	71.6	81.4	83.0	91.7	92.8
PI 76407	Emergence	waxy	68.8	59.3	68.8	68.8	83.1
PI 76407	Survival	waxy	59.8	55.0	64.5	64.5	78.8
PI 76407	Weight (g)	waxy	0.33	0.37	0.24	0.58	0.68
PI 82340	Germination	waxy	77.7	80.4	78.9	81.6	79.6
PI 82340	Emergence	waxy	47.7	62.0	52.5	47.7	62.0

PI 82340	Survival	waxy	39.4	39.4	39.4	39.4	68.0
PI 82340	Weight (g)	waxy	0.38	0.26	0.34	0.21	0.41
PI 87355	Germination	waxy	48.7	59.2	52.3	60.9	69.6
PI 87355	Emergence	waxy	45.2	54.7	49.9	64.2	78.5
PI 87355	Survival	waxy	38.9	43.6	34.1	53.1	72.2
PI 87355	Weight (g)	waxy	0.30	0.29	0.00	0.21	0.29
PI 173971	Germination	wild-type	72.4	85.2	85.1	83.2	82.7
PI 173971	Emergence	wild-type	47.3	66.3	75.8	85.4	90.1
PI 173971	Survival	wild-type	45.5	45.5	55.1	50.3	69.3
PI 173971	Weight (g)	wild-type	0.31	0.28	0.33	0.26	0.41
PI 217897	Germination	waxy	50.2	54.4	55.0	61.3	63.1
PI 217897	Emergence	waxy	59.6	59.6	78.6	59.6	59.6
PI 217897	Survival	waxy	51.3	56.1	56.1	56.1	56.1
PI 217897	Weight (g)	waxy	0.34	0.38	0.22	0.31	0.39
PI 455543	Germination	waxy	90.9	94.2	88.6	88.1	91.3
PI 455543	Emergence	waxy	77.8	96.9	87.4	87.4	73.1

PI 455543	Survival	waxy	59.3	64.1	49.8	87.9	78.4
PI 455543	Weight (g)	waxy	0.32	0.25	0.24	0.57	0.69
PI 547915	Germination	wild-type	61.3	64.8	74.1	60.8	71.1
PI 547915	Emergence	wild-type	49.7	59.3	68.8	78.3	64.0
PI 547915	Survival	wild-type	36.0	50.3	40.7	69.3	64.5
PI 547915	Weight (g)	wild-type	0.23	0.24	0.10	0.32	0.37
PI 562758	Germination	waxy	95.0	95.8	94.4	97.8	93.5
PI 562758	Emergence	waxy	59.3	40.2	64.0	83.1	73.5
PI 562758	Survival	waxy	40.7	7.4	50.3	78.8	55.0
PI 562758	Weight (g)	waxy	0.37	0.26	0.43	0.47	0.47
PI 563015	Germination	waxy	89.7	84.5	92.9	90.4	90.1
PI 563015	Emergence	waxy	68.8	54.5	54.5	68.8	68.8
PI 563015	Survival	waxy	59.8	50.3	40.7	59.8	69.3
PI 563015	Weight (g)	waxy	0.09	0.06	0.00	0.09	0.15
PI 563068	Germination	waxy	59.2	63.6	65.7	64.4	42.7
PI 563068	Emergence	waxy	63.3	53.8	58.5	53.8	77.6

PI 563068	Survival	waxy	45.2	45.2	45.2	54.7	69.0
PI 563068	Weight (g)	waxy	0.33	0.25	0.31	0.44	0.35
PI 563402	Germination	waxy	86.7	84.4	86.5	90.4	92.9
PI 563402	Emergence	waxy	55.3	22.0	55.3	69.6	103.0
PI 563402	Survival	waxy	41.3	17.4	41.3	65.1	79.4
PI 563402	Weight (g)	waxy	0.24	0.10	0.31	0.43	0.44
PI 563576	Germination	waxy	58.1	57.6	58.1	56.1	74.6
PI 563576	Emergence	waxy	76.1	66.6	66.6	76.1	80.9
PI 563576	Survival	waxy	55.2	50.4	55.2	69.5	64.7
PI 563576	Weight (g)	waxy	0.25	0.20	0.27	0.28	0.35
PI 568670	Germination	waxy	39.3	43.5	57.6	50.5	53.7
PI 568670	Emergence	waxy	63.6	63.6	49.4	63.6	73.2
PI 568670	Survival	waxy	57.4	47.9	28.8	57.4	66.9
PI 568670	Weight (g)	waxy	0.32	0.25	0.17	0.56	0.51
PI 563671	Germination	waxy	63.1	64.8	69.6	75.2	78.6
PI 563671	Emergence	waxy	58.9	35.1	30.3	58.9	68.4

PI 563671	Survival	waxy	52.6	24.1	24.1	43.1	47.9
PI 563671	Weight (g)	waxy	0.17	0.00	0.09	0.00	0.37
PI 563672	Germination	wild-type	41.0	59.8	53.3	48.2	55.4
PI 563672	Emergence	wild-type	51.7	70.8	66.0	75.6	94.6
PI 563672	Survival	wild-type	45.2	64.2	40.4	69.0	88.0
PI 563672	Weight (g)	wild-type	0.38	0.39	0.22	0.45	0.59
PI 565116	Germination	wild-type	81.7	88.9	87.7	79.9	61.5
PI 565116	Emergence	wild-type	82.2	67.9	63.2	87.0	101.3
PI 565116	Survival	wild-type	66.5	52.2	61.7	80.8	99.8
PI 565116	Weight (g)	wild-type	0.52	0.30	0.47	0.45	0.64
PI 567811	Germination	waxy	87.7	93.0	88.7	93.9	92.5
PI 567811	Emergence	waxy	69.6	50.6	50.6	45.8	69.6
PI 567811	Survival	waxy	46.0	50.8	41.3	41.3	65.1
PI 567811	Weight (g)	waxy	0.20	0.59	0.24	0.30	0.47
PI 567910	Germination	wild-type	82.0	83.4	83.9	85.8	89.2
PI 567910	Emergence	wild-type	72.1	81.6	72.1	62.6	67.3

PI 567910	Survival	wild-type	60.8	70.3	51.2	60.8	60.8
PI 567910	Weight (g)	wild-type	0.35	0.40	0.36	0.44	0.55
PI 567931	Germination	wild-type	33.5	46.9	49.2	40.6	44.3
PI 567931	Emergence	wild-type	74.4	55.3	50.6	60.1	74.4
PI 567931	Survival	wild-type	69.8	41.3	36.5	55.5	74.6
PI 567931	Weight (g)	wild-type	0.56	0.35	0.22	0.56	0.86
PI 586524	Germination	waxy	79.4	85.9	85.8	77.5	88.8
PI 586524	Emergence	waxy	81.6	76.9	57.8	81.6	91.2
PI 586524	Survival	waxy	56.0	65.5	41.7	60.8	75.0
PI 586524	Weight (g)	waxy	0.58	0.51	0.35	0.45	0.73
Known *waxy* line							
PI 667647	Germination	waxy	56.1	56.0	53.9	55.9	64.2
PI 667647	Emergence	waxy	40.5	31.0	45.3	59.6	64.4
PI 667647	Survival	waxy	27.5	27.5	37.1	51.3	41.8
PI 667647	Weight (g)	waxy	0.15	0.16	0.22	0.33	0.30
Known wild-type lines							

PI 586537	Germination	wild-type	91.2	86.6	91.4	91.9	94.8
PI 586537	Emergence	wild-type	52.5	47.7	62.0	66.7	76.3
PI 586537	Survival	wild-type	48.9	29.9	58.5	63.2	63.2
PI 586537	Weight (g)	wild-type	0.30	0.17	0.37	0.39	0.32
NSL 4346	Germination	wild-type	69.9	71.3	79.3	77.3	79.8
NSL 4346	Emergence	wild-type	79.6	65.3	55.8	84.3	79.6
NSL 4346	Survival	wild-type	73.2	68.5	49.4	78.0	63.7
NSL 4346	Weight (g)	wild-type	0.30	0.36	0.25	0.30	0.30
PI 651495	Germination	wild-type	74.7	68.9	59.7	69.5	71.0
PI 651495	Emergence	wild-type	74.5	55.5	74.5	74.5	74.5
PI 651495	Survival	wild-type	54.1	35.0	54.1	82.7	77.9
PI 651495	Weight (g)	wild-type	0.31	0.24	0.26	0.42	0.42
Grif 652	Germination	wild-type	89.9	86.5	87.8	85.7	88.2
Grif 652	Emergence	wild-type	78.5	92.8	83.3	83.3	92.8
Grif 652	Survival	wild-type	67.4	62.7	62.7	67.4	81.7
Grif 652	Weight (g)	wild-type	0.52	0.08	0.33	0.47	0.47

PI 217672	Germination	wild-type	93.6	93.2	93.8	92.5	83.9
PI 217672	Emergence	wild-type	98.3	93.6	69.8	84.0	107.9
PI 217672	Survival	wild-type	92.2	82.7	63.6	92.2	82.7
PI 217672	Weight (g)	wild-type	0.44	0.31	0.38	0.48	0.37
PI 586540	Germination	wild-type	95.8	90.8	94.5	93.9	93.8
PI 586540	Emergence	wild-type	93.2	64.6	69.4	74.1	74.1
PI 586540	Survival	wild-type	46.9	51.7	51.7	46.9	37.4
PI 586540	Weight (g)	wild-type	0.22	0.29	0.31	0.22	0.22
PI 535783	Germination	wild-type	47.9	47.8	44.2	48.5	51.2
PI 535783	Emergence	wild-type	43.6	53.1	62.6	76.9	91.2
PI 535783	Survival	wild-type	37.4	46.9	56.5	70.8	89.8
PI 535783	Weight (g)	wild-type	0.47	0.28	0.27	0.54	0.54
PI 641836	Germination	wild-type	76.7	68.3	71.9	65.4	76.3
PI 641836	Emergence	wild-type	77.8	77.8	58.8	73.1	96.9
PI 641836	Survival	wild-type	59.3	49.8	35.5	73.6	87.9
PI 641836	Weight (g)	wild-type	0.31	0.22	0.13	0.39	0.42

PI 653616	Germination	wild-type	64.8	63.1	65.0	67.8	81.0
PI 653616	Emergence	wild-type	75.9	61.6	47.3	47.3	52.1
PI 653616	Survival	wild-type	75.1	60.9	46.6	41.8	41.8
PI 653616	Weight (g)	wild-type	0.38	0.35	0.36	0.34	0.33

[y]Grain from PIs were incubated in suspensions of conidia, sterile water, or were germinated without treatment. Number of germinated grains was determined. A subset of germinated grains was grown for three more weeks in soil at which time number of emerged plants and surviving plants were determined. Fresh weights were also determined and analyzed by total weights for row, then divided by number of surviving plants to determine mean weight per plant.

[z]NSL 4346 is maintained at National Center for Genetic Resources Preservation, Fort Collins, CO, while all others are maintained at Plant Genetic Resources Conservation Unit, Griffin, GA.