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Evaluation of sorghum germplasm used in US breeding programmes for sources of sugary disease resistance*

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Ergot or sugary disease of sorghum has become an important constraint in North and South American countries that rely on F_1 hybrid seeds for high productivity. The objective of this research was to determine the vulnerability of various germplasm sources and publicly bred sorghum lines to sugary disease (*Claviceps africana*) in the United States. Flower characteristics associated with sugary disease resistance were also studied. A-/B-line pairs, R-lines, putative sources of resistance and their hybrid combinations with an A_3 cytoplasmic male-sterile source were evaluated using a disease incidence, severity, and dual-ranking system. Trials were planted in a randomized complete block design with three replications and repeated in at least two planting dates. Planting dates and pedigrees had significant effects on overall ranking for resistance. A-lines were most susceptible to sugary disease. R-lines were more susceptible than B-lines with respect to incidence and severity of the disease. Newer releases of A- and B-lines were more susceptible to sugary disease than older releases. Sugary disease reaction of A-lines was a good indicator of disease reaction of B-lines. Tx2737, a popular R-line, was highly susceptible to sugary disease in spite of being a good pollen shedder because the stigma emerged from glumes 2–3 days before anthesis. The combination of flower characteristics associated with resistance were least exposure time of stigma to inoculum before pollination, rapid stigma drying after pollination, and small stigma. An Ethiopian male-fertile germplasm accession, IS 8525, had good levels of resistance. Its A_3 male-sterile hybrid had the highest level of resistance in the male-sterile background. IS 8525 should be exploited in host-plant resistance strategies.

Keywords: *Claviceps africana*, ergot, germplasm, honeydew, resistance, sorghum

Introduction

Sorghum (*Sorghum bicolor*) is the fifth most important cereal crop in the world. Its estimated farm value to the United States agricultural economy was $2·2 billion and $1·7 billion in 1996 and 1997, respectively [Tim Lust, Executive Director, National Grain Sorghum Producers (NGSP), personal communication, 1999]. Ergot or sugary disease is highly destructive on sorghum and formerly subject to quarantine regulations in Australia and countries in North America and South America, since the disease was initially restricted to Asia and Africa. *Claviceps africana*, the ergot pathogen initially described from Zimbabwe (Frederickson *et al*., 1991), was first reported on sorghum in the Americas in Brazil in 1995 (Reis *et al*., 1996). Since then, the pathogen has moved rapidly throughout South America, and northwards to Central America, the Caribbean and continental USA (Bandyopadhyay *et al*., 1998). *Claviceps africana* was also reported from Australia in 1996 (Ryley *et al*., 1996). Estimates of the cost of the disease to the sorghum seed industry vary greatly but current information suggests increases of $5·00–6·00 a bag (22·7 kg) to cover additional chemical controls, cultural practices and processing (Tim Lust, NGSP, personal communication, 1999).

Conidia of the anamorph (*Sphacelia sorghi*) infect through the stigma and replace the ovary with a soft fungal mass, the sphacelium. A spore-laden sweet fluid, called honeydew, exudes from the sphacelium.

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Honeydew-smeared grains become sticky and are difficult to combine-harvest. Infected panicles produce poor quality seed, discoloured by fungal saprophytes growing on the honeydew. Later, a hard sclerotium develops under appropriate environmental conditions.

The susceptible period for infection of florets begins when stigmas are exposed to inoculum and ends when fertilization occurs. A short susceptible period can contribute to reduction in sugary disease risk of sorghum accessions (Bandyopadhyay *et al.*, 1998). The structure of flower components and the flowering process may influence the duration of the susceptible period by affecting the time interval between stigma exposure to inoculum and fertilization of ovary. Flowering behaviour of sorghum has been described in detail (Stephens & Quinby, 1934; Quinby, 1958). The sorghum inflorescence is a raceme consisting of several spikelets or flowers. In a sessile spikelet, stamen and pistil are enclosed within an accessory of inner membranaceous lemmas and paleas that are normally completely covered by outer and inner glumes. The apical part of the glume of some sorghum accessions possesses a bristle-like awn. Anthesis occurs basipetally. During anthesis, the glumes open widely at night or early in the morning, anthers and stigmas emerge, anthers shed pollen to pollinate stigma, and later the glumes close, leaving the anthers and stigma hanging out. Rapid pollination and fertilization preclude infection and colonization (Bandyopadhyay *et al.*, 1998). However, the flowering process and the structure of flowers vary considerably in different sorghum accessions.

Hybrid seed production is most vulnerable to the disease since the ergot pathogen attacks unfertilized ovaries. Epidemics may occur when either nonsynchronous flowering of male-sterile lines (A-lines) and restorer lines (R-lines) or adverse environmental conditions result in poor pollen shedding, reduced pollen viability, and delayed seed set (Bandyopadhyay *et al.*, 1998). Due to the recent entry of sugary disease into the USA, the vulnerability to the disease is not known for seed parents used widely for F_1 hybrid development and seed production.

Disease evaluation techniques and artificial inoculation techniques have been developed to screen germplasm for resistance to sugary disease in the field and greenhouse (Sundaram, 1970, 1971; Khadke *et al.*, 1978; McLaren, 1992; Musabyimana *et al.*, 1995). Tegegne *et al.* (1994) evaluated inoculation techniques and compared quantitative and visual assessment methods to screen germplasm for resistance to sugary disease. Quantitative assessment required counting the number of spikelets with sphacelia, with healthy grain, and without either, in a composite sample of spikelets from one primary branch of each node of the panicle rachis. They also used a 1–5 visual rating scale as follows: 1, no ergot/sphacelia; 2, 1–10%; 3, 11–25%; 4, 26–50%; 5, >50% infected spikelets in a panicle. Both systems have their drawbacks. Quantitative assessment is costly and time-consuming, while the percentage visual rating can be misleading. The effectiveness of a screening technique must rely on relatively quick and efficient evaluation so that large numbers of accessions can be screened across several locations and years in replicated trials. The objectives of this research were to rank various publicly available seed parents and evaluate germplasm sources to identify sources of resistance to the disease for subsequent utilization in breeding programs. Flower characteristics associated with sorghum accessions with different ergot reaction were also studied.

Materials and methods

Materials

Twelve A-/B- (i.e. maintainer line of the A-line) pairs, and 12 R-lines were evaluated for resistance to sugary disease. The A-/B-lines represented important public inbred lines released from the Texas Agricultural Experiment Station, and are widely used in hybrid seed production. Sources of resistance reported in the literature (Bandyopadhyay *et al.*, 1996) and their hybrid combinations with an A_3 cytoplasmic genetic male-sterile line (A_3SC103-12E) (Table 1) were also evaluated. A_3 sterile hybrids were used to observe whether resistance might occur in sterile sorghums with reported resistant sources in their background. In this paper, entries within a type of sorghum (A-, B- and R-lines, sources of resistance and their crosses) are referred to as pedigrees.

Trials

A-/B-line pairs (sown as paired plots, A-line next to the corresponding B-line), with a range of maturities, and 12 R-lines were sown in the winter of 1997/98 at the Isabela Farm (Isabela, Puerto Rico) of the United States Department of Agriculture (USDA)-Agriculture Research Service (ARS). Seeds of each line were sown in a single row plot (5 m long) replicated three times in a randomized complete block design. The trials were established on 9 and 19 December 1997 and 9 and 26 January 1998 in separate but contiguous blocks. A-/B- and R-lines were sown as separate trials within each planting. The third sowing of R-lines was abandoned due to insect damage. Sources of resistance and their hybrid combinations with an A_3 cytoplasmic genetic male-sterile line were planted on 11 and 19 December 1997. Plantings were surrounded by the cytoplasmic male-sterile line ATx623, which was used as a disease spreader. None of the trials was artificially inoculated.

Sugary disease evaluation

The first rating used in this study was a percentage incidence score of panicles affected with sugary disease. At least 20 panicles were observed in each plot for

presence or absence of the disease and percentage disease incidence was calculated. The second rating was based on a 1–5 scale (Tegegne *et al.*, 1994) and represents severity of sugary disease within a representative sample of the plot as follows: 1, no infected florets on the panicle; 2, 1–10 infected florets on the panicle; 3, 11–25 infected florets on the panicle; 4, 26–50 infected florets on the panicle; 5, >50 infected florets on the panicle. Days to 50% anthesis were recorded for each plot.

Weather monitoring and data analysis

An automatic weather station (Campbell Scientific, Inc., North Logan, UT, USA) was used to record temperature, relative humidity, rainfall, solar radiation, and wind speed and direction data. A data-logger captured data every 1 min and computed total rainfall for each day, and daily minimum, maximum and mean values for other weather parameters. Weather indices were established to reflect a 10-day period covering 5-day pre- and 5-day postflowering for each accession. Based on flowering notes, a data set was created for each line in which the daily minimum, maximum and average temperatures, and mean relative humidity were each averaged over the 10-day pre and postflowering period.

Data analysis

Disease incidence and disease severity of each pedigree were ranked separately within each replicate of the three trials (A-/B-line, R-line, and resistant accessions) using PROC RANK of SAS (SAS, 1985). Ranks of disease incidence and disease severity of each pedigree within a replicate of each trial were averaged. The average rank value of each pedigree was further ranked among all pedigrees within a replicate of a trial to obtain an overall ranking, again using PROC RANK of SAS. Disease incidence and severity, and the overall ranking data of pedigrees were analysed in a split-plot analysis (random effects) with planting dates as main plots and pedigrees as subplots. The weather parameters were included to run correlation analyses with time to 50% anthesis, ergot incidence, ergot severity and overall ranking. Correlation analyses were done separately for A-lines, B-lines, R-lines and the resistant sources and the hybrids in A_3 cytoplasm.

Flower characteristics

Flower and pollination characteristics of five R-lines (RTx2737, Tx2862, Tx2783, RTx430, and Tx436) with contrasting sugary disease reaction were recorded in the second sowing of the trials at Isabela, Puerto Rico. Similar observations were recorded for IS 8525 at Lubbock, Texas, in a crossing block under cooler temperatures (minimum night temperature $<10°C$) compared with Isabela. At least five panicles of each accession were observed for the following characteristics: (i) time from pollination to drying of stigma; (ii) size and shape of stigma; (iii) pollen deposition on stigma; (iv) exposure of stigma before pollination; and (v) presence or absence of aperture on flower tip. Glumes of nearly 25 flowers in each panicle were marked with a marker pen a few days before anthesis was anticipated. The marked flowers were observed daily *in situ* in the morning at ×10 magnification to determine if an aperture existed at the tip of the clasping outer and inner glumes through which the stigmas were visible. The day of stigma emergence outside the glumes and the day when anthesis occurred were also recorded. During the morning of the day of pollination, stigmas of five flowers were observed at ×10 magnification in the field to record the extent of pollen deposition on stigma on a 1–3 scale as follows: 1, 1–10 pollen grains; 2, 11–25 pollen grains; 3, >25 pollen grains deposited on stigma feathers, respectively. The stigmas were then excised, and their length and shape measured.

The size of stigma was recorded as short (1–3 mm), medium (3–6 mm) or long (6–12 mm). The shape of stigma was recorded as narrow (stigma $<1{\cdot}5$ mm in width), or feathery (stigma >2 mm in width). From the time of pollination in the morning, stigmas of five flowers were observed at 2-h intervals throughout the day, and then again the next morning to determine the time taken for drying of stigma after pollination. Stigmas were considered dry when they lost turgidity, turned ashy and drooped.

Results

Time to flowering

Planting dates significantly affected days to 50% anthesis within A-/B-, R-lines and resistant sources (Table 2). Flowering was significantly delayed with delay in sowing as the environment moved into longer

Table 1 Pedigrees of reported sources of sorghum germplasm resistant to *Claviceps africana* and hybrid combinations with an A_3 cytoplasmic male-sterile evaluated for resistance to sugary disease in Puerto Rico

Plant introduction or hybrid	Other designation	Country of origin
PI 533835	Birgalli Ahmar	Sudan
PI 533863	740 Oua Berr	Chad
PI 533866	255 Tirter	Sudan
A_3SC103-12E × PI 533866	–	–
PI 533998	Brawley	USA
A_3SC103-12E × PI 533998	–	–
PI 534160	Magune	USA
A_3SC103-12E × PI 534160	–	–
PI569037	IS 19013	Sudan
A_3SC103-12E × PI 569037	–	–
PI 569250	IS 19228	Sudan
–	A53	Japan
PI 563092	IS 8525	Ethiopia
A_3SC103-12E × IS 8525	–	–

Table 2 Analysis of variance for days to 50% anthesis and overall ranking of sugary disease severity and incidence in sorghum A-, B- and R-lines, and reported sources of resistance to sugary disease and their hybrids across various planting dates at Isabela, Puerto Rico 1997–98

		Days to 50% anthesis		Overall ranking	
Sorghum line	Source	d.f.	Mean squares	d.f.	Mean squares
A-lines	Model	55	75·3758	55	254·9630
	Planting date (PD)	3	108·4259**	3	2732·5265**
	Pedigree (PE)	11	331·3384**	11	289·4181**
	PD × PE	33	3·0572	33	66·7879**
	Error	88	3·1654	87	24·5221
B-lines	Model	55	70·7251	55	208·7201
	PD	3	73·7477**	3	1292·3785**
	PE	11	320·8958**	11	487·2841**
	PD × PE	33	3·4245	33	53·0136
	Error	88	2·6540	88	51·1125
R-lines	Model	41	59·2057	41	108·5884
	PD	2	267·8426**	2	847·4088**
	PE	11	151·4032**	11	156·4895**
	PD × PE	22	7·1052	22	42·0929**
	Error	66	4·2525	58	16·5041
Resistant lines/hybrids	Model	30	42·2355	30	102·2341
	PD	1	59·1447**	1	56·8839*
	PE	12	76·0034**	13	205·7253**
	PD × PE	12	16·9542**	12	18·6717*
	Error	49	3·3943	47	9·4705

*, ** Significant at $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively.

days. Day lengths in Puerto Rico range between 11·03 and 13·21 h (Miller *et al*., 1968). Sorghum accessions planted on 9 December 1997 flowered in mid-February when day length averaged 11·6 h. Average day length during the flowering period of last planting was 12·6 h. Days to 50% anthesis increased by 3–5 days as day lengths increased. Pedigrees also had a significant effect on days to 50% anthesis (Table 2), which ranged from 49 to 73 days. Days to 50% anthesis among A-/B-lines were significantly different (Table 2) and ranged from 55 to 73 days (Table 3). In general, newer releases, such as A/BTx631, A/BTx626, A/BTx635 and A/BTx623, flowered significantly later than older releases such as A/BTx399 and others. These newer releases flowered between 64 and 73 days, while the older releases flowered between 55 and 62 days. Such generalizations could not be made of the R-lines. Flowering dates were significantly different among the R-lines. The sources of resistance and their hybrids with A_3 cytoplasm tended to take less time to flower than the other entries, with days to flowering ranging from 49 to 60 days.

Sugary disease incidence and severity

Significant differences were detected among sorghum lines for their susceptibility to sugary disease (Table 2). Among the four categories of sorghum lines, A-lines were most susceptible. R-lines, with a few exceptions, were more susceptible than the B-lines with respect to sugary disease incidence and severity (Table 3). Sugary disease incidence ranged from 72·6 to 100% and severity ranged from 3·2 to 4·7 in the 12 A-lines. ATx631, ATx626, ATx623 and A35 had sugary disease incidences above 95% and severities more than or equal to 4·5. AOK11, ATx2752 and ATx3042 were less susceptible (sugary disease incidences below 80%, and severities below 4·0) among the A-lines. Sugary disease severities in the B-lines ranged from 1·5 to 2·0, while incidences varied from $\approx 5\%$ for BTx378, BTx2752 and BOK11, to $\approx 62\%$ for BTx626 and BTx635 (Table 3). Among the 12 R-lines, Tx2737 was most susceptible to sugary disease in terms of both incidence and severity, whereas Tx7000 had the lowest severity and Tx2880 the lowest incidence.

Overall ranking

The overall ranking is an index that takes both sugary disease severity and incidence into consideration. Lower rankings indicate lower mean sugary disease incidence and severity. Planting dates and pedigrees had significant effects on the overall ranking of all four categories of lines (Table 2). Although all the A-lines are highly susceptible to *C. africana*, ATx631 was most susceptible to sugary disease, while ATx2752 and AOK11 were least susceptible (Table 3). In general, newer releases, such as A/BTx631, A/BTx626, A/BTx635 and A/BTx623, ranked significantly higher than older releases (A/BTx399, A/BTx378, A/BTx3197, A/BTx3042, A/BTx2752 and A/BOK11). The lines BTx2752 and BTx378 ranked significantly lower than all other B-lines except BOK11. A strong positive correlation

Table 3 Time to 50% anthesis, mean disease incidence, disease severity rating and overall sugary disease ranking of sorghum A-/B- and R-lines, and reported sources of resistance and their hybrids across various planting dates at Isabela, Puerto Rico 1997–98

Pedigrees	Time to 50% anthesis (days)	Disease incidence (%)	Disease severity[a]	Mean overall ranking[b]
A-lines				
ATx631	73 a[c]	100·0 a	4·7 a	31·41 a
ATx626	65 c	97·8 a	4·7 ab	29·77 ab
ATx623	66 c	99·1 a	4·5 ab	28·98 ab
A35	66 c	95·4 a	4·6 ab	26·98 abc
A.1	70 b	93·5 ab	4·3 abc	26·96 abc
ATx635	65 c	88·6 abc	4·5 abc	26·58 bc
ATx399	57 f	88·8 abc	4·1 bcd	24·19 cd
ATx378	59 e	88·2 abc	4·0 cd	23·08 cd
ATx3197	62 d	83·3 bcd	3·9 cd	21·96 de
ATx3042	58 f	78·1 cd	3·7 de	18·77 ef
ATx2752	58 f	76·3 d	3·6 de	18·56 ef
AOK11	59 e	72·6 d	3·2 e	15·37 f
B-lines				
BTx626	63 d	62·1 a	2·0 a	34·35 a
BTx635	67 b	62·8 a	2·0 ab	33·94 a
BTx631	70 a	35·4 b	2·0 ab	29·33 ab
BTx623	65 c	23·9 c	2·0 ab	27·37 bc
B.1	68 b	26·6 bc	1·9 ab	26·67 bc
B35	64 c	36·8 b	1·8 abc	24·87 bcd
BTx3042	55 h	17·4 cd	2·2 a	24·69 bcd
BTx3197	61 e	10·2 de	1·9 abc	22·06 cd
BTx399	55 h	12·5 de	1·8 abc	21·90 cd
BOK11	59 f	5·4 e	1·8 abc	18·56 de
BTx2752	57 g	4·4 e	1·6bc	15·48 e
BTx378	58 g	5·4 e	1·5 c	14·77 e
R-lines				
Tx2737	59 g	87·1 a	3·2 a	28·22 a
Tx2862	65 bcd	59·6 b	2·4 b	19·47 b
Tx2908	67 b	60·7 b	2·2 bc	18·97 b
Tx2783	63 ef	54·2 bcd	2·2 bc	17·89 bc
RTAM428	71 a	58·1 bc	2·1 bcd	17·36 bc
Tx2536	65 bc	60·9 b	2·0 cd	17·34 bc
RTx430	63 cde	62·6 b	2·0 cd	16·94 bc
Tx433	63 def	56·7 bc	2·0 cd	16·00 bcd
Tx7000	55 h	56·0 bc	1·8 d	15·00 bcd
R.EON361	66 b	46·6 bcd	1·9 cd	13·56 cd
Tx436	61 f	32·5 cd	1·9 cd	12·42 d
Tx2880	61 f	30·2 d	1·9 cd	12·25 d
Resistant lines/hybrids				
A_3 × PI 534160	54 bc	97·5 a	3·9 a	22·6 a
A_3 × PI 569037	49 e	84·4 ab	3·8 a	22·0 a
A_3 × PI 533998	51 de	100·0 a	3·2 a	21·7 a
A_3 × PI 533866	51 de	75·9 bc	3·7 a	21·3 a
A_3 × IS 8525	49 e	62·0 c	2·3 b	16·3 b
PI 533835	60 a	33·9 d	2·1 bc	13·1 bc
PI 569250	52 cd	31·4 d	2·0 bc	12·3 c
PI 534160	59 a	22·9 de	2·0 bc	12·0 c
PI 533998	58 a	23·4 de	2·1 bc	12·0 c
PI 533863	59 a	18·7 def	2·1 bc	11·7 c
PI 533866	52 cd	10·2 ef	2·0 bc	9·9 cd
A53	53 cd	9·6 ef	1·7 bc	7·3 de
PI 569037	55 b	6·8 ef	1·7 bc	7·2 de
IS 8525	52 cd	1·4 f	1·3 c	3·7 e

[a]Based on a 1–5 scale where 1 = no ergot, and 5 = 51 or more spikelets infected in a panicle.

[b]Ranking index derived from ergot incidence and ergot severity using PROC RANK analysis (SAS, 1985). Susceptible lines have a higher rank value.

[c]For each group of sorghum lines, means within a column followed by a common letter do not differ significantly ($P \leq 0{\cdot}05$) by the Duncan multiple range test criterion.

(Spearman rank correlation coefficient, $r_s = 0{\cdot}722$, $P < 0{\cdot}01$) was found between rankings of the B-lines with their respective A-lines. For example, the same six A- and B-line pairs had the top six overall ranking. Tx2737 had significantly higher ranking than all other R-lines, indicating a high degree of susceptibility to sugary disease. Within the resistant sources and their A_3 hybrids, three lines showed some promise as possible sources of resistance to sugary disease. The most promising line was IS 8525 from Ethiopia, which had the lowest ranking within all the reported sources of resistance tested.

Planting date (PD) × pedigree (PE) interaction was not significant for the B-lines, suggesting that rankings of the B-lines did not change with planting dates; however, PD × PE interactions were significant for A-lines, R-lines and the resistant sources (Table 2), indicating that as inoculum loads increased, rankings within the different groups of lines changed.

Correlation between flowering, severity and incidence of sugary disease, overall ranking and weather indices

Among both the A-lines and B-lines, positive correlations were found between flowering dates and overall rankings, which indicate that date of flowering had some influence on a pedigree's susceptibility to the disease (Table 4). In general, PEs that flowered earlier tended to have less sugary disease. Disease incidence and severity had a high correlation coefficient (except in the case of B-lines), but it was less than their respective correlation coefficients with overall ranking for all four categories of PEs (Table 4).

The minimum, maximum and average temperatures were in the ranges 17·6–21·8°C, 28–30·4°C, and 23·3–24·8°C, respectively, during the 10-day flowering period of all accessions throughout the course of experiments. No rainfall was recorded during the flowering period, but dew occurred on each night and kept panicles wet until 09·00 h. Average relative humidity was positively correlated to overall rankings of A-, B- and R-lines. Many of the correlations observed for the A-, B- and R-lines did not hold true for the resistance sources and their hybrids. In Puerto Rico, disease incidences and ratings seemed to be more dependent upon relative humidity than on temperature, due to low variation in minimum temperature in our experiments.

Flower characteristics

Considerable differences were observed in flower characteristics of the five R-lines with varying susceptibility to sugary disease, and IS 8525 (Table 5). Time to stigma drying varied from <8 h (i.e. stigma dried before 16·00 h on the day of pollination) for the resistant accession IS 8525, 10–12 h for Tx436, and 12–24 h for other accessions. The most bulky stigma belonged to RTx430, whereas IS 8525 had the shortest

Table 4 Correlations among days to 50% anthesis, incidence and severity of sugary disease, overall ranking and ambient relative humidity in A-/B- and R-lines, and resistant lines/hybrids of sorghum at Isabela, Puerto Rico 1997–98

Sorghum lines	Parameters	50% anthesis	Disease incidence	Disease severity[a]	Overall ranking[b]
A-lines	50% anthesis	1·0000			
	Disease incidence	0·3560**	1·0000		
	Disease severity	0·4205**	0·6888**	1·0000	
	Overall ranking	0·4472**	0·8459**	0·9179**	1·0000
	Relative humidity	0·1533	0·2507**	0·2965**	0·3212**
B-lines	50% anthesis	1·0000			
	Disease incidence	0·5404**	1·0000		
	Disease severity	0·1438	0·2846**	1·0000	
	Overall ranking	0·4487**	0·7486**	0·7449**	1·0000
	Relative humidity	0·1076	0·1732*	0·0189	0·1573*
R-lines	50% anthesis	1·0000			
	Disease incidence	0·2177*	1·0000		
	Disease severity	0·0449	0·5032**	1·0000	
	Overall ranking	0·1775	0·8895**	0·8020**	1·0000
	Relative humidity	0·2070*	0·3627**	0·1579	0·2718**
Resistant lines/hybrids	50% anthesis	1·0000			
	Disease incidence	−0·3313**	1·0000		
	Disease severity	−0·2660*	0·7919**	1·0000	
	Overall ranking	−0·2510	0·9134**	0·8836**	1·0000
	Relative humidity	0·1679	0·0572	−0·0658	−0·0249

[a]Based on a 1–5 scale where 1 = no ergot, and 5 = 51 or more spikelets infected in a panicle.

[b]Ranking index derived from ergot incidence and ergot severity using PROC RANK analysis (SAS, 1985). Susceptible lines have a higher rank value.

*, **, significantly different from zero at 0·05 and 0·01 levels of probability, respectively.

Table 5 Flower and pollination characteristics of five sorghum R-lines at Isabela (Puerto Rico) and the sugary disease-resistant sorghum accession IS 8525 at Lubbock (USA) 1998

Pedigrees	Time to stigma drying[a] (h)	Stigma size and shape[b]	Pollen deposition on stigma[c]	Stigma exposure (hours before anthesis)	Aperture at glume tip	Remarks
RTx2737	12–24	Long, narrow	3	72	Present	Stigma emerge 3 days before anthesis
Tx2862	12–24	Short, narrow	2	0	Absent	Anthers trapped in between glumes
Tx2783	12–24	Medium, feathery	3	24	Present	Outer glume awned; small aperture between lemma and palea
RTx430	12–24	Long, feathery	3	0	Absent	Stigma trapped in between glumes
Tx436	10–12	Short, narrow	2	0	Absent	Stigma dries rapidly after pollination
IS 8525	< 8	Short, narrow	3	0	Absent	Stigma dries rapidly after pollination

[a]Time from pollination to drying of stigma of individual flowers.
[b]Stigma size: short = 1–3 mm, medium = 3–6 mm, and long = 6–12 mm; stigma shape: narrow = stigma feathers < 1 mm in width, and feathery = stigma feathers > 1 mm in width.
[c]Evaluated on a 1–3 scale as follows: 1, 1–10 pollen grains; 2, 11–25 pollen grains; 3, > 25 pollen grains deposited on stigma feathers, respectively.

stigma with few stigma feathers. More than 11 pollen grains deposited on the stigma of all the accessions, but Tx2862 and Tx436 had comparatively less pollen compared with other accessions.

In all accessions, except RTx2737 and Tx2783, stigma emergence and anthesis occurred almost simultaneously. RTx2737 had protogynous flowers since stigma emerged from glumes at least 3 days before anthesis. The outer glumes of Tx2783 possessed long awns, which distended the apical part of the outer glumes. As a result, a small gap appeared between the clasping outer glume and inner glume when the stigma and anthers expanded and exerted pressure at the flower tip a day before their emergence.

Discussion

Sorghum germplasm commonly used in F_1 hybrid breeding programmes in the USA were not exposed to sugary disease prior to 1996 since the disease was recently recorded for the first time in North America and South America. This study reports the reaction of several A-, B- and R-lines commonly used in hybrid seed development and production programmes, and sources of putative resistance to sugary disease. None of the material evaluated was free of sugary disease, although various degrees of susceptibilities were observed. IS 8525, a sorghum accession of Ethiopian origin, was highly resistant to the disease. As a group, A-lines were most susceptible, followed by R-lines and B-lines. High susceptibility of A-lines is not surprising due to their male-sterility (Bandyopadhyay *et al*., 1998).

The sources of putative resistance tested in this study were accessions from the gene bank of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and had been previously evaluated in the highlands of Ethiopia (Tegegne *et al*., 1994). Most of the material tested was either bicolour or guinea-type sorghum. IS 8525 from Ethiopia showed the greatest potential for use in a host-plant resistance strategy to combat sugary disease. It also showed some potential for reduced infection in a male-sterile background since F_1 male-sterile hybrid with IS 8525 as a parent had comparatively less disease than other male-sterile hybrids. IS 8525 is now widely used in breeding programmes in the USA (Texas A&M University, Kansas State University, and several private-sector breeding programmes), Australia and South Africa to develop sorghum cultivars resistant to sugary disease.

Newer A-/B-lines were more susceptible to sugary disease than older varieties. A physiological determinant may explain this observation. Newer lines tended to be

more nonsenescent than the older lines. Duncan *et al.* (1981) noted that several lines that were developed out of the Sorghum Conversion programme (i.e. newer lines) remained green and vigorous after physiological maturity. In contrast, senescence in leaves of 'normal' sorghums (SC) starts at the base of the culm. McBee (1984) used 'reduced progressive senescence' (RPSC) to describe sorghums which stay green and vigorous during maturation. RPSC sorghums have been associated with resistance to disease, drought, and insects (Johnson *et al.*, 1976; Foster *et al.*, 1977; Rosenow & Clark, 1981; Rosenow *et al.*, 1983; Wanous, 1989; Tenkouano, 1990). Duncan *et al.* (1981) concluded that RPSC lines had greater stem diameter, maintained greater green leaf area for longer, and had greater leaf area index, leaf area duration and leaf area ratios than SC sorghums. This tendency towards nonsenescence may also contribute to a longer stigma receptivity of these newer lines, thus creating a wider window of opportunity for infection by *C. africana*.

The R-lines varied considerably in their susceptibility to sugary disease with severity scores ranging from 1·9 to 3·2. In general, R-lines with good pollen shed and seed set tended to be less susceptible to the disease. This was not the case in the line Tx2737, one of the most common male-fertility restorers currently in use in the sorghum seed industry. The R-line Tx2737 is an excellent pollen shedder, a desirable flower characteristic for resistance to sugary disease (Bandyopadhyay *et al.*, 1998). On further evaluation of Tx2737, however, a unique floral characteristic was observed. The stigma of Tx2737 emerges between the glume at least 3 days before the anthers, thus providing a time period during which *C. africana* spores have an advantage for infection. Conidia germinate on the stigma 16–24 h after infection and take $\approx$ 36 h to colonize the ovary (Bandyopadhyay *et al.*, 1998). The protogynous flower characteristic of Tx2737 makes it vulnerable to the pathogen on the first few days of stigma emergence and may be the reason for the increase in incidence and severity observed in the field. Similar observations have been recorded in pearl millet (*Pennisetum glaucum*) (Thakur & King, 1988).

Other flower characteristics may also determine reaction of sorghum accessions to sugary disease. Due to the qualitative nature of data recorded in this study for different flower characteristics, descriptive association between flower characteristics and resistance is discussed in this paper, instead of determining their quantitative relationships. Least exposure time of stigma to inoculum before pollination, rapid stigma drying after pollination, and small stigma were the combination of characteristics associated with the resistant accession IS 8525 and the least susceptible R-line Tx436 (Table 5). Rapid stigma drying may be a result of speedy and efficient pollination and fertilization processes, and it leads to a quick transition from susceptibility to resistance since conidia cannot infect stigma of fertilized flowers (Bandyopadhyay *et al.*, 1998). In fact, stigma of IS 8525 dried before sunset of the day of pollination, and became resistant before natural environmental conditions favourable for infection (e.g. dew) could occur at night. Glumes that tightly clasp the pistil may preclude infection of the stigma and the ovary. The presence of an awn in the flower is not a desirable characteristic since it has a tendency to distend the apical part of the glumes, thereby exposing the stigma prior to pollination, as observed in Tx2783 (Table 5). A combination of flower characteristics that reduce opportunities for infection by spores may play an important role in sorghum flower's ability to tolerate high inoculum loads. However, some flower characteristics desirable for sugary disease resistance are contradictory to flower characteristics used to select for good seed set in male-sterile lines (e.g. long stigmas and prolonged stigma receptivity). Floral characteristics are poorly understood in sorghum and this is an area that will require greater study in order to elucidate their role in susceptibility to sugary disease.

Evaluation of sugary disease resistance must be carried out under environmental conditions that favour the disease. Low average temperature (< 15°C), through its indirect effect on pollen viability, predisposes sorghum flowers to infection (McLaren, 1997). Moderate temperatures (14–28°C) combined with high relative humidity ($> 90\%$) are favourable for infection, disease development and spread (Bandyopadhyay *et al.*, 1998). This study was carried out under the latter disease-conducive conditions and not at temperature regimes that affect pollen viability. Sugary disease resistance of the PEs evaluated in this study remains to be examined under situations where pollen viability is likely to be a problem, using methodologies described by McLaren (1992, 1997).

To be useful in host-plant resistance breeding programmes, effective screening techniques must provide a relatively quick and efficient evaluation of a large number of germplasm sources. Quantitative assessment of damage by sugary disease is time-consuming and compounded by difficulties when using a visual percentage assessment scale. Use of ranking is commonly practised in breeding programmes to determine thresholds for selecting desirable genotypes. The dual-purpose ranking scale provides a relatively quick and accurate assessment of incidence and severity of the disease on lines within a breeding programme, hybrids that will be available for commercial use, and germplasm that is needed for host-plant resistance. To date, the scale has been used to evaluate accurately and efficiently differences among nearly 100 commercial hybrids and a representative lot of A-/B-lines for their reaction to sugary disease in two other trials in Texas. In both of these experiments (data not shown), the approach has been reliable and useful.

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