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Research Article
Response of Sorghum Accessions from Three African Countries to Anthracnose, Grain Mould and Rust

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Abstract
Background and Objective: Sorghum is one of the most indispensable crops, especially in the drier tropics where many millions of people rely on it for their daily calories intake. However, the crop is hampered by several biotic stresses, causing annual economic losses estimated at hundreds of millions of dollars. The study aimed to evaluate diverse sorghum germplasm for resistance against pathogens causing anthracnose, grain mould and rust. **Materials and Methods:** A total of 179 accessions from Ethiopia, Gambia and Senegal were evaluated for resistance to anthracnose, grain mould and rust during the 2017 and 2018 growing seasons. Seeds were planted in a randomized complete block design and each accession was replicated 3 times at the USDA Research Station, Isabela, Puerto Rico. Disease assessment and germination rate were based on published procedures. **Results:** Out of the 179 accessions evaluated, 138 were resistant to anthracnose. Among these anthracnose resistant accessions, 41 accessions, including PI276832, PI534001, PI533903 and PI665159 also were resistant to *Puccinia purpurea*, which causes rust. Three accessions PI514411, PI514318 and PI514538 out of the 138 anthracnose resistant accessions were also resistant to grain mould. Two accessions PI514318 and PI514538 from Senegal were resistant to all 3 diseases. Among the anthracnose resistant accessions, 20 recorded a 90% or above germination rate. Germination rate was correlated with the sorghum responses to the 3 diseases, a positive correlation was found between germination rate and severity to rust, while negative correlations were detected between germination rate and severities to anthracnose and grain mould. **Conclusion:** The work is significant because it has identified 2 accessions that are resistant to anthracnose, grain mould and rust. The identified accessions may be useful in breeding programs to introgress the resistance genes they possess into elite or parental lines beyond Puerto Rico.

Key words: Sorghum, anthracnose, rust, grain mould, germination rate, fungal species, disease resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench.), one of the most important cereal crops, plays a critical role in resilient farming and is projected as a smart crop to overcome the food and nutritional insecurity in the developing world[1]. As are other cereal crops, sorghum is consistently exposed to abiotic and biotic stresses. Among biotic stresses, anthracnose, rust and grain mould are major constraints to sorghum production[2]. Anthracnose, caused by *Colletotrichum sublineola* Henn. ex Sacc. and Trotter 1913 (syn. *C. sublineolum*) damages all aerial parts of sorghum including leaf and stalk[3-6]. A recent study showed a significant negative correlation between leaf anthracnose severity and grain yield, for every percent increase in leaf anthracnose, there is a 27-85 kg ha^{-1} loss in grain yield[7]. Rust, caused by *Puccinia purpurea* cooke, is known to occur wherever sorghum is grown and under environmental conditions favouring rust, grain yield losses can be up to 65%[8]. Anthracnose, rust, leaf blight (*Exserohilum turcicum*), zonate leaf spot (*Gloeocercospora sorghi*) and oval leaf spot (*Ramulispora sorghicola*) were among the most observed sorghum diseases in western Kenya according to Ngugi *et al.*[9]. Grain mould is a major biotic, yield-reducing constraint and losses ranging from 30-100% based on the susceptibility of the cultivar, time of flowering, developmental stage and soil type have been reported[10,11]. The sorghum grain mould pathosystem is exceedingly dynamic, as grain mould is innately multifarious, consisting of a multitude of fungi demonstrating various trophic lifestyles: Necrotrophic, saprophytic and hemibiotrophic[9]. Many fungal genera, including *Fusarium thapsinum* Klittick, Leslie, Manassas, *Fusarium incarnatum* (Desk.), Sacc. (syn. *Fusarium semitectum* Berk. and Ravenel) (https://nt.ars-grin.gov/fungaldatabases/), *Curvularia lunata* (Wakk.) Boedijn, *Colletotrichum sublineola* Henn. ex Sacc. and Trotter, *Alternaria alternata* (Fr.) Keissl., *Alternaria* spp. and *Epicoccum sorghinum* (Sacc.) Aveskamp, Gruyter and Verkley, *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, and Van Kesteren) (https://nt.ars-grin.gov/fungaldatabases/) are associated with sorghum grain mould disease complex[11-15]. Most genetic improvement programs in sorghum have relied upon classical breeding approaches, but traits determining biotic and abiotic stress tolerance remain among the most challenging to identify[16]. The first step to improving sorghum protection against pathogens is a large-scale evaluation for diseases by phenotyping sorghum collections either inoculated with the respective pathogens or planting them in 'hot spots'.

To identify new sources of resistance, it is important to evaluate diverse sorghum germplasm against economically important diseases. Thus, the study aimed to evaluate sorghum accessions from Ethiopia, Gambia and Senegal for resistance to anthracnose, grain mould and rust.

MATERIALS AND METHODS

Study area: Experiments for the disease evaluation were carried out at the USDA-Tropical Agriculture Experiment Station in Isabela, Puerto Rico, during the 2017 and 2018 growing seasons. The site is located at longitude 67.3 W, latitude 18.3 N and 128 m above sea level[17]. The soil type in the station is an oxisol series Coto[17].

Field trial: A total of 179 accessions from Ethiopia, Gambia and Senegal obtained from the sorghum collections maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia, were evaluated for resistance to anthracnose, grain mould and rust. Accessions were planted in a randomized complete block design at the experiment station. Each accession was replicated 3 times. Fertilizer application and other agronomic managements were followed according to local recommendations.

Anthracnose trial: Inoculum preparation, inoculation and disease assessment methods for the anthracnose studies have been previously described[18]. Briefly, a mixture of 2 isolates of *C. sublineola* was used to inoculate sterilized sorghum in bottles and allowed to completely colonize the grain. Plants were inoculated at growth stages 4 and 5 by placing *C. sublineola* colonized grain in the plant whorls. Disease assessment was based on a 1-5 rating scale, where (1) No symptoms or chlorotic flecks on leaves, (2) Hypersensitive reaction on leaves but no acervuli formation, (3) Lesions on lower and bottom leaves with acervuli, (4) Necrotic lesions with acervuli observed on leaves and not yet on the flag leaves and (5) Most leaves dead due to infection with the infection on the flag leaf containing abundant acervuli. The symptom types were then categorized into 2 reaction classes, resistant is rated as 1 or 2 and susceptible is rated as 3, 4, or 5.

Grain mould trial: Inocula preparation and inoculation method were as previously described by Prom *et al.*[19]. Briefly, *F. thapsinum*, *F. semitectum*, *F. proliferatum* and *C. lunata* were grown separately in Petri plates containing half-strength Potato Dextrose Agar (PDA) medium and incubated at 25°C for 10 days under a 12 hrs photoperiod. Conidia from the pathogens were harvested by flooding the plates with sterilized water and suspensions were filtered through layers of sterile cheesecloth into separate beakers and mixed then diluted with sterile water to final concentrations of 1×10^{6} mL^{-1}. Tween 80 was added to the mixture at a rate of 1.0 mL L^{-1}. At 50 % bloom, 3 sorghum panicles per accession/replicate were randomly selected, tagged and then inoculated with 5-7 mL spore suspension/panicle using a hand-held spray bottle at different dates in June and July, for the experiments. The panicles were bagged to prevent bird damage. At maturity, panicles were harvested and threshed. The hand threshed kernels were assessed for grain mould severity using a 1-5 scale, where 1 = no mould observed on the seeds, 2 = 1-9%, 3 = 10-24%, 4 = 25-49% and 5 = 50% or more of the seeds exhibiting characteristics of grain mould infection such as decreased seed size or shrivelled, different discolourations from light whitish, salmon-orange, black, pinkish, to greyish black and the presence of fungal fruiting bodies[20,21]. Germination rates were obtained by placing 100 randomly selected seeds per replicate on Anchor seed germination paper (Anchor Paper CO, St. Paul, MN) and evaluating the number of seeds that germinated in 7 days.

Rust trial: Seeds were planted in the field at the USDA Research Station, Isabela, Puerto Rico, with a history of rust pressure, so the plants were infected naturally. The rust assessment scale noted in Cuevas *et al.*[22] was modified in this study, in that 6 leaves were removed from 3 plants per row and evaluated and the average score as 1 = no rust, 2 = 1-15% leaf area infected, 3 = 16-30% leaf area infected, 4 = 31-50% leaf area infected, 5 = 51-75% leaf area infected and 6 = above 75% leaf area infected. Infection was then further categorized into 4 classes, 1 or 2 are considered as resistant, 3 is moderately resistant, accessions rated 4 are moderately susceptible and rated 5 or 6 are considered susceptible.

Statistical analysis: For statistical analysis, Tukey's HSD (honestly significant difference) for all possible pair tests was conducted with JMP Pro 15 for comparing scores of the 3 diseases and germination rate. In this way, the overall error rate was protected. Spearman's rank correlation coefficient was performed to analyze correlations between the 4 traits with JMP Pro 15 as well. Since evaluated scores for the diseases are nonparametric, Spearman's rank correlation was used instead of the Pearson correlation.

RESULTS

Anthracnose (*C. sublineola*): Among 179 accessions, 167 accessions were scored <3 which is considered resistant and 138 accessions were scored as 2 (mean = 2.17±0.03 (SE) among the population) (Fig. 1). The positive (BTx 623, susceptible rated a 5) and negative (SC 748-5, resistant rated a 2) control lines showed clear resistance and susceptible response. The data in Table 1 listed 138 accessions rated as resistant to anthracnose and their corresponding mean scores for grain mould, rust and germination rate as examples without the control lines. A total of 41 accessions, including PI329697, PI514583, PI514502, PI534131, PI576344 and PI576379 also were resistant to rust. Three accessions PI514411, PI514318 and PI514538 were resistant to both anthracnose and grain mould (Table 1). Among the anthracnose resistant accessions, 20 had germination rates of 90% and above.

Rust (*P. purpurea*): Among the 179 accessions evaluated for rust, 88 accessions scored <3 and 14 accessions were scored as 1, the lowest score (mean = 2.84±0.09 (SE) across the population). These 14 accessions, including PI330821, PI576344 and PI533800 are considered highly resistant to rust (Table 2). Unlike anthracnose, which was extremely skewed to resistance, scores for rust were more uniformly distributed (Fig. 2). Forty-four accessions were scored above 4. The data in Table 2 lists the most resistant accessions as examples, along with their corresponding scores for anthracnose, grain mould and germination rate. Out of the 14 highly rust resistant accessions, 12 were also resistant to anthracnose, while one accession PI514318 was resistant to grain mould. The data in Table 2 also shows that the germination rate for these accessions ranged from 79.1-26.9% (PI514318-PI534131).

Grain mould: Only 28 accessions among 123 tested accessions were scored <3 and 28 accessions were scored above 4 (mean = 3.34±0.06 (SE), indicating that these accessions were very susceptible to grain mould. As data in Fig. 3 depicted that grain mould ratings for the accessions tested ranged between 2 and 5. The data in Table 3 shows the accessions (scores <3) within 123 tested accessions along with their scores for the other traits. Accessions PI514411, 514318 and PI514538 with a score of 2 were grain mould resistant and may possess genes for resistance to anthracnose, while PI514318 and PI514538 also were resistant to rust. The germination rate among the 28 accessions ranged from 96-49.7% (PI514583-PI514365).

Germination rate: Like grain mould, seed germination rates for the 123 accessions were determined, germination rates of 90% and above were detected in 20 accessions. Forty accessions showed higher than 80% germination, while 44 accessions had less than 60% germination rate. The

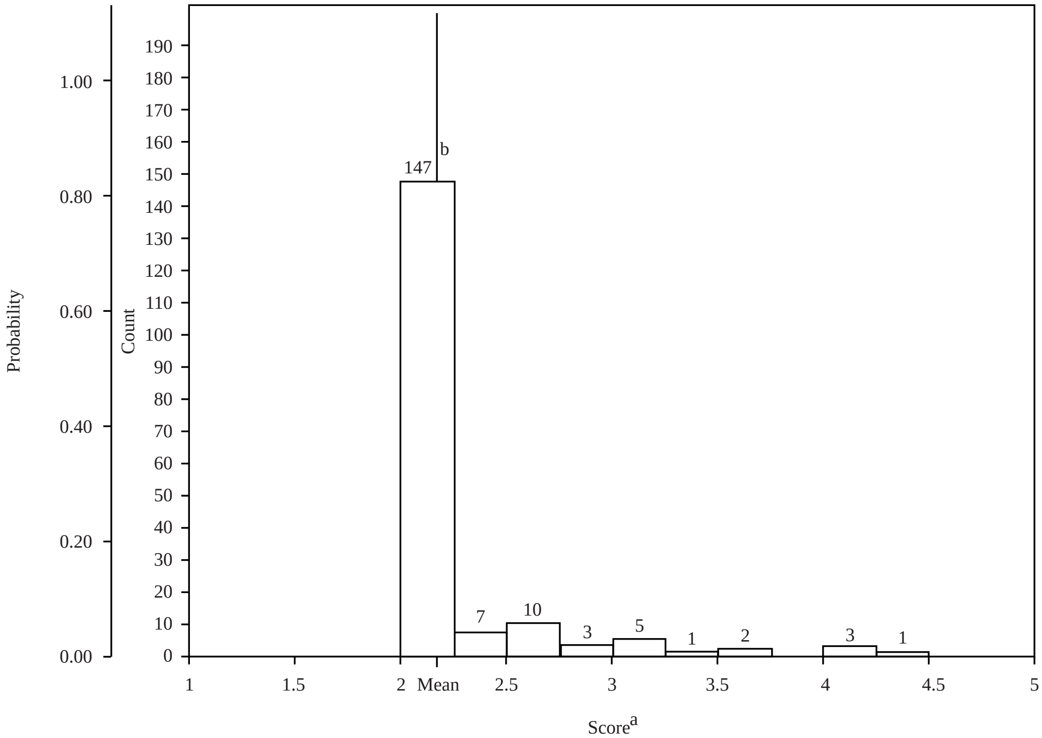

Fig. 1: Phenotype distribution of response levels for anthracnose among 179 tested sorghum accessions

[a]X axis indicates average scores based on disease evaluations and [b]Y axis is the number of sorghum accessions and probability

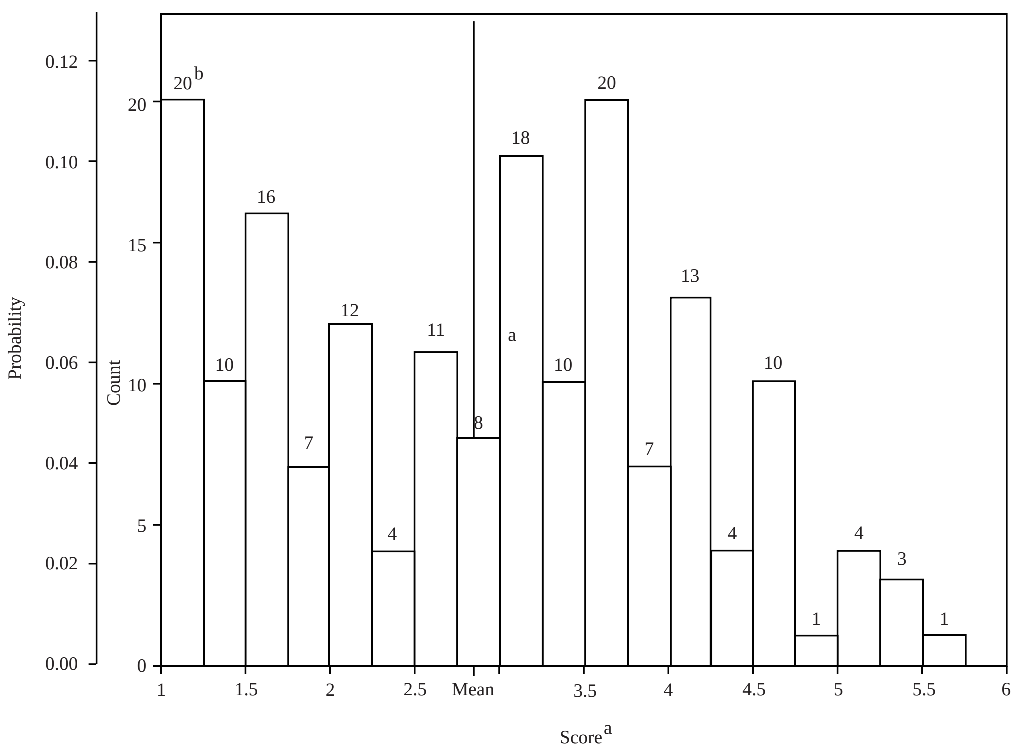

Fig. 2: Phenotype distribution of response levels for rust

[a]X axis indicates average scores based on disease evaluations and [b]Y axis is the number of sorghum accessions and probability

Table 1: One hundred thirty-eight resistant accessions against *C. sublineola* and their corresponding reactions to grain mould, rust and germination rate[1]

Accessions	Anthracnose	Grain mould	Rust	Germination rate
PI 267557	2.0	3.2^{abcdef}	$3.0^{abcdefghi}$	$73.5^{abcdefghi}$
PI 647797	2.0	-	$1.8^{cdefghi}$	-
PI 514437	2.0	2.8^{bcdef}	4.8^{abcde}	$80.1^{abcdefghi}$
PI 267565	2.0	2.8^{bcdef}	1.7^{defghi}	82.8^{abcdi}
PI 330230	2.0	3.0^{abcdef}	5.0^{abcd}	$52.3^{abcdefghi}$
PI 514415	2.0	-	$3.5^{abcdefghi}$	-
PI 330261	2.0	3.6^{abcdef}	$2.0^{bcdefghi}$	$74.8^{abcdefghi}$
PI 514398	2.0	2.8^{bcdef}	1.7^{defghi}	82.9^{abcd}
PI 514348	2.0	3.0^{abcdef}	$3.0^{abcdefghi}$	$90.7^{abcdefghi}$
PI 514484	2.0	-	$3.5^{abcdefghi}$	-
PI 514502	2.0	-	1.2^{ghi}	-
PI 576376	2.0	4.6^{abf}	$3.3^{abcdefghi}$	27.0^{efgh}
PI 534131	2.0	4.0^{abcdef}	1.0^{h}	26.9^{efgh}
PI 647732	2.0	-	1.5^{efghi}	-
PI 514474	2.0	2.5^{abcdef}	$2.7^{abcdefghi}$	$90.0^{abcdefghi}$
PI 514519	2.0	3.0^{abcdef}	$3.2^{abcdefghi}$	$79.7^{abcdefghi}$
PI 329697	2.0	-	1.0^{h}	-
PI 514413	2.0	-	$2.8^{abcdefghi}$	-
PI 514409	2.0	3.7^{abcdef}	$3.5^{abcdefghi}$	$94.4^{abcdefi}$
PI 330004	2.0	-	$2.2^{bcdefghi}$	-
PI 647721	2.0	-	4.7^{abcdef}	-
PI 514393	2.0	2.7^{cde}	$2.0^{bcdefghi}$	$74.0^{abcdefghi}$
PI 647745	2.0	-	$2.2^{bcdefghi}$	-
PI 514558	2.0	-	$2.7^{abcdefghi}$	-
PI 514485	2.0	-	$3.0^{abcdefghi}$	-
PI 533918	2.0	3.5^{abcdef}	$3.0^{abcdefghi}$	$59.0^{abcdefghi}$
PI 514578	2.0	-	$2.5^{abcdefghi}$	-
PI 514440	2.0	3.0^{abcdef}	$3.7^{abcdefghi}$	$91.1^{abcdefgi}$
PI 514581	2.0	-	$3.2^{abcdefghi}$	-
PI 514507	2.0	2.8^{bcdef}	$2.0^{bcdefghi}$	89.4^{abcd}
PI 514583	2.0	2.7^{bcdef}	1.7^{defghi}	96.0^{abcd}
PI 665166	2.0	3.0^{bcdef}	$2.2^{bcdefghi}$	$62.7^{abcdefghi}$
PI 576431	2.0	3.7^{abcdef}	$3.2^{abcdefghi}$	$64.7^{abcdefghi}$
PI 514496	2.0	3.0^{abcdef}	$2.2^{bcdefghi}$	$87.6^{abcdefghi}$
PI 514467	2.0	-	$2.3^{abcdefghi}$	-
PI 644717	2.0	4.0^{abcdef}	4.7^{abcdef}	24.0^{gh}
PI 251637	2.0	2.8^{bcdef}	$4.5^{abcdefgi}$	81.9^{abcefi}
PI 257601	2.0	3.1^{abcdef}	1.8^{cdefgi}	$52.5^{abcdefghi}$
PI 514606	2.0	2.5^{abcdef}	$3.5^{abcdefghi}$	$83.4^{abcdefghi}$
PI 514347	2.0	3.0^{abcdef}	4.7^{abcdef}	96.4^{abcd}
PI 514609	2.0	-	$3.7^{abcdefghi}$	-
PI 576344	2.0	4.0^{abcdef}	1.0^{h}	$73.6^{abcdefghi}$
PI 514611	2.0	3.0^{abcdef}	$3.8^{abcdefghi}$	$89.9^{abcdefghi}$
PI 514612	2.0	3.0^{abcdef}	$2.8^{abcdefghi}$	$94.2^{abcdefi}$
PI 514392	2.0	3.0^{abcdef}	1.5^{efghi}	$95.0^{abcdefi}$
PI 514614	2.0	3.7^{abcdef}	$1.8^{cdefghi}$	$88.7^{abcdefghi}$
PI 522145	2.0	-	1.7^{defghi}	-
PI 669703	2.0	2.8^{bcdef}	$3.3^{abcdefghi}$	$81.6^{abcdefghi}$
PI 533799	2.0	3.8^{abcdef}	1.3^{fghi}	$36.4^{abcdefghi}$
PI 329968	2.0	-	$3.5^{abcdefghi}$	-
PI 533828	2.0	4.3^{abcdf}	$3.5^{abcdefghi}$	24.0^{gh}
PI 668717	2.0	3.0^{bcdef}	1.3^{fghi}	$57.9^{abcdefghi}$
PI 533903	2.0	3.8^{abcdef}	1.0^{h}	$56.4^{abcdefghi}$
PI 514582	2.0	-	$2.7^{abcdefghi}$	-
PI 514414	2.0	3.1^{abcdef}	$2.8^{abcdefghi}$	$93.6^{abcdefi}$
PI 533923	2.0	4.0^{abcdef}	$4.3^{abcdefghi}$	$62.5^{abcdefghi}$
PI 534001	2.0	3.7^{abcdef}	1.0^{h}	$28.6^{cdefghi}$
PI 514577	2.0	-	$4.0^{abcdefghi}$	-
PI 534116	2.0	3.3^{abcdef}	1.5^{efghi}	92.7^{a}
PI 534121	2.0	4.5^{abcf}	1.3^{fghi}	$67.2^{abcdefghi}$
PI 663869	2.0	-	$3.0^{abcdefghi}$	-
PI 576379	2.0	4.2^{abcdef}	1.0^{h}	$64.0^{abcdefghi}$
PI 514394	2.0	3.2^{abcdef}	5.3^{ab}	$51.3^{abcdefghi}$

Table 1: Continue

Accessions	Anthracnose	Grain mould	Rust	Germination rate
PI 576381	2.0	4.5^{abcf}	1.3^{fghi}	63.0^{abcdefghi}
PI 514286	2.0	4.4^{abcdf}	4.3^{abcdefghi}	59.4^{abcdefghi}
PI 576432	2.0	4.0^{abcdef}	3.2^{abcdefghi}	19.0^{abcdefghi}
PI 194355	2.0	4.0^{abcdef}	3.5^{abcdefghi}	85.3^{abcdei}
PI 514386	2.0	-	4.0^{abcdefghi}	-
PI 514506	2.0	2.1^{cdef}	2.5^{abcdefghi}	96.5^{abc}
PI 665159	2.0	-	1.0^{h}	-
PI 514338	2.0	-	3.5^{abcdefghi}	-
PI 564779	2.0	4.2^{abcdef}	2.7^{abcdefghi}	28.1^{defghi}
PI 564780	2.0	-	1.5^{efghi}	-
PI 330764	2.0	3.5^{abcdef}	4.7^{abcd}	76.0^{abcdefghi}
PI 576375	2.0	4.5^{abcf}	5.7^{a}	36.6^{abcdefghi}
PI 330821	2.0	-	1.0^{h}	-
PI 669704	2.0	3.5^{abcdef}	3.3^{abcdefghi}	62.4^{abcdefghi}
PI 669699	2.0	3.5^{abcdef}	4.3^{abcdefghi}	77.3^{abcdefghi}
PI 665169	2.0	2.8^{bcdef}	3.2^{abcdefghi}	86.4^{abcd}
PI 514406	2.0	-	3.2^{abcdefghi}	-
PI 514562	2.0	3.0^{abcdef}	3.2^{abcdefghi}	78.3^{abcdefghi}
PI 514287	2.0	3.7^{abcdef}	3.5^{abcdefghi}	50.0^{abcdefghi}
PI 660638	2.0	2.8^{bcdef}	2.7^{abcdefghi}	91.7^{abcdefgi}
PI 665167	2.0	3.2^{abcdef}	4.0^{abcdefghi}	70.1^{abcdefghi}
PI 514385	2.0	-	4.0^{abcdefghi}	-
PI 534152	2.0	4.1^{abcdef}	1.7^{defghi}	46.2^{abcdefghi}
PI 514534	2.0	2.3^{cdef}	1.3^{fghi}	84.5^{abcdefghi}
PI 514589	2.0	-	3.0^{abcdefghi}	-
PI 564776	2.0	4.7^{abcdf}	1.2^{ghi}	43.7^{abcdefghi}
PI 514546	2.0	-	1.8^{cdefghi}	-
PI 534151	2.0	4.0^{abcdef}	2.8^{abcdefghi}	36.8^{abcdefghi}
PI 514505	2.0	2.7^{bcdef}	1.7^{defghi}	83.3^{abcdefghi}
PI 514603	2.0	-	1.3^{fghi}	-
PI 514605	2.0	4.2^{abcdef}	3.3^{abcdefghi}	63.5^{abcdefghi}
PI 514551	2.0	2.7^{bcdef}	3.5^{abcdefghi}	83.1^{abcdefghi}
PI 514531	2.0	-	4.7^{abcdef}	-
PI 514613	2.0	3.0^{abcdef}	2.3^{abcdefghi}	49.8^{abcdefghi}
PI 564778	2.0	5.0^{ab}	3.7^{abcdefghi}	47.2^{abcdefghi}
PI 514533	2.0	-	1.5^{efghi}	-
PI 514453	2.0	-	3.3^{abcdefghi}	-
PI 514608	2.0	-	3.8^{abcdefghi}	-
PI 514610	2.0	-	3.4^{abcdefghi}	-
PI 330271	2.0	-	1.2^{cdefghi}	-
PI 514521	2.0	3.0^{abcdef}	2.7^{abcdefghi}	92.2^{abcdefgi}
PI 514455	2.0	-	3.2^{abcdefghi}	-
PI 514411	2.0	2.0^{cdef}	2.3^{abcdefghi}	86.0^{abcdefghi}
PI 330255	2.0	3.7^{abcdef}	4.0^{abcdefghi}	57.0^{abcdefghi}
PI 514318	2.0	2.0^{cdef}	1.0^{cdefghi}	79.1^{abcdefghi}
PI 514365	2.0	2.5^{abcdef}	4.7^{abcdefghi}	50.0^{abcdefghi}
PI 514516	2.0	-	1.3^{bcdefghi}	64.5^{abcdefghi}
PI 533861	2.0	5.0^{ab}	1.7^{abcdefghi}	-
PI 514538	2.0	2.0^{cdef}	1.3^{bcdefghi}	58.0^{abcdefghi}
PI 533792	2.0	-	1.5^{abcdefghi}	36.5^{abcdefghi}
PI 669636	2.0	3.5^{abcdef}	2.0^{abcdefghi}	63.0^{abcdefghi}
PI 267606	2.0	3.3^{abcdef}	2.8^{abcdefghi}	76.8^{abcdefghi}
PI 267655	2.0	-	1.0^{hi}	-
PI 276822	2.0	-	1.8^{bcdefghi}	-
PI 276832	2.0	-	1.0^{cdefghi}	-
PI 329313	2.0	3.0^{abcdef}	3.4^{abcdefghi}	93.4^{abcdefghi}
PI 514279	2.0	3.0^{abcdef}	4.4^{abcdefghi}	55.2^{abcdefghi}
PI 514280	2.0	3.3^{abcdef}	3.2^{abcdefghi}	79.9^{abcdefghi}
PI 514281	2.0	3.0^{abcdef}	3.8^{abcdefghi}	94.7^{abcdefghi}
PI 514285	2.0	5.0^{abcdf}	4.2^{abcdefghi}	21.3^{abcdefghi}
PI 514310	2.0	4.0^{abcdef}	4.5^{abcdefghi}	53.5^{abcdefghi}

Table 1: Continue

Accessions	Anthracnose	Grain mould	Rust	Germination rate
PI 514429	2.0	3.0^{abcdef}	3.4^{abcdefghi}	94.2^{abcdefghi}
PI 514431	2.0	3.0^{abcdef}	4.6^{abcdefghi}	91.9^{abcdefgi}
PI 514454	2.0	-	4.2^{abcdefghi}	-
PI 514456	2.0	3.0^{abcdef}	3.8^{abcdefghi}	74.7^{abcdefghi}
PI 514457	2.0	3.0^{abcdef}	4.2^{abcdefghi}	93.2^{abcdefghi}
PI 514460	2.0	3.0^{abcdef}	3.6^{abcdefghi}	93.0^{abcdefghi}
PI 514471	2.0	-	3.4^{abcdefghi}	-
PI 514472	2.0	-	3.5^{abcdefghi}	-
PI 514595	2.0	-	1.8^{bcdefghi}	-
PI 514597	2.0	2.0^{abcdef}	4.2^{abcdefghi}	76.3^{abcdefghi}
PI 514601	2.0	3.1^{abcdef}	3.8^{abcdefghi}	91.9^{a}
PI 514602	2.0	-	3.0^{abcdefghi}	-
PI 563484	2.0	4.1^{abcdef}	5.3^{abcdefg}	65.0^{abcdefghi}
PI 669702	2.0	3.0^{bcdef}	2.4^{abcdefghi}	77.1^{abcdefghi}

[1]Means within a column with the same letter(s) are not significantly different at the probability level of 5% based on Tukey's HSD test. -: Indicates not tested

Table 2: Mean score for selected resistant accessions against *P. purpurea* and their corresponding mean scores for anthracnose, grain mould and germination rate[1]

Accessions	Rust	Anthracnose	Grain mould	Germination rate
PI 330821	1.0	2.0^{g}	4.0^{abcdef}	45.0^{abcdefghi}
PI 576344	1.0	2.0^{g}	4.0^{abcdef}	73.6^{abcdefghi}
PI 534001	1.0	2.0^{g}	3.7^{abcdef}	28.6^{cdefghi}
PI 330271	1.0	2.0^{g}	-	-
PI 534131	1.0	2.0^{g}	4.0^{abcdef}	26.9^{efgh}
PI 534146	1.0	2.8^{efg}	3.2^{abcdef}	55.5^{abcdefghi}
PI 514318	1.0	2.0^{g}	2.0^{cdef}	79.1^{abcdefghi}
PI 267655	1.0	2.0^{g}	-	-
PI 329697	1.0	2.0^{g}	-	-
PI 665159	1.0	2.0^{g}	-	-
PI 276832	1.0	2.0^{g}	-	-
PI 576379	1.0	2.0^{g}	4.2^{abcdef}	64.0^{abcdefghi}
PI 533903	1.0	2.0^{g}	3.8^{abcdef}	56.4^{abcdefghi}
PI 533800	1.0	4.2^{abcd}	3.8^{abcdef}	44.2^{abcdefghi}

[1]Means within a column with the same letter(s) are not significantly different at the probability level of 5% based on Tukey's HSD test. -: No test results

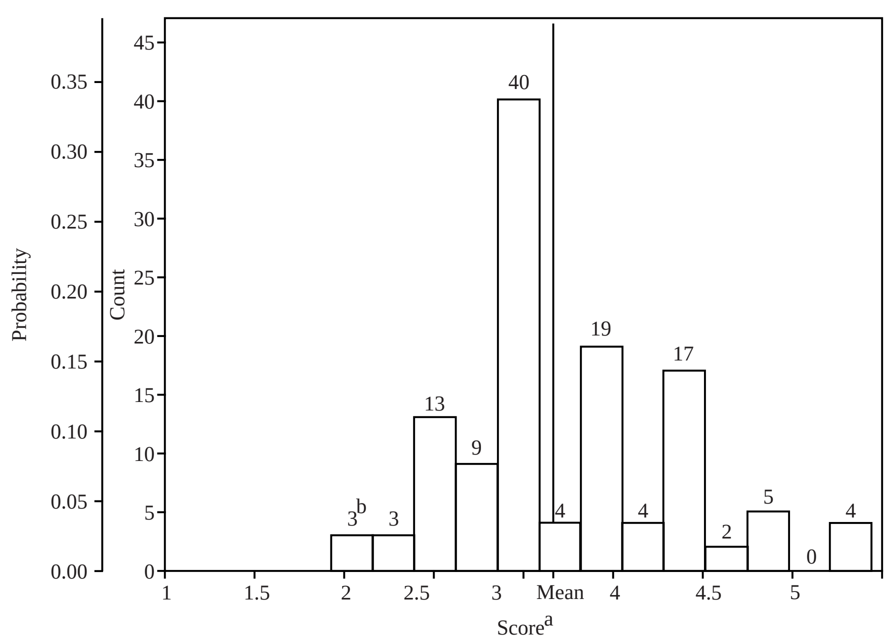

Fig. 3: Phenotype distribution of the reaction or response levels for grain mould

[a]X axis indicates average scores based on disease evaluations and [b]Y axis is the number of the sorghum lines and probability

Table 3: Mean score for the most grain mould resistant accessions and their corresponding mean scores for anthracnose, rust and germination rate[1]

Accessions	Grain mould	Anthracnose	Rust	Germination rate
PI 514411	2.0	2.0^{g}	$2.3^{abcdefghi}$	$86.0^{abcdefghi}$
PI 514538	2.0	2.0^{g}	$1.3^{bcdefghi}$	$58.0^{abcdefghi}$
PI 514318	2.0	2.0^{g}	$1.0^{cdefghi}$	$79.1^{abcdefghi}$
PI 514506	2.33	2.0^{g}	$2.5^{abcdefghi}$	94.5^{abc}
PI 514534	2.33	2.0^{g}	1.3^{fghi}	$84.5^{abcdefghi}$
PI 563482	2.33	3.2^{cdefg}	$2.2^{bcdefghi}$	$73.4^{abcdefghi}$
PI 514474	2.5	2.0^{g}	$2.7^{abcdefghi}$	$90.0^{abcdefghi}$
PI 514606	2.5	2.0^{g}	$3.5^{abcdefghi}$	$83.4^{abcdefghi}$
PI 514365	2.5	2.0^{g}	$4.7^{abcdefghi}$	$49.7^{abcdefghi}$
PI 660637	2.6	2.7^{fg}	$2.0^{bcdefghi}$	87.7^{ab}
PI 267618	2.6	4.0^{abcde}	$4.0^{abcdefghi}$	$68.7^{abcdefghi}$
PI 267624	2.6	2.2^{g}	$3.2^{abcdefghi}$	$79.8^{abcdefghi}$
PI 514308	2.6	2.2^{g}	5.0^{abcd}	$54.8^{abcdefghi}$
PI 656032	2.7	3.0^{edfg}	1.5^{efghi}	$78.1^{abcdefghi}$
PI 514412	2.7	2.2^{g}	5.3^{ab}	$63.4^{abcdefghi}$
PI 514505	2.7	2.0^{g}	1.7^{defghi}	$83.3^{abcdefghi}$
PI 514551	2.7	2.0^{g}	$3.5^{abcdefghi}$	$83.1^{abcdefghi}$
PI 514583	2.7	2.0^{g}	1.7^{defghi}	96.0^{abcd}
PI 514393	2.7	2.0^{g}	$2.0^{bcdefghi}$	$74.0^{abcdefghi}$
PI 660638	2.8	2.0^{g}	$2.7^{abcdefghi}$	$91.7^{abcdefgi}$
PI 514437	2.8	2.0^{g}	4.8^{abcde}	$80.1^{abcdefghi}$
PI 514507	2.8	2.0^{g}	$2.0^{bcdefghi}$	89.4^{abcd}
PI 514398	2.8	2.0^{g}	1.7^{defghi}	82.9^{abcd}
PI 669703	2.8	2.0^{g}	$3.3^{abcdefghi}$	$81.6^{abcdefghi}$
PI 665169	2.8	2.0^{g}	$3.2^{abcdefghi}$	86.4^{abcd}
PI 514399	2.8	2.2^{g}	$3.7^{abcdefghi}$	88.4^{a}
PI 251637	2.8	2.0^{g}	$4.5^{abcdefgi}$	81.9^{abcdef}
PI 267565	2.8	2.0^{g}	1.7^{defghi}	82.8^{abcd}

[1]Means within a column with the same letter(s) are not significantly different at the probability level of 5% based on Tukey's HSD test

Table 4: Most germinated accessions with average germination rate, average scores for anthracnose, grain mould and rust were listed for each accession[1]

Accessions	Germination rate	Anthracnose	Grain mould	Rust
PI 514506	96.5	2.0^{g}	2.3^{cdef}	$2.5^{abcdefghi}$
PI 514347	96.4	2.0^{g}	3.0^{abcdef}	4.7^{abcdef}
PI 514583	96.0	2.0^{g}	2.7^{bcdef}	1.7^{defghi}
PI 514392	95.0	2.0^{g}	3.0^{abcdef}	1.6^{efghi}
PI 514409	94.4	2.0^{g}	3.7^{abcdef}	$3.5^{abcdefghi}$
PI 514612	94.2	2.0^{g}	3.0^{abcdef}	$2.8^{abcdefghi}$
PI 514429	94.2	2.0^{g}	3.0^{abcdef}	$3.4^{abcdefghi}$
PI 514414	93.6	2.0^{g}	3.1^{abcdef}	$2.8^{abcdefghi}$
PI 329313	93.4	2.0^{g}	3.0^{abcdef}	$3.4^{abcdefghi}$
PI 514457	93.2	2.0^{g}	3.0^{abcdef}	$4.2^{abcdefghi}$
PI 514460	93.0	2.0^{g}	3.0^{abcdef}	$3.6^{abcdefghi}$
PI 534116	92.7	2.0^{g}	3.3^{abcdef}	1.5^{efghi}
PI 514521	92.2	2.0^{g}	3.0^{abcdef}	$2.6^{abcdefghi}$
PI 514431	91.9	2.0^{g}	3.0^{abcdef}	$4.6^{abcdefgi}$
PI 514601	91.9	2.0^{g}	3.1^{abcdef}	$3.8^{abcdefghi}$
PI 660638	91.7	2.0^{g}	2.8^{bcdef}	$2.7^{abcdefghi}$
PI 329968	91.3	2.0^{g}	3.0^{abcdef}	$3.5^{abcdefghi}$
PI 514440	91.1	2.0^{g}	3.0^{abcdef}	$3.7^{abcdefghi}$
PI 514348	90.7	2.0^{g}	3.0^{abcdef}	$3.0^{abcdefghi}$
PI 514474	90.0	2.0^{g}	2.5^{abcdef}	$2.7^{abcdefghi}$

[1]Means within a column with the same letter(s) are not significantly different at the probability level of 5% based on Tukey's HSD test

average germination rate was 66.72±1.91 (SE) within the tested accessions (Fig. 4). The data in Table 4 lists the accessions with 90% and above germination rate and their scores for the other traits. PI514506 had the highest germination rate (96.5%), followed by PI514347, PI514583 and PI514392. All the 20 accessions were resistant to anthracnose. Accessions PI514583 and PI514392 with 96.0 and 95.0% germination rates were also resistant to rust.

Correlation analysis: Based on correlation analysis, anthracnose score showed a slight negative significant correlation with rust score with Spearman ρ = -0.16 (Table 5).

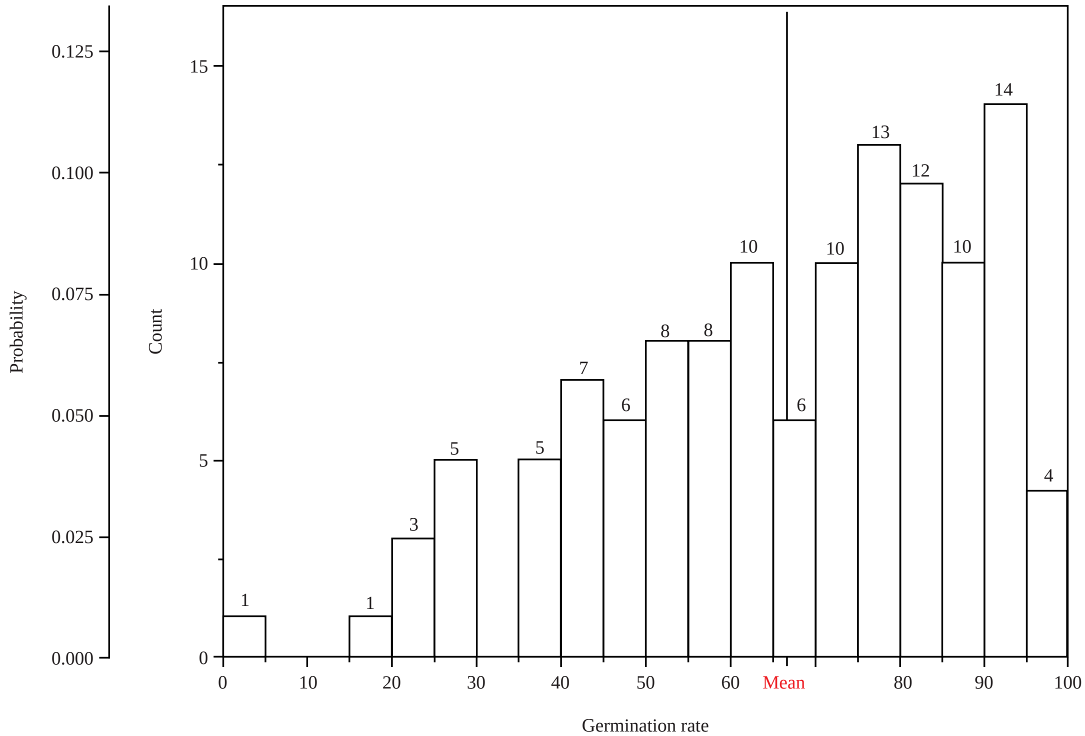

Fig. 4: Phenotype distribution of seed germination rate
[a]X axis indicates the average germination rate and [b]Y axis is the number of sorghum accessions and probability

Table 5: Correlations between two traits (spearman ρ) and p-value

Pairs	Spearman ρ	Probability
Rust-anthracnose	-0.16	<0.0001
Grain mould-anthracnose	-0.10	0.01
Grain mould-rust	-0.05	0.17
Germination rate-anthracnose	-0.18	<0.0001
Germination rate-rust	0.21	<0.0001
Germination rate-grain mould	-0.37	<0.0001

Anthracnose response level was also negatively correlated with grain mould response level (Spearman ρ = -0.10 and p-value = 0.01).

Germination rate was correlated with the sorghum responses to the 3 diseases, a positive correlation was found between germination rate and severity to rust (Spearman's ρ = 0.21 with p-value = <0.0001). In contrast, significant negative correlations were detected between germination rate and severities to anthracnose (Spearman's ρ = -0.18 with p-value = <0.0001) and grain mould (Spearman's ρ = -0.37 with p-value = <0.0001), indicating that the higher the infections due to anthracnose and grain mould the lower the germination rate.

DISCUSSION

The results indicate that the sorghum accessions (Table 1) from various locations tested against the *C. sublineola* isolates common to Isabela, Puerto Rico, may provide good sources for anthracnose resistance, as a majority of the sorghum accessions were not infected. Cuevas *et al.*[23,24] found a large number of anthracnose resistant sources among Ethiopia and Senegal in Puerto Rico. Anthracnose resistant sources have also been documented from different sorghum growing regions[25-27]. Although anthracnose resistant sources have been documented, the hyper-variable nature of the pathogen warrants continuous evaluation from diverse sorghum germplasm to broaden the genetic base[28-33]. The *Puccinia purpurea* disease assay also revealed that several accessions were resistant to rust as well. Over two growing seasons, Cuevas *et al.*[22] reported 12 accessions that were resistant to rust while PI482787 exhibited zero infection. Sharma *et al.*[27] also reported 3 accessions IS 473, IS 23684 and IS 23521 from the sorghum mini-core collection that was resistant to rust, anthracnose and leaf blight. In this study, several accessions, including PI534131, PI576344, PI534001, PI665159 and PI533903 were resistant to both anthracnose and rust (Table 1 and 2). However, many accessions tested in this study were susceptible to grain mould with scores greater than 3. Grain mould resistant sources have been identified in different locations around the world[23,34-38].

Sorghum genotypes ((SV1*Sima/IS23250)-CG1-BGBK-CCBK, R.9732_(adn55*Tx430)-B10 and SRN39_Striga Res. from Southern Africa were shown to be resistant to *F. thapsinum*[34]. Kumar *et al.*[35] identified several grain mould resistant hybrids that included ICSA 101×PVK 801, ICSA 382×GD 65055 and ICSA 400×GD 65028. Over the years, 1000's of photoperiod sensitive accessions had been evaluated at ICRISAT and 156 coloured-grain accessions were shown to possess high levels of resistance to grain mould[38]. Accessions PI533871, PI576130 and PI656036 were resistant when challenged with either *Alternaria alternata* alone or a mixture of *A. alternata*, *F. thapsinum* and *Curvularia lunata*[39]. Histograms that represented the distributions for the 4 traits presented in this study revealed that these phenotypic traits are almost evenly distributed throughout the tested accessions except for *C. sublineola* resistance. Recently, various sorghum populations were evaluated for anthracnose and grain mould resistance and GWAS analyses revealed potential candidate resistance-related genes[24,37,39-42]. For example, it has been applied for anthracnose resistance in Sorghum Association Panel (SAP)[41,42]. mini core lines[43], Ethiopian sorghum lines[24] and Senegal sorghum collections[23,40] and for grain mould SAP responses by Prom *et al.*[39]. Similarly, there are many studies of sorghum grain mould associated with molecular markers to reveal resistance genes in sorghum[37,39,44]. For rust in sorghum, Quantitative Trait Locus (QTL) and association mapping methods were implemented to study rust resistance[45]. Significant QTL and rust resistance QTL were identified and classic rust resistance QTL regions were enriched with the defence-related NBS-encoding gene family[45]. In contrast, multi-trait effect rust resistance QTL wasn't enriched with the defence-related gene[45]. Most of the accessions tested in this study don't have publicly available genotyping information, but once it is available, the phenotypic data presented in this study is expected to serve as a fundamental source for sorghum resistance-related research.

Seed germination rate is an important trait for crop production. This study revealed a moderately negative correlation between germination rate and grain mould severity in sorghum (ρ = -0.37, p-value = <0.0001). Similarly, Prom *et al.*[46] detected a significant negative correlation between grain mould and percent germination rate. Erpelding and Prom[47] noted a significant negative correlation between seed germination and the incidence of *F. semitectum, C. lunata* and *Bipolaris* spp., while Garud *et al.*[48] reported a significant negative association between *Fusarium* spp. infection and seed germination. Furthermore, a weakly negative correlation was detected between germination rate and anthracnose scores (ρ = -0.18). Intriguingly, a moderately positive correlation was found between germination rate and rust response (ρ = 0.21) which could mean rust pathogens can infect sorghum accessions with high germination rates more easily than others and further investigation is needed.

CONCLUSION

This study investigated the response of sorghum accessions from three African countries. The work identified several accessions, including PI576344, PI533903, PI534001 and PI665159 as potential resistant sources against *C. sublineola* and *P. purpurea*, causing anthracnose and rust on sorghum, respectively. Also, accessions PI514318 and PI514538 both from Senegal were found to be resistant to anthracnose, rust and grain mould. In conclusion, these accessions can be used in breeding programs to introgress their resistant genes into elite, parental and in hybrid seed development.

SIGNIFICANCE STATEMENT

Globally, anthracnose, grain mould and rust are major constraints to sorghum production and profitability and the best management strategy is the use of resistant cultivars/lines. This study identified many sorghum accessions that were resistant to anthracnose and rust. Two accessions PI514318 and PI514538 were found to be resistant to anthracnose, rust and grain mould. In addition, many of the identified resistance sources also exhibited high percent germination rates, indicating that these lines will be useful in breeding programs.

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