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Multi-Location and Multi-Year Field Trials Revealed Broad-Spectrum Resistance of Sorghum (Sorghum bicolor (L.) Moench) to Anthracnose (Colletotrichum sublineola)

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ABSTRACT

Ethiopia is one of the centers of origin for sorghum (Sorghum bicolor (L.) Moench), where distinct agro-ecological zones have significantly contributed to its genetic diversity. Although the magnitude varies among the country's regions, sorghum production is severely constrained by anthracnose caused by Colletotrichum sublineola, causing significant grain and biomass losses. This study was conducted to identify sorghum landraces grown in Ethiopia with broad-spectrum resistance and to assess the effect of environments on sorghum anthracnose interaction. In the 2022 cropping season, 285 sorghum accessions, the vast majority of which were landraces, were evaluated at five diverse locations, i.e., Assosa, Bako, Chiro, Haramaya, and Pawe in 2015, 2016, and 2022. Accessions were evaluated according to their initial, final, and mean anthracnose severity scores and the area under the disease progress curve. Analysis of variance (ANOVA) revealed significant differences among accessions, locations, and genotype-by-environment interactions. Based on their reaction to the disease, accessions were categorised into different resistance classes. Most of the accessions were susceptible to anthracnose in the western sites of the country (Assosa, Bako and Pawe) than in the eastern part (Chiro and Haramaya). Among the tested accessions, landraces ETSL100267, ETSL100152, ETSL100388, ETSL100090, ETSL100284, ETSL100107, IS38279, and ETSL101249, and the variety Bonsa were resistant across all locations in the 2022 field trials, suggesting that these landraces might harbour genes with broad-spectrum resistance or have accumulated multiple resistance genes. This study provided insights into the sources of anthracnose resistance and how environmental conditions affect it, which is highly useful for breeders to select germplasm to develop anthracnose-resistant sorghum varieties that are suitable for both specific environments and a broad adaptation.

1 | Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most produced cereal crop globally, following maize, wheat, rice, and barley with a

total production of 58 million tons in 2022 (Faostat 2023). Its versatility and tolerance to different abiotic stresses make it of paramount importance for subsistence farming under low-input conditions in Africa and Asia (Martin 2016). The most common

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producers are small-scale subsistence farmers with minimal access to production inputs (Bantilan et al. 2004). In Africa, sorghum is grown in a large belt that spreads from eastern Africa, bordering the Sahara in the north, to the equatorial forest in the south (Charles et al. 2009). This area extends through the drier parts of eastern and southern Africa, where rainfall is too low for the successful cultivation of maize (Taylor 2003). Sorghum is mainly produced as food and animal feed, as well as a source of fibre (Venkateswaran et al. 2018). In Ethiopia, sorghum is produced in diverse agro-ecologies that vary in rainfall, elevation, temperature, and production constraints. The majority of sorghum grains produced in the country are consumed in the form of traditional bread locally called Enjera. Other food types made from sorghum grains include Nifro (boiled grain), Genfo (porridge), and soups. It is also used to make local drinks such as Tella, Areke, and Borde/Cheka (FAO 2014). About 75% of sorghum produced in the country is consumed at the household level as Enjera, homemade alcohol, and animal feed (USDA 2020).

Despite its role as a major food and feed crop, the production of sorghum is affected worldwide by an array of constraints, among which sorghum anthracnose disease caused by the fungal pathogen (Colletotrichum sublineola) is the major one, especially in the warm and humid regions of the world (Thakur, Rao, et al. 2007; Girma et al. 2021). Although the magnitude varies among regions of the world, the pathogen causes significant losses in sorghum grain yield and quality. The yield loss may reach up to 86% (Prom et al. 2022). The eastern highlands and western regions of Ethiopia are regarded as the most sorghum anthracnose-prone areas in the country. This is primarily due to their favourable environmental conditions that promote pathogen infection, reproduction, and dissemination. C. sublineola has a hemibiotrophic nature of infection (Wharton and Julian 1996). At the initial stage, it exhibits a biotrophic phase of infection that lasts from 24 to 72h (Wharton and Julian 1996; Munch et al. 2008). The biotrophic phase is followed by a necrotrophic phase after the development of secondary hyphae, which involves a cell death (Wharton and Julian 1996). The disease begins with a small red flick at the plants' vegetative stage, which gradually grows into a leaf blight, although the symptoms may vary according to the plants susceptibility and environmental conditions (Chala, Alemu, et al. 2010). The disease causes leaf blight, stalk rot, leaf sheath rot, and grain mould (Thakur, Rao, et al. 2007).

So far, crop rotation, planting date adjustment, cultivar mixture, intercropping, biological control, fungicide applications, and resistant cultivars are the major strategies taken as effective options to manage the disease (Perumal et al. 2009). Due to its economic, environmental, and health impacts, fungicide application is less popular. Currently, the use of resistant varieties is considered as an effective, durable, and sustainable management strategy for this pathogen (Stutts and Vermerris 2020). However, developing resistant varieties against sorghum anthracnose is challenging due to the pathogen's infection variability that requires continuous evaluation and assessment to come up with effective and durable resistant genes (Xavier et al. 2018). Due to the diversity of the crop, Ethiopian sorghum landrace collections are serving as an important source of sorghum improvement (Cuevas et al. 2019). The ability of sorghum varieties to resist the pathogen is often heavily influenced by environmental factors, such as temperature, rainfall, and relative humidity (Xavier et al. 2017). For instance, temperature can affect how different sorghum genotypes respond, potentially making the crop more susceptible to infection if temperatures rise beyond specific thresholds (De Silva et al. 2017; Mewa et al. 2023). Moreover, the pre-penetration and post-penetration stages of infection differ among cultivars (Wharton and Julian 1996). This is likely due to the pathogens virulence genes and their roles during adhesion, infection, and colonisation on the one hand, and the host's growth stage and defence mechanisms on the other hand (Stutts and Vermerris 2020). In both cases, favourable environmental conditions dictate the hostpathogen relationship (Chala, Brurberg, et al. 2010). Therefore, this study was proposed to evaluate the reaction of Ethiopian sorghum landraces against C. sublineola in multi-location and multi-year field trials involving contrasting environments and to come up with a durable source of resistance.

2 | Materials and Methods

2.1 | Plant Material

Two-hundred eighty-five sorghum accessions were used in this study, of which 277 and eight were landraces and improved varieties, respectively. Two-hundred thirty-seven of the 277 landrace accessions were obtained from the Ethiopian Biodiversity Institute (EBI), while 40 were obtained from the Ethiopian Institute of Agricultural Research (EIAR). According to the sorghum racial classifications (Doggett 1988), 102 (35.8%), 82 (28.8%), 62 (21.8%), 25 (8.8%) and 14 (4.9%) of the 285 landraces belong to *dura*, *guinea*, *caudatum*, *kafir* and *bicolor* races, respectively.

2.2 | Experimental Sites and Weather Data

The sorghum landraces were evaluated for their reaction to C. sublineola at five experimental sites in Ethiopia under natural infestation conditions. The experimental sites were Chiro and Haramaya in the eastern part of Ethiopia and Assosa, Bako, and Pawe in the western part. Assosa, Bako, and Pawe are lowlands with hot and humid weather conditions, while Chiro and Haramaya are relatively cool weather conditions, representing major sorghum producing agroecologies of Ethiopia (Table 1). Assosa, Bako, and Pawe are characterised by silt soil, and Chiro and Haramaya by clay soil. The field experiments were conducted in 2015, 2016, and 2022. Two hundred sixty-eight of the 285 accessions were evaluated at Bako in 2015 and 2016, and at Pawe in the 2015 cropping seasons. In the 2022 cropping season, all 285 landraces including PML981476 (a resistant check) and TAM428 (a susceptible check) were evaluated at four locations (Assosa, Chiro, Haramaya, and Pawe). Therefore, 268 accessions were evaluated at two locations in 2015, at one location in 2016, and at four locations in 2022. During the experimental periods, over half of the cultivated land in the districts hosting the experimental sites was planted with sorghum, except for Bako district, where the majority of the area was covered with

TABLE 1 | Geographic descriptions of experimental sites, their weather conditions during experimental years and number of accessions and replications.

Site	Year	Lat (N)	Long (E)	Alt (M)	RF	Max T (°C)	Min T (°C)	RH	# LR	Rep
Assosa	2022	10.02.620	034.33.997	1552	1912.77	27.95	17.40	70.31	285	1
Chiro	2022	09.03.845	040.52.510	1970	684.77	27.84	15.91	56.45	285	2
Haramaya	2022	09 24.991	042 02.164	2010	567.17	28.32	15.27	55.49	285	2
Pawe	2022	11.19.000	036.24.000	1120	1254.00	32.3	17.14	76.50	285	1
Bako	2015	09.05.514	037.02.762	1638	1606.16	24.95	12.55	66.19	268	1
Bako	2016	9.05.514	037.02.762	1638	1924.52	24.23	12.57	69.58	268	1
Pawe	2015	11.19.000	036.24.000	1120	1148.40	32	17.06	84.92	268	1

Abbreviations: #LR, Number of landraces; Alt, altitude in meters above sea level; Lat, latitude; long, longitude; Max *T*, maximum temperature; Min *T*, minimum temperature; Rep, number of replications; RF, annual rainfall; RH, relative humidity.

maize. Records of rainfall, temperature, and relative humidity for Assosa, Pawe, and Haramaya experimental sites were obtained from the Ethiopian meteorological agency, which has meteorological stations near the experimental sites. For Bako and Chiro, the weather data were obtained from the NASA power website (https://registry.opendata.aws/nasa-power, Accessed in October 2024) due to lack of functional weather stations in the areas (Table 1).

2.3 | Experimental Design, Planting and Data Collection

Each landrace was planted in a single 3 m long row. The spacing between rows and between plants within a row was 75 and 15 cm, respectively. The experiments were conducted without within-site replications except for two replications that were used at Chiro and Haramaya in 2022 (Table 1). Planting was done based on local experiences, which was from June 5 to 10 at Assosa, Bako, and Pawe and on May 25 at Haramaya and Chiro across the experimental years. Regular agronomic practices for sorghum cultivation were applied at all sites. Disease assessment was commenced at the booting stage and done four times at 15-day intervals at all sites except Chiro. Due to the absence of the disease until the dough stage at the Chiro site, disease data were collected only once at the dough stage. The modified 1-5 scoring scale (Cota et al. 2017) was used for disease scoring. The scales are as follows: 1 = no chlorotic flecks on the leaf, 2 = flecks on the leaf with no acervuli, 3 = necrotic lesions with acervuli in the lower leaf, 4 = necrotic lesions with acervuli in the lower and middle leaves, and 5 = most leaves are dead due to infection. Scales 1 and 2 were regarded as resistant (R) while 3, 4, and 5 were regarded as susceptible (S) (Prom et al. 2022).

2.4 | Data Analysis

Area under disease progress curve (AUDPC) was calculated from the disease severity data as described by (Madden et al. 2007).

AUDPC =
$$\sum_{i=1}^{n-1} \left[0.5 (x_{i+1} + x_i) \right] [t_{i+1} - t_i]$$

where, x_i is the disease severity percentage at the *i*th observation, t_i is the time of the *i*th observation (time was recorded as a 15 day interval between recordings started from first observation of the disease in most of the landraces at booting stage), and *n* is the total number of observations.

Arcsine transformation was used to normalise the disease severity data. The analysis of variance (ANOVA) on the transformed data was conducted using the PROC GLM statistical procedure of SAS software version 9.4 (SAS Institute). Heatmaps for correlations between plant traits, weather data, and plants' disease reactions were constructed using the "Corrplot" and "gplots" functions in R version 4.2.2 (R Core Team 2018). Graphs that visualise disease progress and weather data were generated using Microsoft Excel.

3 | Results

3.1 | Reaction of Sorghum Landraces

ANOVA showed significant differences (p < 0.05) in final anthracnose severity (FAS) score among landraces, locations, and their interactions at Assosa/Pawe, Chiro and Haramaya in the 2022 cropping season (Table 2). Among the 285 landraces, the number of susceptible landraces varied from 6.67% to 80%. About 228 (80%) of 285 landraces showed a susceptible reaction at Assosa/Pawe and 209 (73.33%) landraces at Haramaya, whereas only 19 (6.67%) landraces showed a susceptible reaction at Chiro. In general, based on FAS, In the 2022 cropping season nine Ethiopian Sorghum landraces (ETSL) (ETSL100267, ETSL100152. ETSL100388, ETSL100090, ETSL100284. ETSL100107, IS38279, ETSL101249, PML981476, and the variety Bonsa) were consistently resistant across the four environments. Most of the landraces, including the susceptible check, TAM428, showed resistance reaction at Chiro. Therefore, at Chiro, most likely the environment could not support the pathogen to infect the landraces.

The mean disease severity score was also used to analyse the field performance of 268 landraces at Bako in 2015 and 2016 and at Pawe in 2015 along with the field performance of landraces at Assosa, Chiro, Haramaya and Pawe in 2022 cropping season

(Figure 1). Among the 268 landraces, the number of susceptible landraces ranged from 6.34% to 92.9%. The most conducive area and year for sorghum anthracnose was Pawe in 2015 where 249 (92.9%) of the landraces showed susceptible reaction. Conversely, at Chiro in 2022 only 17 (6.34%) of the landraces showed susceptible reaction. However, at Assosa and Haramaya in 2022, 63 (23.5%) and 59 (22%) of the landraces showed resistance reaction respectively. This suggests the existence of a good level of genetic variation among the sorghum landraces to *C. sublineola* and enough disease pressure in the experimental sites to evaluate the landraces.

The disease reaction of landraces was assessed by using initial anthracnose severity (IAS), FAS, mean anthracnose severity (MAS), AUDPC and disease incidence. All the parameters revealed a significant difference (p < 0.05) among landraces and locations (Table 3). In the 2022 cropping season, the highest mean disease severity was recorded at Assosa/Pawe (32.6%) followed by Haramaya (24.6%). The lowest mean disease severity was recorded at Chiro (10.6%) (Figure 2). There were also substantial differences in disease severity among the 268

TABLE 2Analysis of variance of 285 landraces based on FAS at Assosa/Pawe, Chiro and Haramaya in the 2022 cropping season. Assosa andPawe locations were used as a replica of each other.

Source	DF	Sum of square	Mean square	F	р
Locations	2	60.34	30.17	397.18	< 0.0001
Landraces	284	45.51	0.16	2.11	< 0.0001
Locations × Landraces	568	48.93	0.086	1.13	< 0.0491

Abbreviation: df, degree of freedom.



FIGURE 1 | Field performance of sorghum landraces (a) at Haramaya in the 2022 cropping season, with Susceptible (ETSL101161), resistant (ETSL101337) and resistant (ETSL101719) reactions, left to right direction, and (b) at Pawe in the 2022 cropping season, susceptible (IS 38353) and moderately resistant (ETSL100310) reactions, left to right direction, and (c) the number of resistant and susceptible landraces out of 268, across seven environments. AS, Assosa; BK, Bako; CH, Chiro; HU, Haramaya; LRs, landraces; PW, Pawe.

TABLE 3	Combined Analysis of variance of	anthracnose severity at Assosa/Pawe and	Haramaya in the 2022 cropping season.
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Parameters	Source	DF	Sum of square	Mean Square	F	р
IAS	AS Locations		9719.40	9719.40	143.97	< 0.0001
	Landraces	284	41,747.11	147.00	2.18	< 0.0001
	Locations×Landraces	284	28,720.09	101.13	1.50	< 0.0001
FAS	Locations	1	18,276.98	18,276.98	262.85	< 0.0001
	Landraces	284	46,831.81	164.90	2.37	< 0.0001
	Locations×Landraces	284	27,592.37	97.16	1.40	< 0.0004
MAS	Locations	1	13,656.00	13,656.00	265.68	< 0.0001
	Landraces	284	39,795.42	140.12	2.73	< 0.0001
	Locations×Landraces	284	22,501.05	79.23	1.54	< 0.0001
Disease incidence	Locations	2	1,437,428.492	718,714.246	3872.08	< 0.0001
	Landraces	284	538,873.289	1897.441	10.22	< 0.0001
	Locations×Landraces	568	884,578.508	1557.357	8.39	< 0.0001
AUDPC	Locations	1	39,371,586.65	39,371,586.65	402.84	< 0.0001
	Landraces	284	89,880,434.74	316,480.40	3.24	< 0.0001
	Locations×Landraces	284	52,688,655.54	185,523.43	1.90	< 0.0001

Note: Disease incidence is computed based on the final disease score at Assosa/Pawe, Chiro, and Haramaya in 2022.

Abbreviations: AUDPC, area under disease progress curve; df, degree of freedom; FAS, final anthracnose severity; IAS, initial anthracnose severity; MAS, mean anthracnose severity.



FIGURE 2 | Mean comparison of (a) final anthracnose severity (FAS) and (b) disease incidence over three locations. Means covered by the same bar are not significantly different at (p < 0.05) (AsPw, Assosa and Pawe; CH, Chiro; HU, Haramaya).

sorghum landraces across the seven locations. The highest mean disease severity 64.8% was recorded at Pawe in 2015, followed by Bako in 2016 (45.6%) and Pawe in 2022 (36.4%). The lowest mean disease severity was exhibited at Chiro in 2022 (11.7%) followed by Haramaya in 2022 (19.5%) and Bako in 2015 (34.3%).

In the 2022 cropping season, disease incidence also significantly (p < 0.05) varied between landraces and locations (Figure 2b). The highest disease incidence was observed at Assosa/Pawe (77.3%) followed by HU (27.4%) and CH (8.6%).

Due to onetime anthracnose score at Chiro, AUDPC was computed only in two locations (Assosa/Pawe and Haramaya). In general, AUDPC showed significant variation (p < 0.05) among landraces and locations (Table 3). The AUDPC values varied from 494 (landrace ETSL100388) to 1925 (landrace ETSL101388) at Assosa/Pawe and from 69 (variety Bonsa) to 2290 (landrace ETSL101388) at Haramaya in the 2022 cropping season. The mean anthracnose score rate, which was determined by calculating the slope of the regression line for disease severity over 15-day intervals, also revealed a significant and positive correlation at both locations (Figure 3).

3.3 | Environment and Disease Pressure

Total amount of rainfall and its distribution, temperature and relative humidity considerably varied across locations and years (Figure 4). The highest annual rainfall was recorded at Bako in 2016 and Assosa in 2022, which was 1924.5 and 1912.8 mm, respectively. On the other hand, the lowest rainfall (567.17 mm) was received by Haramaya in 202. Relative humidity ranged from 55.49% at Haramaya in 2022% to 84.92% at Pawe in 2015.



FIGURE 3 | The disease progress curves of sorghum anthracnose recorded in 15 days interval at Haramaya (red line) and Assosa/Pawe (blue line) in 2022 cropping season.



FIGURE 4 | Trends showing weather conditions: (a) Temperature over locations and years, (b) rainfall intensity over locations and years, (c) relative humidity over locations and years and (d) mean annual relative humidity of each location and year. AS, Assosa; BK, Bako; CH, Chiro; HU, Haramaya; PW, Pawe.

The disease pressure was also high in all the environments except Chiro in 2022, at which disease was observed only at dough stage. The disease severity varies in accordance with temperature and relative humidity, and as temperature increases, the disease severity increases. For instance, at Pawe in 2015, more than 92.9% of the landraces showed susceptible reactions and, in that year and location, the temperature and relative humidity of the season were higher than all locations and years (Figure 4a,c). In 2016 at Bako, the rainfall was higher than Pawe in 2015; however, the mean disease severity at Bako in 2016 was 45.6% while the mean disease severity at Pawe in 2015 was 64.8%. Therefore, rainfall alone could not influence the interaction between the host and the pathogen. Moreover, in the main cropping season (May to November) the highest monthly mean

rainfall was recorded at Bako in 2016 (248.85mm) followed by Bako in 2015 (211.27mm) and Assosa in 2022 (201.96mm) (Table 3). Whereas, the highest relative humidity was recorded at Pawe in 2015 (90%) followed by Pawe in 2022 (80.43%) and Bako in 2016 (78.89%). However, the highest mean disease severity was recorded at Pawe in 2015 (64.79%) followed by Bako in 2016 (45.59%). Here, Relative humidity played a significant role in disease development than rainfall and temperature. This substantial impact of environmental factors on the observed variation in disease severity suggests that the sorghum landraces and the pathogen interaction were highly dependent on environmental conditions, particularly relative humidity and temperature (Figure 4a,c).

3.4 | Correlation Between Disease Severity and Weather Data

Anthracnose severity showed a significant positive correlation with relative humidity (r=0.92, p < 0.01) while it had a significant negative correlation with days to flowering (r=0.78, p < 0.05) and the number of resistant landraces (r=0.75) (Figure 5). A significant negative correlation was also exhibited between relative humidity and days to flowering (r=0.89, p=0.05) in which landraces with a long maturity period could counter higher disease severity. Relative humidity was also negatively correlated with the number of resistant landraces (r=0.61).

3.5 | Temporal Dynamics of Sorghum Anthracnose Over Years and Locations

The disease reaction majorly varies in two contrasting environments (Chiro and Haramaya) in the east and (Assosa, Bako and Pawe) in the west. Resistant reaction scored 1 and 2 (Blue), medium resistance reaction 3 (white) and susceptible reaction 4 and 5 (Red) (Figure 6). In the overall disease assessment, the highest disease severity was observed at Pawe in 2015, followed by Bako in 2016 and Pawe in 2022. Inconsistent numbers of resistant landraces were recorded at the same locations but in different years. For instance, at Bako in 2015, 121 (45.2%) landraces exhibited resistance reaction, whereas only 38 (14.2%) landraces exhibited the same (resistant) reaction at the same location in 2016. Similarly, at Pawe in 2015 and 2022, 19 (7%) and 54 (20%) of the landraces exhibited resistance reaction, respectively. There was no visible relationship between sorghum race types and disease reaction of the landraces. Based on the race grouping, guinea was the most susceptible race type at Pawe in 2015, for which only 5% of the landraces were resistant compared to race caudatum, which had 9.3% resistant landraces. However, at Pawe in 2022, race group caudatum was the most susceptible race with 35.2% resistant landraces compared to kaffir, from which 59.1% of the landraces showed resistance reaction. At Bako in 2015, races bicolor, guinea and kaffir showed nearly similar and better resistance reaction compared to caudatum and durra. A similar manner of reaction was also observed at



FIGURE 5 | Correlation between plant traits, disease severity, environmental factors, and number of susceptible and resistant genotypes PH, plant height; R_GTs, number of resistant genotypes; S_GTs, number of susceptible genotypes; RH, relative humidity; *T*, temperature; and DTF, days to flower.



sorghum_Anthracnose



FIGURE 6 | A heat map showing the reaction of sorghum Landraces against sorghum anthracnose over locations and years. The severity score is shown as a gradient colour, with resistant reactions marked as Blue (1 and 2) and susceptible reactions marked as white (3) and red (4 and 5). AS22, Assosa 2022; BK15, Bako 2015; BK16, Bako2016; CH22, Chiro 2022; HU22, Haramaya 2022; PW15, Pawe 2015; PW22, Pawe 2022.

Bako in 2016, where the disease was more severe than at Bako in 2015. At Haramaya in 2022, 71.4% of bicolor and 92.5% of guinea landraces were resistant.

4 | Discussion

In the past several decades, various studies have been conducted on sorghum anthracnose caused by C. sublineola, and corresponding resistance genes in sorghum (Mohan et al. 2010; Mace et al. 2014; Burrell et al. 2015; Stutts and Vermerris 2020). Furthermore, sorghum landraces and wild relatives have been evaluated and QTL regions related to sorghum anthracnose resistance were mapped (Cuevas et al. 2019; Ahn et al. 2021, Girma et al. 2021). However, only a few of the sorghum landraces were reported as consistently and stably resistant against sorghum anthracnose (Prom et al. 2023). This could be due to the coevolution of the pathogen strains with the host leading to selection pressure of the pathogen in response to diverse hosts (Tesso et al. 2012). These kinds of selection pressures could allow the pathogen to overcome a defence from the host, which is a complex process that occurs on many levels, at which environmental pressure affects each level of change (de Crecy et al. 2009; Tesso et al. 2012). On top of that, most of the identified markers are linked to QTL regions, making it challenging to validate them across contrasting environments (Abreha et al. 2021).

In this study, 285 Ethiopian sorghum landraces and cultivars were evaluated over years and locations. Results revealed significant variations across landraces, locations, and their interactions in terms of anthracnose incidence and severity. In general, anthracnose incidence was higher at Assosa and Pawe than at Haramaya and Chiro. In addition, the highest disease severity was recorded at Pawe in 2015 (64.8%) followed by Bako in 2016 (45.6%). The considerable disease severity and incidence variation recorded between years and locations could be attributed to differences in weather conditions (Chala, Brurberg, et al. 2010). It has also attributed variation in anthracnose incidence and severity to the variation in the pathogen virulence level and/or the variation of environmental factors, particularly high relative humidity and temperature (Romero et al. 2022). For instance, the relative humidity at Pawe in 2015 and Assosa in 2022 was 84.9% and 70.3%, respectively, whereas at Chiro and Haramaya in 2022 it was 56.5% and 55.5%, respectively. On the other hand, the mean annual temperature at Pawe in 2015 and Assosa, Chiro, and Haramaya in 2022 was 32°C, 27.95°C, 27.8°C, and 28.3°C respectively. On the other hand, there was a variation over years at Bako and Pawe, suggesting the need for breeding towards durable resistance (Thakur et al. 2009).

Association of the weather towards the resistance or susceptibility of the landraces were clearly visible in the two contrasting environments. In the 2022 cropping season the average relative humidity and temperature in the western part of the country (Assosa and Pawe) was 73.4% and 30.13°C, whereas, in the eastern part of the country (Chiro and Haramaya) exhibited 56% and 28.08°C respectively. In line with the variation of disease severity in the two environments (32.6% in the west and 17.5% in the east parts of the county). Changes in gene expression is one of the most rapid host responses early after infection and could suggest critical genetic players in resistance or susceptibility to infection (Herman and Williams 2015; Fu et al. 2020). And a battery of defence responses of sorghum is transcriptionally regulated resulting in the accumulation of pathogenesis related proteins, secondary metabolites and signalling components and these resistance pathways are affected by environmental factors (Velásquez et al. 2018; Fu et al. 2020). Mewa et al. (2023), reported that when temperature raised from a certain level, the sorghum crop becomes susceptible to the *C. sublineola*. The sorghum landraces reacted differently in the seven environments along with environmental variation and may be also pathogen population structure. The population structure of *C. sublineola* varies across distinct geographic regions even in the same location and changes with crop growing seasons, Consequently, improved varieties resistant to *C. sublineola* should possess broad-spectrum resistance (Abreha et al. 2021; Mekonen et al. 2024).

Due to the change of environment, a similar manner of variation could be observed in the pathogen population structure and the pathogenicity pathways. In addition, the initial inoculum at the experimental site could play an important role as it could affect the next generation. For example, warm temperatures shorten the pathogen incubation period and increase the number of generations of the pathogen, leading to increased abundance of the pathogen over a growing season (Singh et al. 2023). Similarly, Moraes et al. (2015) suggest that temperature and wetness duration could increase conidial germination and appressorial melanization during the pre-penetration stage of *Colletotrichum gloeosporoides* on Guava. In general, the dynamics of interactions between the host and the pathogen change with the change of moisture and temperature (Burrell et al. 2015).

Anthracnose severity showed a significant positive correlation with relative humidity. These results suggest that relative humidity may play a more crucial role in disease development and severity than temperature or rainfall, as it creates an environment conducive for the pathogen to infect landraces. Consequently, this favourable humidity could also enhance the production of proteins associated with pathogenicity (Buiate et al. 2017). On top of that, the level of relative humidity and temperature during infection could impact the plant to susceptibility (Prom et al. 2015). For instance, a 5°C temperature fluctuation in the interaction between Phytophthora infestans and potato made the potato more susceptible than daily constant temperatures (Shakya et al. 2015). Conversely, Chen et al. (2024) reported that the infection rate decreased with the increase of temperature in anther smut fungi. In such cases, the level of tolerance of the crop and pathogen to temperature should be well-defined. A susceptible host will not be infected by a virulent pathogen if the environmental conditions are not conducive to the pathogen. This might be due to the pathogen's virulence-related proteins, which are directly affected by changes in the environment (Shao et al. 2021; Kumar et al. 2024). Furthermore, The successful establishment as well as the overall reproduction and survival of the pathogen is primarily determined by its effector proteins (Lu et al. 2022), which are significantly influenced by temperature and humidity (Xavier et al. 2017; Velásquez et al. 2018). Moreover, pathogen infection could be limited by leaf surface wetness and humidity. In previous studies conducted on C. acutatum isolates, conidia germination and formation of appressoria was favoured by an increase in wetness period (Moral et al. 2012). On the other hand, Salotti et al. (2022) reported that C. sublineola mycelial growth, conidial germination, infection, and sporulation were highly dependent on temperature. In addition, Sorghum utilises secondary metabolites to defend against

pathogens by accumulating phytoalexins in the area of infection (Liu et al. 2010) and the effectiveness and regulation of these metabolites can be influenced by humidity and water availability (Velásquez et al. 2018).

In the 2022 cropping season, landraces ETSL100267, ETSL100152, ETSL100388, ETSL100090, ETSL100284, ETSL100107, IS38279, ETSL101249, and PML981476 and the variety Bonsa were resistant against sorghum anthracnose at four locations. So, those lines were fairly adapted to test all locations and exhibited that they could be used for resistance breeding for a wider adaptation and could be used as a source material for the development of varieties against sorghum anthracnose. However, the reaction of most landraces varied with the variation of the environment and the significant interaction between landraces and environments supports breeding for specific adaptation (Thakur et al. 2009). The five botanical sorghum races (102 Dura, 82 Guinea, 62 Caudatum, 25 Kafir and 14 Bicolor) were included in the study materials. Environmental conditions significantly influenced the host-pathogen interaction, leading to disease development. However, there was no relation between the race type and level of disease reaction. In all the experimental sites, usually the main cropping season stays for 6–7 months, which starts from the end of May to the end of November. Disease severity varied across experimental sites and years, as environmental factors potentially affected the pathogen's ability to regenerate, infect, proliferate, and spread. On top of that, the effect could be extended beyond the main cropping season, as a pathogen inoculum survives overwinter in soil and crop residue (Munch et al. 2008). The level of primary inoculum sources from the crop residues in the soil and environmental conditions before the cropping season could also influence the intensity of the disease in the main cropping season (Behnke 2023).

In this study, the impact of the environment on the hostpathogen interactions was evaluated across three locations during a 1-year observation period and two locations over a 2year period. Overall, climate change could increase outbreak risks by altering pathogen evolution, affecting host-pathogen interactions, and facilitating the emergence of new pathogenic variants (Singh et al. 2023). However, to gain a comprehensive understanding of the response of landraces and the pathogen population, additional factors may need to be considered. For example, Conditions that are adverse to the crop, like weather and nutrients, could weaken the crop to the pathogen (Tesso et al. 2012). Furthermore, the effect of soil and plant microbiomes could also have a significant contribution towards changing the host response as they prime the plant immune system (Andersen et al. 2018; Singh et al. 2023). The presence of wild relatives in the surroundings of experimental sites might also aggravate the situation. On the other hand, Mewa et al. (2023) reported that nucleotide-binding leucine-rich repeat mediated resistance is modulated by temperature and confirmed the stability of genes at high temperatures. While the environment significantly influenced host-pathogen interactions, it may be even more crucial during the pathogen's initial contact and establishment with the host. Furthermore, the pathogen could lead to disease epidemics and total crop loss in rainy and humid weather conditions (Salotti et al. 2022). In conclusion, for the development of disease, a conducive environment could have a significant influence than a virulent pathogen and a susceptible host (Buiate et al. 2017).

5 | Conclusions

The 285 accessions studied in multi-location field trials over years exhibited differential reactions to anthracnose. However, accessions consistently demonstrated resistance across environments, suggesting that these landraces may possess broad-spectrum resistance genes or have accumulated multiple resistance genes. Temperature and relative humidity were major environmental factors that affect the plants response to the pathogen and increased the severity of the disease in this study. Therefore, developing anthracnose-resistant varieties for specific environments could be the most effective breeding strategy to achieve stable and durable resistance. In addition, climate change will likely affect temperature and relative humidity in sorghum growing regions where anthracnose is a serious problem, potentially facilitating pathogen strain diversification and virulence. Therefore, continuous evaluation of sorghum landraces and wild relatives would be imperative to identify sources with resistance genes and the introgression of these genes into elite varieties. In future research that involves the evaluation of host-pathogen interactions at specific locations, it is essential to examine not only the genetic diversity of landraces and the target pathogen but also environmental changes, soil and plant microbiomes, and the crop's developmental stages. Furthermore, research on how environmental factors influence sorghum defence mechanisms and virulence-promoting proteins of C. sublineola may provide valuable insights into effective management strategies for this pathogen, thereby promoting sustainable sorghum production as stable crops for millions.

Author Contributions

M.M.: conceptualization, resource, supervision, experimentation, data collection, data analysis, preparation of the draft manuscript, review and editing. A.C., K.T. and Ti.M.: conceptualization, supervision, experimentation, review and editing. Te.M., H.N., A.T., G.G. and Z.M.: conceptualization, resource, supervision, experimentation, data analysis, review and editing. H.A. and B.K.: supervision, experimentation, data collection and review and editing. M.G.: resource, supervision, data analysis, preparation of the draft manuscript, review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original datasets analysed and generated in this study are included in the article; further inquiries can be directed to the corresponding author.

Peer Review

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