STUDIES ON THE *COLLETOTRICHUM SUBLINEOLUM* (P. HENN)-*SORGHUM BICOLOR* (L. MOENCH) PATHOSYSTEM IN ETHIOPIA

STUDIER AV PATOSYSTEMET *COLLETOTRICHUM SUBLINEOLUM* (P. HENN) -*SORGHUM BICOLOR* (L. MOENCH) I ETIOPIA

Philosophiae Doctor (PhD) Thesis

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Ås 2010

Thesis number 2010: 19
ISSN 1503-1667
ISBN 978-82-575-0930-9

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Acknowledgments

This PhD study has given me the chance to meet several people, who were very much collaborative and willing to help at times of difficulties. I would like to express my deepest gratitude to Professors Anne Marte Tronsmo and May Bente Brurberg for their excellent supervision of the thesis and kind help throughout my four year study period. I am very grateful to Dr. Arne Stensvand for his valuable comments and suggestions. I also thank Dr. Leif Sundheim for his kind help in translating the title and abstract of the thesis to Norwegian. Drs. Ferdu Azerefegne and Tameru Alemu have given me useful advice during the field work for which I am grateful. Dr. Ferdu Azerefegne also provided me with logistic support and additional funding for field experiments. I thank Alemayehu Getachew, Dawit Kassa, Paulos Dana, Simon Atsbaha and Tesfaye Shiferaw, who helped me a lot during the field work.

The laboratory experiments reported in this thesis were conducted at the Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division. I acknowledge the staff members of the institute in general and the biotechnology section in particular for welcoming me and stepping in to help whenever I was in need of one. Special thanks to Drs. Abdelhameed Elameen and Xiaoren Chen, Aruppillai Suthaparan, Jafar Razzaghian, Grete Lund, Heidi Udens Aamot, Hege, Jihong Liu Clarke, Monica Skogen and Sonja Klemsdal for all the help they rendered me throughout my stay at the institute.

I thank Dr. Louis K. Prom, Mr. Asfaw Adugna and the various centers of the Ethiopian Agricultural Research Institute for providing seeds of different sorghum accessions used in the field experiments. Ethiopian sorghum farmers in different regions also deserve my sincere appreciation for allowing me to enter their field for data collection and harvest leaves from the plants that are dearest to them.

I also acknowledge the International Crops Research Instutite for the Semi-Arid Tropics for allowing me to use the picture on various sorghum heads. I thank Lånkassen, Norwegian Agency for Development and Cooperation, Development Innovation Fund and the Research and Extension Office of Hawassa University for financial support during my PhD studies.

My colleagues in Norway and Hawassa: Ajebu Nurfeta, Ambachew Demisse, Ayana Angassa, Belachew Asalif, Birhanu Abate, Etana Debela, Ermias Deribe, Fassill Bekele, Fitsum Teklay, Solomon Eyob, Tamerat Andarge, Waktole Tiki and Yibrah Beyene, I thank

you very much for the nice time we spent together. I really have enjoyed our being together and learnt a lot from your good personalities for which I am grateful.

My late father, Chala Heyie, and my mother, Meaza Lemma, gave all what they have to see their little boy grow up. So I sincerely acknowledge them for the indispensable role they played in my life.

My wife, Firehiwot Hailu, and my kids (Hilina and Yonas) inspired me all the time with a truely great love and heartfelt good will. I sincerely thank them for their patience and persistent encouragement.

Abstract

This thesis covers the assessment of anthracnose incidence and severity in Ethiopia, characterisation of *Colletotrichum sublineolum* isolates from different sorghum regions of Ethiopia, studying the impact of four selected host genotypes and weather variables on the severity and temporal dynamics of anthracnose, and an evaluation of Ethiopian sorghum accessions for resistance to anthracnose. For these purposes, field surveys were carried out, *C. sublineolum* isolates were characterised using phenotypic and molecular markers, and two separate field experiments were conducted.

The field surveys were conducted in the cropping seasons 2005 and 2007, in 49 districts of six geographic regions. Anthracnose was observed in 41 (84%) of the surveyed districts but both disease incidence and severity varied significantly among the survey districts, geographic regions, altitude groups and climatic zones. Anthracnose incidence ranged from 0 to 77% while severity varied between 0 and 59% across the survey areas. Disease incidence and severity were significantly higher in the lowland (<1500 masl (meters above sea level)) and intermediate altitude areas (1500-2000 masl) as compared to the highlands (>2000 masl). Anthracnose severity was the highest (ca. 40%) in areas characterised by high rainfall (>1200 mm/annum) and intermediate temperature (16-30°C). Areas with moderate rainfall (800-1200 mm/annum) and intermediate or high (>30°C) temperature had a much lower anthracnose severity (<20%). Correlation analysis revealed a significantly positive relationship between rainfall and anthracnose development while temperature did not have a significant effect.

In order to study the diversity within *C. sublineolum* from Ethiopia, sorghum leaves showing anthracnose symptoms were collected from five sampling sites (North, East, South, Southwest 1 and Southwest 2) in four geographic regions, and single spore isolates were categorised into five groups based on their sampling origin. For phenotypic characterisation 50 randomly selected single spore isolates (10 per sampling site) were cultured on PDA plates at 25ºC with four replications. There was significant variation among the isolates in growth rate (1.7-5.8 mm/day, P = 0.0023). However, colony colour and margin showed little variations.

For molecular characterisation, amplified fragment length polymorphism analysis (AFLP) was conducted on 102 isolates using six primer combinations. The isolates were highly diverse as revealed by Dice similarity coefficients between individual isolates (0.32-0.96, mean 0.78), average gene diversity for each isolate-group (0.14-0.23, mean 0.19) and

proportion of polymorphic peaks per isolate-group (44-68%, mean 58%). Pair-wise genetic distances, and cluster and principal coordinate analyses suggested the genetic separation of the Southern and Eastern isolates from the other groups and also from each other. Overall there was high genetic variation (F_{ST} = 0.42) and limited gene flow (Nm = 0.34), and hence such variations should be given due considerations in future sorghum breeding programmes.

The impact of four selected host genotypes and weather variables on anthracnose development was studied in a two year field experiment in southern Ethiopia. The four sorghum genotypes (AL70, BTx623, 2001 HararghieColl No. 12 and 2001 PWColl No. 022) were planted in a randomised complete block design with four replications. Initial, final and mean anthracnose severities; and area under the disease progress curves were used as evaluation criteria. Highly significant variations were found among the tested genotypes regardless of the evaluation criteria and experimental year confirming the impact of host genotypes on anthracnose. Anthracnose appeared earlier and progressed rapidly on the susceptible genotype (BTx623) while it appeared late and progressed slowly on the resistant genotype (2001 PWColl No. 022). Initial, final and mean anthracnose severities were all lowest (0.88, 7.56 and 3.57 %, respectively) on 2001 PWColl No. 022 and highest (16.13, 78.38 and 46 %, respectively) on BTx623 across the years. The remaining two genotypes (2001 HararghieColl No. 12 and AL70) had intermediate responses. Correlation and regression analyses revealed a significant impact of rainfall on anthracnose development while temperature appeared to have a non significant effect further confirming the results from the field survey.

In another field experiment, a total of 56 Ethiopian sorghum accessions and two susceptible checks (AL70 and BTx623) were evaluated for resistance to anthracnose. Final anthracnose severity (FAS) at 140 days post planting, relative area under the disease progress curve (rAUDPC) and disease progress rate (R) were used as evaluation parameters, and they all varied significantly among the tested accessions. FAS varied between 6.6 and 77.7% while rAUDPC and R ranged from 0.1 to 1.7 and from 0.01 to 0.06 units/day, respectively, over the two years. Analysis of variance revealed significant impact of experimental years, and accession by year interaction on anthracnose development suggesting lack of stable reaction by some accessions. Correlations between the evaluation parameters were high and hence final anthracnose severity was recommended as an efficient and time saving parameter in anthracnose resistance evaluations.

All the Ethiopian accessions had significantly lower disease level compared to the susceptible check (BTx623) regardless of the evaluation parameter and experimental year. Fifteen and 18 accessions were rated resistant to anthracnose in 2007 and 2008, respectively, of which six accessions were consistently resistant in both years. The current results suggest the potential Ethiopian sorghum genotypes may have in serving as sources of resistance to anthracnose.

Sammendrag

Avhandlingen inneholder en kartlegging av forekomst og angrepsgrad av antraknose i Etiopia, en alvorlig sjukdom på sorgum. Isolater fra den patogene soppen *Colletotrichum sublineolum* samlet i ulike etiopiske sorgumregioner ble karakterisert. Effekter av fire utvalgte sorgum genotyper og klima på antraknose ble studert og resistens mot sjukdommen i etiopiske sorgumlinjer ble bestemt. Soppen ble kartlagt i felt og isolater ble karakterisert ved hjelp av fenotype og molekylære markører. To feltforsøk ble gjennomført.

I vekstsesongene 2005 og 2007 ble sjukdommen kartlagt i 49 distrikter lokalisert til seks geografiske regioner. Antraknose ble funnet i 41 (84 %) av de kartlagte distriktene, men det var signifikante forskjeller i både forekomst og angrepsgrad mellom distrikter, geografiske regioner, høydenivåer og klimasoner. Forekomsten av antraknose varierte fra 0 til 77 %, mens angrepsgraden varierte mellom 0 og 59 % i distriktene som ble kartlagt. Forekomsten av antraknose var signifikant høyere i lavlandet (<1500 moh (meter over havet)) og i middels høgder (1500 – 2000 moh) sammenlignet med høglandet (>2000 moh). Angrepene av antraknose var sterkest (ca 40 %) i nedbørrike distrikter (>1200 mm årlig) og middels temperatur (16 – 30°C). Distrikter med middels nedbør (800 – 1200 mm) og middels eller høg (>30°C) temperatur hadde mye svakere angrepsgrad (<20%). Det var signifikant positiv korrelasjon mellom nedbør og antraknoseangrep, mens det ikke var signifikant virkning av temperatur.

For å studere variasjonen innen *C. sublineolum* i Etiopia, ble sorgumblad med antraknose-symptomer samlet inn fra fem lokaliteter (nord, øst, sør, sørvest 1 og sørvest 2) i fire geografiske regioner. Ensporeisolater ble delt i fem grupper basert på geografisk opprinnelse.
For fenotypisk karakterisering ble 50 tilfeldig valgte ensporeisolater (10 fra hver prøvetatt lokalitet) dyrket på PDA ved 25°C med fire gjentak. Det var signifikante forskjeller i vekstrate mellom isolatene (1.7-5.8 mm/daglig, P=0.0023). Men det var liten forskjell mellom kulturene i farge og veksttype.

"Amplified fragment length polymorphism" (AFLP) med seks primerkombinasjoner ble brukt til molekylær karakterisering av 102 isolater. Den store diversiteten mellom isolatene ble vist ved Dice likhetskoeffisient mellom enkelt isolater (0.32-0.96, gjennomsnitt 0.78), gjennomsnittlig gendiversitet for hver isolatgruppe (0.14-0.23, gjennomsnitt 0.19) og andelen polymorfe topper for hver isolatgruppe (44-68%, gjennomsnitt 58%). Parvis genetisk distanse,

samt kluster og prinsippiell koordinatanalyse indikerte genetisk separasjon både mellom sørlige og østlige isolater, og mellom disse isolatene og isolater fra andre geografiske områder. Det ble påvist stor genetisk variasjon (F_{ST} = 0.42) og begrenset genflyt (Nm = 0.34). Denne variasjonen bør vektlegges i framtidig foredling for resistens mot antraknose.

Virkningen av fire sorgum genotyper og klimavariabler på antraknose ble studert i feltforsøk gjennom to vekstsesonger i Sør-Etiopia. De fire genotypene (AL70, BTx623, 2001 HararghieColl No. 12 og 2001 PWColl No. 022) ble plantet i randomiserte, komplette blokkforsøk med fire gjentak. Antraknoseangrepene tidlig og sent i sesongen samt gjennomsnitt ble bestemt. Arealet under kurven for sjukdomsutvikling ble også brukt til vurdering av genotypene. Det var sterk signifikant variasjon mellom genotypene uavhengig av vurderingskriterier og forsøksår. Dette bekreftet betydningen av genotypene for resistens mot antraknose. Sjukdommen startet tidligere og utviklet seg raskere på den mottakelige genotypen (BTx623), mens den kom senere og utviklet seg saktere på den resistente genotypen (2001 PWColl No. 022). Tidlige, sene og gjennomsnitt angrep av antraknose var svakest (henholdsvis 0.88, 7.56 og 3.57%) på 2001 PWColl No. 022 og sterkest (henholdsvis 16.13, 78.38 og 46%) på BTx623 over år. Angrepsgraden på de to andre genotypene 2001 HararghieColl No 12 og AL70) lå mellom de to ytterpunktene. Korrelasjons- og regresjonsanalyser viste signifikant virkning av nedbør på utviklingen av antraknose, mens temperaturen ikke hadde signifikant virkning. Disse resultatene samsvarer med data fra kartleggingen i felt.

I et annet feltforsøk ble 56 etiopiske sorgumlinjer og to mottakelige kontroller (AL70 og BTx623) bedømt for resistens mot antraknose. Angrepsgrad ved slutten av sesongen (FAS) 140 dager etter planting, relativt areal under kurven for sjukdomsutvikling (rAUDPC) og fart på sjukdomsutviklingen (R) ble brukt som parameter ved vurderingen. Det var signifikant variasjon mellom linjene for alle disse parametrene. Gjennom to år var FAS mellom 6.6 og 77.7%, rAUDPC var mellom 0.1 og 1.7 og R varierte mellom 0.01 og 0.06 enheter/dag. Variansanalyse viste signifikant virking av forsøksår og samspill mellom år og sorgumlinje. Dette tydet på ustabil reaksjon hos noen linjer. Det var sterk korrelasjonen mellom vurderte parmetre. Derfor kan vurdering ved slutten av vekstsesongen tilrås som effektiv og tidsbesparende evaluering av resistens mot antraknose.

Alle etiopiske linjer hadde signifikant svakere angrep av antraknose enn den mottakelige kontrollen (BTx623) ved alle vurderingskriterier og i begge forsøksår. I 2007 ble 15 linjer

vurdert som resistente, mens i 2008 ble 18 linjer bedømt som resistente. Seks linjer var resistente begge år. Resultatene viser at etiopiske sorgumlinjer har potensial som kilder for resistens mot antraknose.

List of papers (I-IV)

I. A. Chala, M.B. Brurberg and A.M. Tronsmo. 2010a. Incidence and severity of sorghum anthracnose in Ethiopia. Plant Pathology Journal 9 (1): 23-30.

II. A. Chala, A.M. Tronsmo and M.B. Brurberg. 2010. AFLP analysis revealed a high level of genetic differentiation and low gene flow in *Colletotrichum sublineolum* in Ethiopia, the center of origin and diversity of sorghum. (Manuscript).

III. A. Chala, T. Alemu, L.K. Prom and A.M. Tronsmo. 2010b. Effect of host genotypes and weather variables on the severity and temporal dynamics of sorghum anthracnose in Ethiopia. Plant Pathology Journal 9 (1): 39-46.

IV. A. Chala and A.M. Tronsmo. 2010. Evaluation of Ethiopian sorghum accessions for resistance to anthracnose caused by *Colletotrichum sublineolum*. (Manuscript).

1. General introduction

1.1. Ethiopia from a crop production perspective

Ethiopia is an east African nation located between 3-15ºN and 33-48ºE. It is among the least developed countries in the world with 85% of the population earning its livelihood directly from agriculture. The country is divided into 18 major- and 49 sub- agro-ecological zones (MoA, 1998), which are depicted in Figure 1, making it one of the most diverse countries in terms of topography and weather conditions.

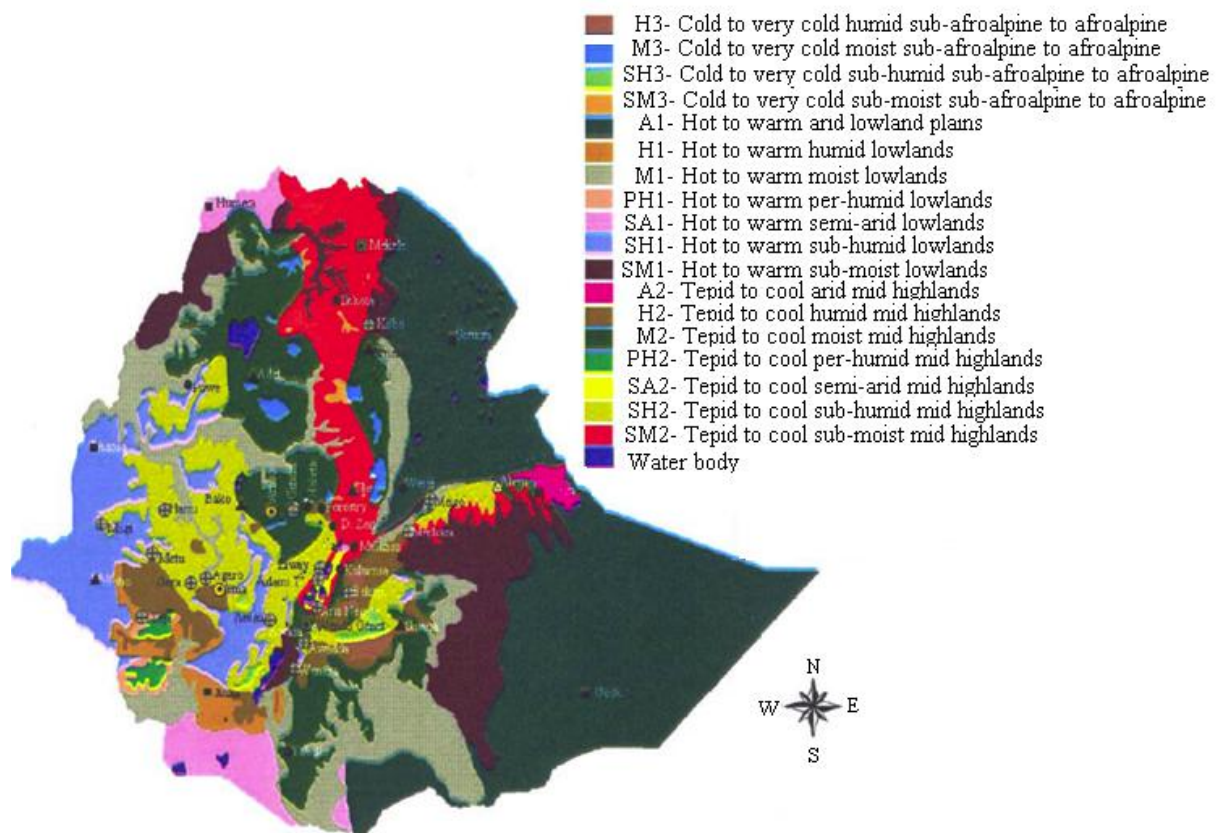

Fig. 1. Agro-ecological zones of Ethiopia. Source: EARO/ARTP, 1999; Tsegaw, 2006.

Ethiopia has a diverse climate ranging from hot and dry deserts to cold and high rainfall areas, with altitudes varying from 400 meters below sea level to more than 3700 meters above sea level (masl) (Hurni, 1986; USDA, 2009). The country lies within one of the 12 mega-centres of cultivated plants (Zeven and Zhukovsky, 1975) and is one of the eight major centres of origin and diversity for the major crop species as identified by Vavilov (1951). Owing to its diverse agro-ecological and climatic conditions, Ethiopia grows diverse crops and is known to be the centre of origin and/or diversity for several wild and cultivated plants, including barley

(*Hordeum vulgare*), chickpea (*Cicer arietinum*), arabica coffee (*Coffea arabica*), ensete (*Enset ventricosum*), finger millet (*Eleusine coracana*), flax (*Linum usitatissimum*), lentil (*Lens culinaris*), niger (*Guizotia abyssinica*), pea (*Pisum sativum*), sesame (*Sesamum indicum*), sorghum (*Sorghum bicolor*), teff (*Eragrostis tef*), and tetraploid wheat (*Triticum turgidum*) (Esquinas-Alcázar, 1983; Sleper and Poehlman, 2006).

Some areas in the country have a uni-modal rainy season while others are characterised by bi-modal rainy season named Meher (long) and Belg (short). According to the Central Statistical Agency (CSA, 2009), about 11.3% (12.4 million ha) of the country's total area (1.1 million km^2) was covered by major crops in the 2008/09 main cropping season. Grain crops were cultivated on 11.21 million ha of land (10.2% of the country's total area), which accounted for 91.4% of the area under crop cultivation and 83.7% of total crop production (Fig. 2). Coffee, vegetables, fruit crops and chat were also among the major crops grown in the country. Cereals accounted for 78.2% (8.8 million ha) of the total area devoted for grain crops and 84.7% (about 14.5 million tons) of the total grain production.

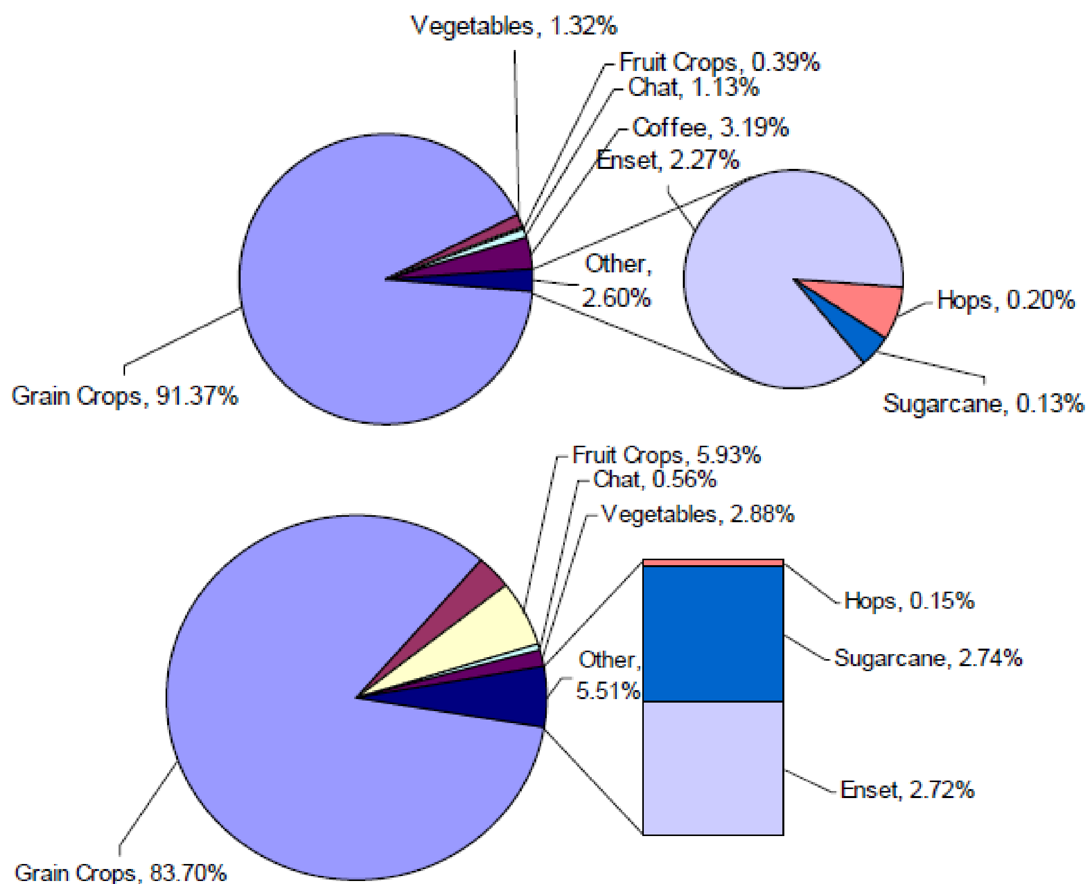

Fig. 2. Proportion of area coverage (above) and production (percentage of total tonnage) (below) of major crops in Ethiopia during the 2008/09 main rainy season.
Source: CSA, 2009.

Major cereals grown in Ethiopia include teff, maize (*Zea mays*), sorghum and wheat that took up to 65.3% of the grain crop area, which is equivalent to 7.3 million ha. Other grain crops that are grown in Ethiopia include pulses [14.2% of the grain crop area (1.59 million ha.), and 11.5% of total grain production (about 1.96 million tons)], and oilseeds [7.6% of grain crop area (about 855 thousand ha.), and 3.9% of total grain production (0.66 million tons)] (CSA, 2009).

1.2. The crop Sorghum

1.2.1. Origin and geographic distribution

Sorghum is an old crop believed to be initially domesticated in sub-Sahara Africa, in the region between Ethiopia and Chad, as early as 5,000-7,000 years ago. From this area of early domestication the crop disseminated to other parts of Africa, Near East, India and China 3,000 years back, and later to USA and Australia. Figueiredo et al. (2008) conducted a phylogeographic analysis of sequence diversity on sorghum varieties for six genes related to grain quality and found 10 RFLP groups classified according to their geographic origin (Fig. 3). The groups also reflect the races (accessions) of sorghum that might have been

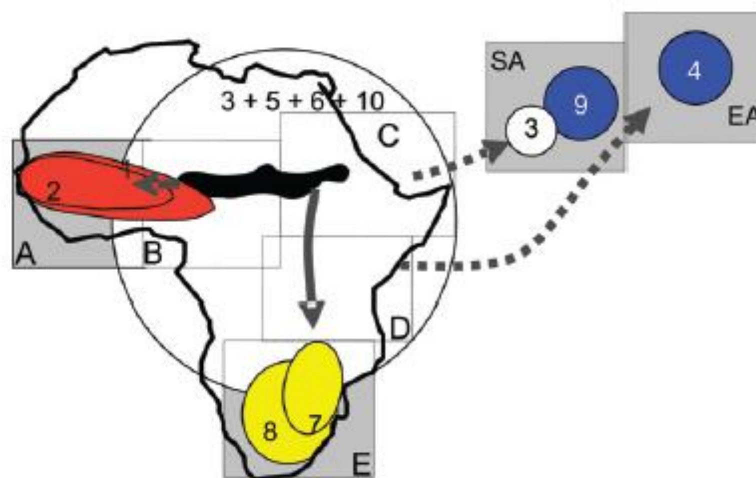

Fig. 3. Geographic distribution of RFLP groups of 10 sorghum accessions.

Source: Figueiredo et al., 2008. Numbers represent sorghum accessions:- 1: guinea from western Africa; 2: guinea margaritiferum from western Africa; 3: durra from central and eastern Africa and from Asia; 4: bicolor and caudatum from China; 5: caudatum from Africa; 6: transplanted caudatum from the Lake Chad region; 7: kafir from southern Africa; 8: guinea from southern Africa; 9: guinea from Asia; 10: caudatum and durra from Asia.

Letters refer to geographic regions: A:- Extreme West Africa; B: North-central Africa; C: Northeast Africa; D: Southeast Africa; E: Southern Africa; SA: South Asia; EA: East Asia.

disseminated from the centre of origin (Northeast Africa) to the different parts of Africa and Asia where they might have served as founder populations.

Nowadays sorghum is widely cultivated in more than 98 countries across the globe including Africa, North- and South- America, Asia, Australia and to some extent in Europe. USA, Nigeria, India, Mexico, Sudan, Australia, Argentina, China, Ethiopia and Brazil were the ten leading producers of sorghum in the 2008 cropping season (FAO, 2009).

1.2.2. Importance

With about 66 million tons of annual production on around 45 million ha of land, sorghum ranks fifth in importance among the world's cereals (FAO, 2009). Worldwide, sorghum production has increased by about six million tons from 1999 to 2008 (Fig. 4). However, total production has been fluctuating between individual years.

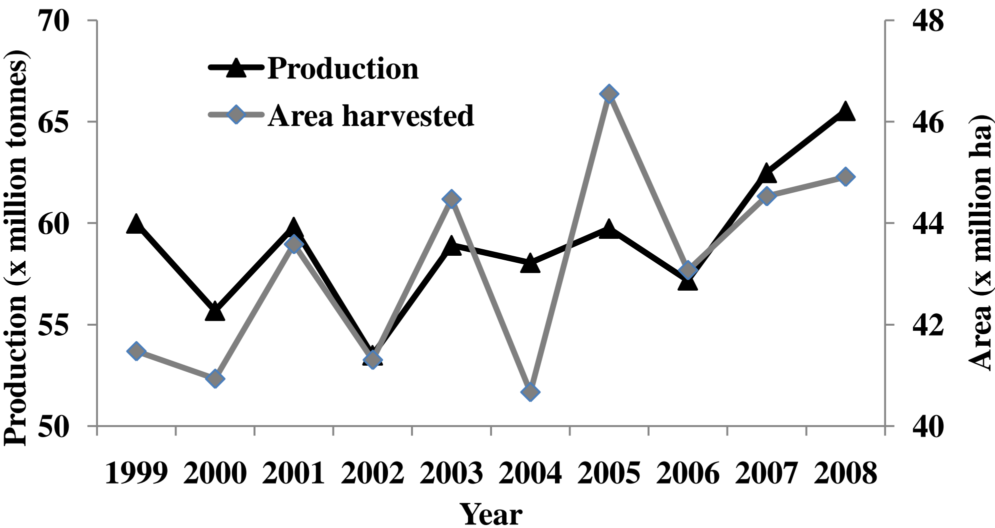

Fig. 4. Area harvested and total production of sorghum worldwide over a ten year period. Data source: FAO, 2009.

Total area covered by sorghum has also increased from 41 million ha. in 1999 to 45 million ha. in 2008 (Fig. 4) but it also had shown fluctuations between years. The variation between production seasons is also evident in terms of yield (data not shown), and this coupled with the variation in area harvested might have contributed to the fluctuations in total production between years. For the first time in the past ten years, an uninterrupted increase in sorghum production was registered from 2006 through 2008, and there was a considerable increase in sorghum production worldwide, productivity per unit area and area harvested from 2007

through 2008 production season (Table 1). However, a few countries, including USA, Sudan and Yemen have witnessed a decline in sorghum production. On the other hand, Australia

Table 1. Worldwide sorghum production[1] and productivity in the 2007 and 2008 cropping seasons for the 20 leading sorghum producers.

Rank	Country	Area harvested (ha)[2]		Yield (t[3]/ha)		Production (t)	
		2007	2008	2007	2008	2007	2008
1	USA	2,747,910	2,942,170	4.5983	4.0779	12,635,715	11,997,875
2	Nigeria	7,812,000	7,617,000	1.1159	1.2233	8,717,411	9,317,876
3	India	8,472,500	7,764,000	0.8440	1.0208	7,150,790	7,925,491
4	Mexico	1,774,975	1,838,130	3.4946	3.5965	6,202,828	6,610,835
5	Sudan	6,522,920	6,619,330	0.7663	0.5845	4,998,514	3,868,998
6	Australia	613,000	845,000	2.0929	3.6355	1,282,948	3,071,998
7	Argentina	594,410	618,625	4.7020	4.7473	2,794,916	2,936,798
8	China	501,131	580,649	4.8587	4.3098	2,434,845	2,502,481
9	Ethiopia	1,464,318	1,533,537	1.4843	1.5102	2,173,487	2,315,948
10	Brazil	662,994	811,662	2.1730	2.4220	1,440,686	1,965,845
11	Burkina Faso	1,607,741	1,901,776	0.9374	0.9859	1,507,096	1,874,961
12	Niger	2,838,847	3,055,251	0.3435	0.3506	975,144	1,071,171
13	Mali	1,090,244	986,367	0.8262	0.9433	900,760	930,440
14	Tanzania	900,000	900,000	1.0000	1.0000	900,000	900,000
15	Egypt	148,660	148,660	5.6763	5.6763	843,839	843,839
16	Chad	900,000	873,295	0.6406	0.7848	576,540	685,362
17	Cameroon	550,000	550,000	0.9090	0.9090	499,950	499,950
18	Uganda	314,000	321,000	1.4522	1.4859	455,991	476,974
19	Venezuela	165,804	165,804	2.3046	2.3046	382,112	382,112
20	Yemen	654,250	442,819	0.9186	0.8507	600,994	376,706
	World	44,528,642	44,911,877	1.4033	1.4591	62,487,043	65,530,920

[1]Data sorted according to 2008 production. Source: FAO, 2009.

[2]ha: hectare

[3]t: tons

registered the highest increase (139%) in sorghum production while the highest reduction (37%) was recorded in Yemen.

The bulk of sorghum (77% or more) is produced in the developing world (Berenji and Dahlberg, 2004). In Africa it is a native and one of the 10 leading crops. Sorghum production in Africa accounts for 14% of the total cereal production (Chantereau and Nicou, 1994) and was estimated to reach more than 22 million tons in the 2008 production season (FAO, 2009). In Ethiopia, the crop grows on more than 1 million ha of land making it one of the five major crops cultivated throughout the country (CSA, 2009). The country's grain yield of sorghum is estimated to be 1.74 t/ha (CSA, 2009). This figure is higher than the FAO estimate (1.51 t/ha) for the country and that of the world average yield of sorghum, which is estimated to be 1.46 t/ha (FAO, 2009).

Sorghum is used for different purposes in different parts of the world. In the developing countries, sorghum grains are used as source of food and local beverages for human consumption while the leaves and stalks are used as feed for animals, source of fuel and to construct rural houses and fences. In the developed world, on the other hand, sorghum is mainly produced to serve as source of feed for animals.

Recently interest has also grown in using sorghum fiber for paper and furniture manufacturing, and extensive work is underway to utilize sorghum as a source of ethanol.

1.2.3. Botanical classification and speciation

Sorghum belongs to the grass family, Poaceae and subfamily Panicoideae. Earlier studies classified the genus Sorghum into five major subgenera/sections, i.e. *Eu-sorghum*/*Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum*, and *Stiposorghum* (Garber, 1950; de Wet, 1978). Owing to its complex and diverse morphological traits, species designations under the genus *Sorghum* is rather controversial. Deu et al. (1994) classified *Sorghum* into four species i.e. *S. bicolor* (annual cultivated sorghum, 2n=2x=20), *S. propinquum* (a perennial rhizomatous wild sorghum, 2n=2x=20), *S. halpense* (a perennial rhizomatous wild sorghum, 2n=40), and *S. almum* (2n=2x=40). Sleper and Poehlman (2006) considered *S. bicolar*, *S. propinquum* and *S. halpense* as the only species of the genus *Sorghum*. On the other hand, Murty and Renard (2001) reported the description of 48 different species of cultivated, wild and weedy *Sorghum*. More than 50 species of *Sorghum* were also listed by the USDA-ARS

(2009). All the cultivated sorghums belong to the species *S. bicolor*, which is an annual plant that includes different wild and weedy taxa in addition to the cultivated sorghum. *S. bicolar* is further divided into 15 races (Harlan and de Wet, 1972).

Five of these, i.e. Bicolor, Guinea, Caudatum, Kafir and Durra, are considered as primary races and the remaining 10 are intermediate races as a result of hybrid combinations among the primary races. These include: Guinea-Bicolar, Caudatum-Bicolar, Kafir-Bicolar, Durra-Bicolar, Guinea-Caudatum, Guinea-Kafir, Guinea-Durra, Kafir-Caudatum, Durra-Caudatum, and Kafir-Durra. All the five basic races are grown in Africa, and all of them but Kafir are found in Ethiopia (Fig. 5).

Additionally five races: Arundinaceum, Virgatum, Verticilliflorum, Aethiopicum and Anomalous were also described by IBPGR and ICRISAT (1993). Among these, Arundinaceum, Verticilliflorum and Aethiopicum are found in Africa with the race Verticilliflorum covering most parts of Africa including Ethiopia.

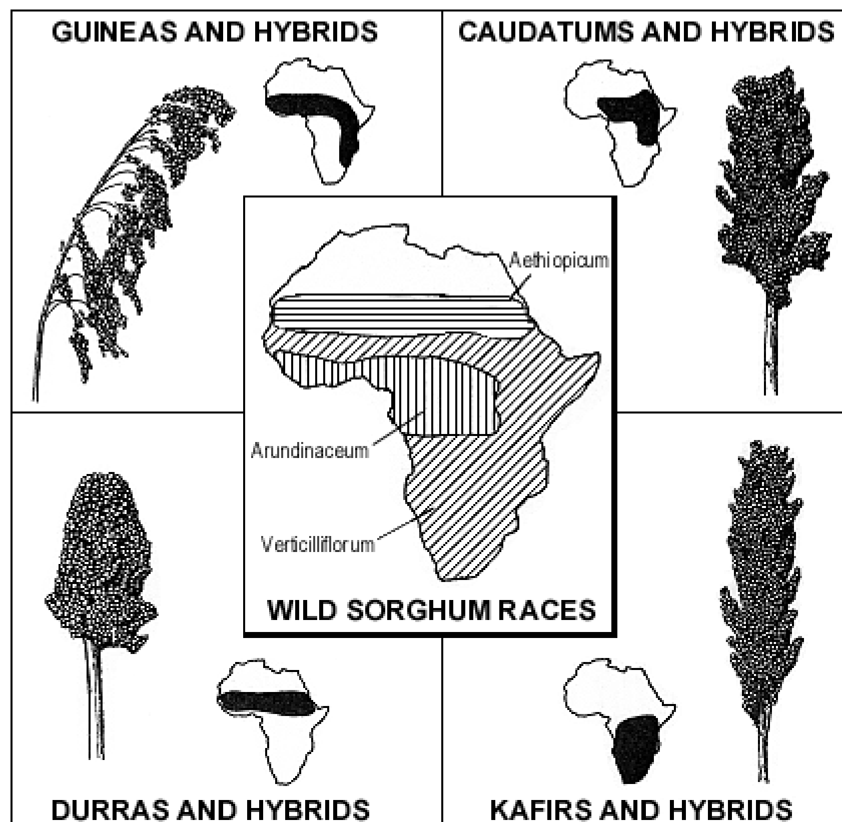

Fig. 5. Geographic distribution of different races of Sorghum in Africa.
Source: Wharton, 2009.

Sorghum bicolor is mainly a self pollinating (inbreeding), diploid (2n=2x=20) plant. Cross fertilization is generally low, around 6% on average (Dogget, 1988; Sleper and Poehlman, 2006). The flowering part of sorghum is characterised by spikelets borne in pairs: one is bisexual and fertile while the other is sterile spikelet (Fig. 6).

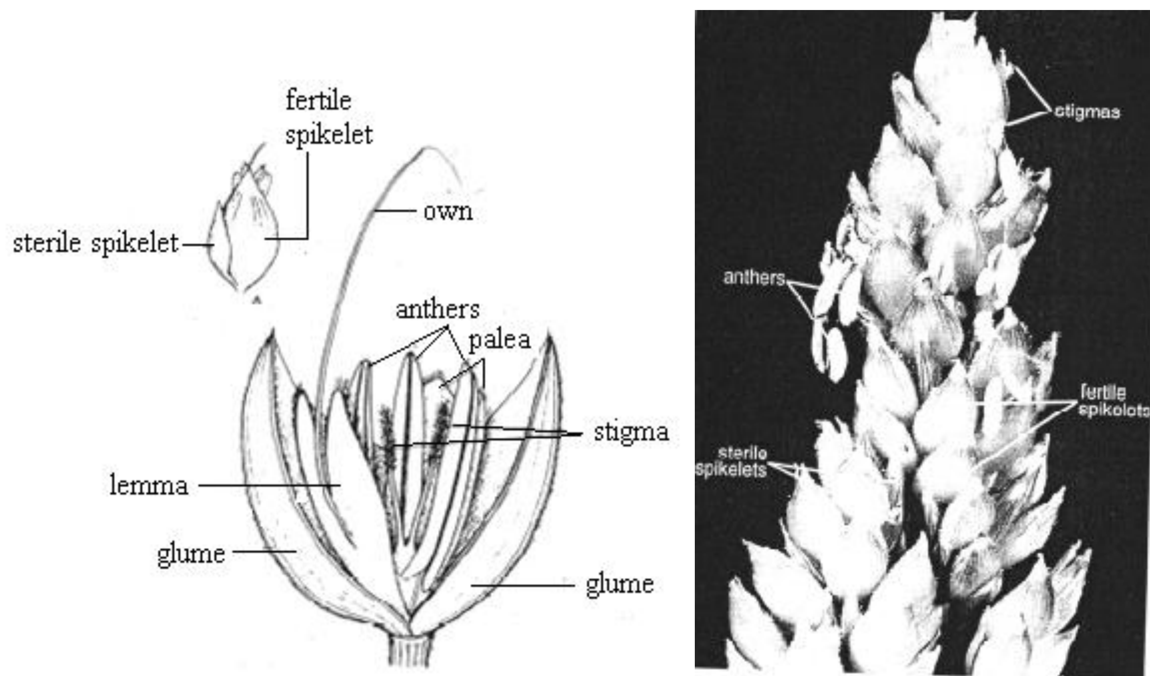

Fig. 6. Spikelets of sorghum (left) and panicle branch from sorghum head (right). The panicle branch shows exposed stigmas and exserted anthers. Source: Sleper and Poehlman, 2006.

1.2.4. Diversity in sorghum

Sorghum is a plant with immense diversity and this has enabled it to adapt to various environmental conditions. The greatest genetic diversity in sorghums is found in Ethiopia and neighbouring areas (Sleper and Poehlman, 2006). Diversity studies are important for the following reasons: i) they enable us to identify genotypes/accessions with novel traits that can be used in sorghum improvement programs; ii) diversity studies, if accomplished at different time intervals in different parts of the world, would help to elucidate the impact of man and the environment (biotic and abiotic factors including climate change) on the local plant population. Such a knowledge is of paramount importance to single out genotypes with unique adaptability to various environmental conditions and even to understand the mechanisms that lie behind it; and iii) such studies can also help us detect landraces that are threatened by genetic loss/drift due to natural and human selection, and as a results of the

introduction of improved varieties, which can serve as inputs in designing proper conservation schemes.

Different approaches have been used to unravel the diversity of sorghum. However, only some of the most commonly used methods are given brief account in the sections below.

1.2.4.1. Morphological diversity

Morphologically sorghum is a very diverse plant. It is one of the tallest grasses with plant height ranging from 0.5 to 5m (Fig. 7). IBPGR and ICRISAT (1993) classified sorghum plants into different groups based on plant color (pigmented [(grey brown or brown) vs. tan (greyed yellow)], leaf midrib color (white, yellow, brown, dull green, purple and others), glume color (white, red, purple, black, grey, etc), grain color (white, yellow, red, brown and others), grain covering by glumes and presence or absence of awns. Inflorescence shape and compactness also show considerable variations among sorghum genotypes as shown in Figure 8. One of the most comprehensive characterizations of sorghum accessions from Ethiopia was conducted by Ayana and Bekele (1998). The researchers characterized 391 sorghum land races from different geographic regions of Ethiopia and Eritrea, eight varieties and 16 introduced elite breeding lines based on 10 morphological traits, i.e. leaf midrib color, waxy bloom, panicle compactness and shape, awn at maturity, grain covering, glume color, seed color, grain plumpness, grain sub-coat and endosperm texture. Results of this study suggested the presence of high and comparable levels of phenotypic variation between the regions of origin and between the adaptation zones. Significant levels of variation were also found within the regions of origin and within the adaptation zones. Additional studies by the same authors found a more pronounced discrimination of sorghum accessions based on adaptation zones rather than regions of origin (Ayana and Bekele, 1999; 2000).

Diversity studies based on morphological features are relatively easy to visualise and are cheap to perform. The morphology of plants may reflect their genetic makeup and hence can have practical significances in identifying heritable traits for breeding programs. Variations in morphological traits like plant height and inflorescence shape of sorghum cultivars are also known to relate to their adaptability to various environmental conditions, including resistance to such adverse conditions as biotic stresses. For example, taller plants have higher competitive advantage over non-parasitic weeds as they can easily smother shorter plants by out competing for light.

Fig. 7. Some morphological types of sorghum from Ethiopia. Photos: A. Chala.
Top: Taller sorghum plants, $\geq$ 4m in height, and with either compact oval (left) or compact elliptic (right) inflorescence; Middle: Plants with intermediate height (ca. 3 m), and compact (left) and loose (right) erect inflorescence; Bottom: Short plants (< 2m) with loose (left) or compact (right) inflorescence.

Another morphological feature that can be related to better adaptability to the environment and thus disease resistance, is head compactness. Sorghum plants with loose inflorescence are more resistant to grain mould than plants with compact heads (Sharma et al., 2010). Hard grains, dark glumes and coloured seeds were also associated with increased resistance to diseases (Bandyopadhyay et al., 1988; Audilakshmi et al., 1999; Sharma et al., 2010).

Differential anthracnose resistance has also been documented among different morphological groups (leaf architecture, and plant and seed color) of sorghum (Bergquist et al., 1974; Frederiksen and Franklin, 1980). Karcher et al. (2008) reported increased drought tolerance in tall Fescue populations with high root/shoot ratios. Root-shoot ratio was also related to increased uptake of soil nutrients in other plants (Trehan and Sharma, 2003). But it should be noted that increase in plant height may not necessarily lead to increased or decreased root length.

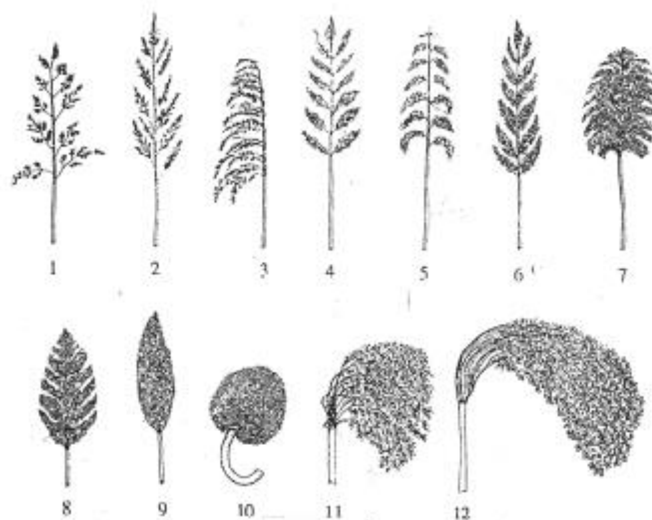

Fig. 8. Different shapes of sorghum heads. Source: IBPRG-ICRISAT, 1993.
1: very lax panicle; 2: very loose erect primary branches; 3: very loose dropping primary branches; 4: loose erect primary branches; 5: loose dropping primary branches; 6: semi-loose erect primary branches; 7: semi-loose dropping primary branches; 8: semi-compact elliptic; 9: compact elliptic; 10: compact oval; 11: half broom corn and 12: broom corn.

Despite all their importance in capturing the diversity of sorghum, morphological traits could sometimes be unreliable or not sufficient to tap the actual diversity of plants as they are subject to human bias and environmental impacts. In addition, some complex quantitatively inherited traits could prove difficult to trace by morphological means only. Such drawbacks of diversity studies based on morphological features solely call the need to supplement them with other more reliable methods including those that employ molecular markers.

1.2.4.2. Isozymes

Isozymes are enzymes that differ in amino acid sequence but catalyze the same reaction. Isozymes differ in biochemical properties such as electrophoretic mobility and are encoded by

different genetic loci. Isozymes that are products of different alleles at the same gene locus are termed allozymes (Markert and Moller, 1959).

Isozymes have been used to provide valuable information into genetics and population diversity of plant genetic resources (Beer et al., 1993; Matus and Hucl, 1999; Bartish et al., 2000; Premoli et al., 2001; Azeqour et al., 2002; Bimb et al., 2004; Cheniany et al., 2007). Isozymes have also been used to characterise sorghum plants and study their genetic diversity. Phul et al. (2006) observed differences in presence/absence and intensity of bands for the isozyme patterns of five male sterile lines in *S. bicolor*. Zongo et al. (2005) described the genetic relationship among 50 Sahelian sorghum landraces from Burkina Faso. Results of this study revealed the following: i) genetic variation was evident both between and within landraces; ii) genotypic frequencies in most of the landraces deviated markedly from Hardy–Weinberg proportions due to a major heterozygote deficit; iii) the landraces are homozygous or a mixture of homozygotes; iv) alleles occurred in a random pattern and unrelated to external selection pressures; and v) the major diversity among landraces appears to be from genetic shift caused by farmers' selection of their seeds. Additional studies that employ isozymes/allozymes in studying sorghum diversity include Morden et al. (1989; 1990); Aldrich et al. (1992); Djè et al. (1998; 1999).

Isozymes are co-dominant markers, cheap and relatively easy to use. However, they show variation only at protein level and hence may underestimate the actual genetic variation. As described by Zhang et al. (2002), isozyme studies are limited by the numbers of enzymes and loci that can be resolved, and reveal only genetic changes in coding regions of the genome that have resulted in an altered amino-acid sequence. Besides, assays are time consuming and hence isozymes were extensively used before the advent of DNA-based techniques (Cooke and Lees, 2004). Nowadays the use of isozymes has diminished dramatically due to the discovery and wider use of DNA-based techniques.

1.2.4.3. Molecular diversity

The use of molecular markers is becoming a standard practice in studying the diversity of plant genetic resources. Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance (Zidenga, 2004). Unlike morphological features, molecular markers are not exposed to the influence of the external environment, hence, they are considered as more reliable tools for diversity studies.

Different markers have been used to study the diversity of sorghum at the genetic/molecular level and some of the most commonly used ones are briefly described below. It should be noted that there exist a range of different methods in the field other than those mentioned below.

Restricted fragment length polymorphisms (RFLPs)

RFLP is one of the first DNA based molecular techniques which came into use after the discovery of the polymerase chain reaction (PCR) (Saiki et al., 1988). The method involves the use of restriction enzymes to digest DNA strands at a specific location (recognition site), resulting in a number of DNA fragments, and separation of the fragments by gel electrophoresis (Dowling et al., 1990 as quoted by Imsiridou et al., 2003). Differences result from base substitutions, additions, deletions or sequence rearrangements within restriction enzymes recognition sequences (Dowling et al., 1990). Such differences in the pattern of the fragments between individuals are called RFLPs, and the patterns are associated with inter- and intra-species relatedness or variation.

RFLPs have been used in genetic comparisons and cultivar identification (Gebhardt et al., 1989; Corniquel and Mercier, 1994; Sakka et al., 2004), and to construct genetic maps that help in tracking and tagging of genes of agronomic importance, including disease and heat resistance (Bentolila et al., 1991; Louie et al., 1991; Ottaviano et al., 1991). Genetic diversity studies in sorghum have also benefited from the invention of RFLPs (Tao et al., 1993; Vierling et al., 1994). Aldrich and Doebley (1992) used RFLP diversity studies of 56 sorghum accessions to confirm the hypothesis that central-northeastern Africa is the most likely principal area of domestication for sorghum. Deu et al. (1994) performed RFLP analysis on 94 sorghum accessions representing different races and geographic origins. Results of this experiment revealed high variability and presence of rare markers within the bicolor race, higher similarity between races localized in southern Africa, and association between neutral markers and traits highly subjected to human selection. They also found out that grouping of the accessions to be in conformity with their racial differentiation and geographic origin. Other studies that employ RFLPs for molecular characterisation of sorghum include Chittenden et al. (1994); Cui, et al. (1995); Ahnert et al. (1996); Yang et al. (1996); Uptmoor et al. (2003).

RFLPs have the advantage of being co-dominant markers and hence they can differentiate between homozygous and heterozygous. In addition, they are reproducible. Despite their wider application in taxonomy studies and population genetics, RFLPs have their own problems. Large amounts of high quality DNA are needed for restriction and southern blotting making the technology laborious, time-consuming and expensive (Elameen, 2009). RFLP technique involves the use of isotopes, which further increase its cost and make it more hazardous to humans. Nevertheless, RFLPs are reliable in linkage analysis and useful in detecting coupling phase of DNA, and hence continue to play a major role in population genetics studies.

Random amplified length polymorphisms (RAPDs)

RAPD techniques are PCR-based reactions that amplify segments of DNA, which are essentially random. The researcher designs a primer with an arbitrary sequence, carries out a PCR reaction and runs an agarose gel to detect and score amplified DNA-fragments.

These molecular markers have been used in the genetic studies of plants, including taxonomy and cultivar identification (Devos and Gale, 1992; Waugh et al., 1992; Dawson et al., 1993; Corniquel and Mercier, 1994; Rani et al., 1995; Wei and Wang, 1995; Sedra et al., 1998; Bartish et al., 2000; Zhang et al., 2002).

RAPDs have also been extensively used in studying the genetic diversity of sorghum. Ayana et al. (2000) described what they called an intermediate level of overall variation (Shannon-Weaver diversity index, H = 53) among 80 sorghum accessions from Ethiopia and Eritrea. They attributed the largest proportions (77%) of the variation to within the regions of origin of the accessions and the remainder (23%) among regions of origin. Jaiswal et al. (1998) used RAPD profiles to distinguish restorers of cytoplasmic male-sterile (CMS) lines in sorghum, which enabled them to conclude that the different restorer lines possess specific nuclear-cytoplasm combinations and suggest that the RAPD techniques can have the potential in identifying markers for different cytoplasms used in CMS. Results of this study have important contribution in sorghum breeding and hybrid seed production that may require CMS parents. In another experiment involving 46 sorghum accessions from South Africa Uptmoor et al. (2003) used RAPDs along with amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) to study the genetic relatedness of sorghum landraces and cultivars grown in low-input conditions of small-scale farming systems. Their

results revealed a clear separation between landraces and breeding varieties, and also a similar level of genetic diversity was detected within both subgroups. RAPD markers also successfully identified races of sorghum and regions with maximum genetic diversity (Menkir et al., 1997). Dahlberg et al. (2002) used RAPD profiling of 94 sorghum accessions representing four of the five races to evaluate different morphological groupings (developed based on different agronomic descriptors) as means of elucidating sorghum diversity. RAPDs have also been used in gene tagging and QTL analysis (Tuinstra et al., 1996).

RAPDs have the advantages of being quick (Colombo et al., 1998) and well adapted for non-radioactive DNA fingerprinting of genotypes (Cao et al., 1999). But they have proved not reproducible, and especially minor bands can be difficult to repeat due to the random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier et al., 1999). In addition, RAPDs are dominant markers and hence cannot distinguish between homozygote and heterozygote individuals, and the resulting bands could be difficult to score. Working with RAPDs may require high quality DNA but reveal much less polymorphism as compared to AFLPs and SSRs.

However, the fact that they are cheap and easy to use makes RAPDs still useful and applicable in genetic diversity studies especially in low funded laboratories at least for the near future.

Amplified fragment length polymorphisms (AFLPs)

AFLP, amplified fragment length polymorphism, is a novel and powerful technique of DNA fingerprinting that play crucial roles in taxonomy and genetic studies. As described by Vos et al. (1995), the AFLP technique is based on selective PCR amplification and involves the following steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments.

AFLPs have now become one of the most commonly utilized markers in assessing the genetic diversity within sorghum. Perumal et al. (2007) studied 46 sorghum lines representing all the five major and nine intermediate races using AFLPs and also SSRs, and classified the lines into three major- and four sub- clusters. Results of this experiment also suggested that molecular classification of the races kafir, guinea, caudatum, durra and their intermediates

were concordant with their morphological groupings. Furthermore, the genetic relatedness within and between these four races and their intermediates was closer than what the phenotype would suggest. On the other hand, the race bicolor and its intermediates were found to be more heterogeneous and their molecular diversity was not reflective of their morphological groupings. In another experiment, Menz et al. (2004) investigated the diversity of 50 sorghum inbred lines based on AFLP and SSR markers and suggested a classification in line with the sorghum working groups, Zera-zera, Kafir, Kafir-Milo, Durra, and Feterita. Comparison of the two markers also enabled the researchers to determine the impact of the distribution of the markers and the coverage of the genome by the markers on the classification of genotypes. They also noted that *Pst*I/*Mse*I AFLP markers, or a set of markers spaced at 1- to 2-cM intervals across the genome, produced clusters that were in better agreement with pedigree information than the analysis based solely on the *Eco*RI/*Mse*I AFLP or SSR markers used in their study. AFLPs have also been successfully utilized in studying sorghum diversity by other researchers including Uptmoor et al. (2003), Geleta et al. (2006) and Wu et al. (2006).

AFLPs have also been used to construct genetic and physical maps, which are essential for map-based gene isolation, comparative genome analysis, and as sources of sequence-ready clones for genome sequencing projects (Boivin et al., 1990; Klein et al., 2000; Menz et al., 2002; Wen et al., 2002).

Owing to their ability to yield many loci per primer combination, AFLPs are considered to be one of the most powerful molecular markers (Milbourne et al., 1997). In addition, they do not need sequence information, and if performed with care, AFLP techniques yield highly reproducible results.

Nevertheless, AFLPs are not without their own short comings. They are dominant markers and hence cannot distinguish between homozygote and heterozygote individuals. Besides, the AFLP technique is more costly and technically demanding compared to others like RAPD. However, the advantages clearly outweigh the shortcomings and hence AFLPs are likely to continue as the markers of choice in many laboratories.

Simple sequence repeats (SSRs)- Microsatellites

SSRs also known as microsatellites or short tandem repeat are short sequences of 1-6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes (Litt and Luty, 1989; Edwards et al.,1991; Jacob et al., 1991). SSRs are becoming very important markers in the taxonomy of plants and genetic studies as they are more variable than many other molecular markers (Powell et al., 1996; Pejic et al. 1998), and hence they have been widely utilized in studying sorghum diversity in recent days. Djè et al. (2000) studied the genetic diversity of 25 sorghum accessions from the international sorghum collection using SSRs and found the sorghum collection to be highly genetically structured. They were also able to identify individual accessions with a high relative contribution to the overall allelic diversity of the collection and suggested that accessions from East Africa and those representing the race bicolar were the most diverse group. Ghebru et al. (2002) reported an exceptionally high genetic diversity (in terms of number and size range of SSR alleles) within the Eritrean sorghum germplasm after comparing 28 Eritrean landraces to other germplams using SSRs. Both within- and between- population diversity and heterozygosity were found to be high and cluster analysis classified the Eritrean sorghum into 7-10 major groups, mostly in conformity with farmers' descriptions.

Dean et al. (1999); Djè et al. (1999); Smith et al. (2000); Agrama and Tunistra (2003); Uptmoor et al. (2003); Menz et al. (2004); Folkertsma et al. (2005), have also used SSRs alone or in combination with other markers to investigate sorghum diversity. The use of SSRs, however, is not limited to diversity studies. Like the other molecular markers, SSRs are also playing a crucial role in molecular mapping of genes (Agrama et al., 2002; Haussmann et al., 2002; Menz et al., 2002; Nagaraj et al., 2005).

Generally, SSRs are co-dominant, highly informative and reproducible markers that generate high level of polymorphism, and hence considered to be a high-throughput approach in studying genetic diversity (Senior et al., 1998; Gherbu et al, 2002; Agrama and Tunistra, 2003). On the other hand, the microsatellite technique is laborious and costly due to the need to identify new markers for each and every species, and the markers may also result in deficiency of heterozygotes and false equal identity of alleles due to independent mutation of the same size (Viard et al., 1998; Varshney et al., 2005; Elameen, 2009).

Additional molecular markers

The above are the most commonly used markers in the study of the diversity of sorghum. Other markers including simple nucleotide polymorphisms (SNPS), array technology and sequencing of different regions across the genome have also been employed in the genetic studies of sorghum (Dillon et al., 2004; Zidenga, 2004; Price et al., 2005; Figueiredo et al., 2008; Calviño et al., 2009). But the search for an ideal marker that combines efficiency with time and cost effectiveness seems never to end.

Molecular markers are nowadays considered as essential tools in modern breeding activities, including in the construction of linkage maps (Haussmann et al., 2002). As noted by Zidenga (2004), genetic distance estimates determined by molecular markers help in identifying suitable germplasm for incorporation into plant breeding programs, and hence the use of molecular markers accelerate the selection process for desirable genotypes and contribute in the manipulation of quantitative trait loci (QTLs). Rao et al. (2007) revealed the potential of molecular markers in identifying salt tolerant sorghum accessions. Besides, molecular markers have been used to detect and characterize QTLs linked to different traits in sorghum, including plant height and maturity (Pereira and Lee, 1995), disease resistance (Gowda et al., 1995; Boora et al., 1998; 1999; Perumal et al, 2009), and drought tolerance (Tuinstra et al., 1997; 1998). Generally, molecular markers are considered to be the most robust and reliable ways of tapping genetic diversity, and they help us understand the evolution and adaptability of plant genetic resources. However, they are not without limitation, and hence should be further improved and supplemented with each other and even with other methods including physiological and morphological characterizations.

1.2.5. Environmental requirements, production and genetic improvement of sorghum

Sorghum can be grown in diverse agro-ecological zones. It grows in areas with altitude range of less than 500 to more than 2000 masl but is mostly cultivated in tropical lowlands with annual rainfall between 500 and 1500 mm. In Ethiopia, the crop is also grown in areas with annual rainfall well above 1500 mm. In terms of temperature, sorghum grows best at 28±3ºc. It can tolerate higher temperatures and moisture stress compared to other cereals but frost will kill the crop. However, the crop is also known to thrive in areas with temperature well below and above the optimum.

Developing countries account for the majority of the total area devoted for world sorghum production. Farmers in these regions mainly produce local landraces that are subjected to both natural and man driven selection. Formal genetic improvements in sorghum began after farmers in Texas observed dwarf and early maturing mutants (Sleper and Poehlman, 2006). Before 1950, sorghum production was based on self-pollinated cultivars, and germplasm improvement activities were limited to a few photoperiod insensitive accessions (Manz et al., 2004). Hybrid seed production started in the USA in the mid 1950s with the identification of the cytoplasmic-male-sterility: fertility-restorer-gene system (Sleper and Poehlman, 2006). Since then sorghum hybrids with improved yielding potential and other desirable traits including short height and resistance to biotic stresses have been released and disseminated worldwide. In addition to national research programs and breeding companies, the International Crops Research Institute for the Semiarid Tropics (ICRISAT) and the International Sorghum and Millet Collaborative Research Support System (INTSORMIL) have taken the mandate of sorghum breeding and germplasm conservation.

In Ethiopia, sorghum research began in 1957 at Haramaya University and this led to the initiation of Ethiopian Sorghum Improvement Program (Mekbib, 2009). Currently sorghum germplam collection and conservation is primarily conducted by the Institute of Biodiversity Conservation, while researches on sorghum agronomy and improvement, and insect, diseases and weed management are mainly conducted by the Institute of Agricultural Research with some involvement of higher learning institutions including Haramaya University.

1.2.6. Production constraints

Though sorghum is a crop with unique adaptability to a wide range of environmental conditions and with an efficient growth rate, its production is constrained by different abiotic and biotic factors worldwide. Among the biotic constraints, bird damage, competition from weeds, insects and diseases of different kinds are the leading factors limiting sorghum production and productivity worldwide. These biotic constraints are responsible for causing more than 70% of total yield loss in sorghum (Berenji and Dahlberg, 2004). This problem is exacerbated by inability of subsistence farmers in the developing countries to apply pesticides owing to their higher costs. To date more than 40 plant diseases are registered on sorghum worldwide among which anthracnose is one (Thakur and Mathur, 2000).

1.3. Sorghum anthracnose

1.3.1. Causal agent

Sorghum anthracnose is caused by a fungal pathogen *Colletotrichum sublineolum*, a member of the *Colletotrichum* complex known to cause several diseases on various plants ranging from grasses to trees.

1.3.1.1. The genus *Colletotrichum*

The genus *Colletotrichum* belongs to the group Coelomycetes, fungi that produce asexual spores (conidia) inside pycnidia or in a bag of hyphae (acervuli; stromata). As noted by Sutton (1992), the first description of fungi that belong to the present day *Colletotrichum* was given by Tode (1790) under the genus *Vermicularia*. The genus name *Colletotrichum* was introduced by Corda (1837) and both names were used interchangeably during the 19th and early 20th centuries (Sutton, 1992).

Colletotrichum is known to be one of the most diverse groups of plant pathogenic fungi. Species designation and diversity studies within *Colletotrichum* make use of various taxonomic tools, including i) phenotypic traits i.e. size and shape of conidia and appresoria; colony color; growth rate; and presence or absence of setae, sexual stage and formation of sclerotia (von Arx, 1957; Sutton, 1992; Photita et al., 2005; Nguyen et al., 2009b), ii) host range or pathogenicity (Liu et al., 2007; Nguyen et al., 2009b), iii) sensitivity to fungicides (Kaboré et al., 2002; Peres et al., 2004), iv) biochemical markers (isozymes) and various molecular markers including RAPD, RFLP, AFLP and microsatellites (Bonde et al., 1991; Abang et al., 2002; 2006; Martínez-Culebras et al., 2002; Heilmann et al., 2006; Liu et al., 2007; Bridge et al., 2008). In addition, sequencing of various regions in the genome and studies on mating type genes have also improved characterization of the genus (Johnston and Jones, 1997; Moriwaki at al., 2002; Talhinhas et al., 2002; Du et al., 2005; Crouch et al., 2006; Zanette et al., 2009).

However, the taxonomy of *Colletotrichum* still remains controversial, mainly because of the short comings associated with each taxonomic tool. Characterization based on morphological features is cheap cost wise and relatively easy to conduct, but such features are inadequate and prone to environmental impacts. Besides, some *Colletotrichum* species are genetically unstable (Fávaro et al., 2007) and hence their phenotypic features may change with time (Crouch et al., 2006; Rivera-Vargas et al., 2006; Paper II of this thesis), leading to unreliable

conclusions. Although they have a practical significance, classification of *Colletotrichum* spp. on the basis of their host range/specificity is also problematic as some species can infect more than one plant species, while a single plant species can also be infected by more than one *Colletotrichum* spp. For example, *Colletotrichum gloeosporioides* is known to infect several fruit crops including almond (Freeman et al., 1996), chili (Than et al., 2008), coffee (Nguyen et al., 2009a), mango (Sanders and Korsten, 2003) and strawberry (Garrido et al., 2008); while each plant species can be infected by other *Colletotrichum* spp. i.e. almond, mango and strawberry by *C. acutatum* (Adaskaveg and Hartin, 1997; Rivera-Vargas et al., 2006; Garrido et al., 2008), chili by *C. capsici* (Than et al., 2008), and coffee by *C. kahawae* (Bedimo et al., 2010). Molecular and biochemical markers, on the other hand, are considered as more reliable means of diversity studies as they reflect the genetic basis behind the classification or characterization of the pathogens. As noted by Thaung (2008), molecular characterization of coelomycetes started around 1990 when Braithwaite et al. (1990) used rDNA to study genetic variations within *C. gloeosporioides*. Since then the use of molecular markers has become a very common and much trusted practice in the characterization of *Colletotrichum* spp. But molecular markers are not without their own drawbacks too. As described under sub-section 1.2.4.3., some of them could be less reliable and less reproducible, while the extent of genome coverage could be questionable with almost all the markers. Lack of agreement among the different tools is also becoming problematic in several instances. Nevertheless, each method has its own strengths, and a continuous effort must be exerted to find possible ways of combining the different taxonomic tools to better understand the diversity of *Colletotrichum* spp.

Colletotrichum spp. are cosmopolitan pathogens causing a wide array of diseases in the tropics, subtropics and temperate regions of the world (Freeman et al., 1998; 2001; Abang et al., 2002; Afandor-Kafuri et al., 2003; Crouch and Beirn, 2009). In some *Colletotrichum* spp. the teleomorph state has been identified and named as *Glomerella*. These include *C. graminicola* (*G. graminicola*), *C. gloeosporioides* (*G. cingulata*), *C. lindemuthianum* (*G. lindemuthiana*) and *C. falcatum* (*G. tucumanensis*). But sexual reproduction has not yet been discovered for many other *Colletotrichum* spp. that infect different plants all over the world. A brief description of major *Colletotrichum* species has been given by Sutton (1992).

Anthracnose on both maize and sorghum was considered to be caused by *Colletotrichum graminicola* (Holliday, 1980). It was suggested that pathogen variability from these two hosts

was limited to pathotype level. However, subsequent works have distinguished between the *Colletotrichum* pathogens causing anthracnose on maize and sorghum based on morpholigcal features i.e. size and structure of appresoria and conidia (Sutton, 1980; 1992), DNA sequence analysis (Sheriff et al., 1995), compatibility tests and DNA based molecular markers (Vaillancourt and Hanau, 1992).

Presently, it is generally accepted that maize anthracnose is considered to be caused by *C. graminicola* while *Colletotrichum* isolates causing the same disease on sorghum are grouped under a different species, *C. sublineolum* (Sutton, 1980; Vaillancourt and Hanau, 1992; Sheriff et al., 1995). Host specificity is another factor used to distinguish these two species (LeBeau, 1950). Jamil and Nicholson (1987) reported *C. sublineolum* as being able to cause stalk rot and leaf blight on sorghum but not on maize. This was in agreement with a recent report by Crouch and Beirn (2009), which treated *C. sublineolum* as the cause of sorghum anthracnose and *C. graminicola* as the causative agent of anthracnose on maize. On the other hand, Venard and Vaillancourt (2007) have given a mixed signal with regard to host specificity of *C. sublineolum*. In an experiment aimed at investigating the penetration and colonization mechanisms of the maize anthracnose pathogen, they observed that *C. sublineolum* was able to infect maize stalks although to a lesser extent as compared to *C. graminicola*. They also noted that *C. sublineolum* was unable to infect intact roots and leaves of maize while *C. graminicola* efficiently colonized both suggesting specialization at the tissue level.

Throughout this thesis, the sorghum anthracnose pathogen is consistently referred as *C. sublineolum*.

1.3.1.2. *Colletotrichum sublineolum*

Biology

C. sublineolum is a plant pathogenic fungus, which has no known sexual stage in nature. It produces two kinds of conidia (falcate or oval). However, *C. sublineolum* is mainly reproduced by means of the falcate conidia produced terminally on sickle shaped conidiophores among the setae (Warren, 1986). As reported by Souza-Paccola et al. (2003), the falcate conidia are uninucleate and produced in solid media, while the oval conidia are uni- to multi-nucleate (with one to three nuclei) and have been produced in liquid media. The oval conidia, though variable in size, are generally smaller than the falcate (Panaccione et al.,

1989). There are conflicting reports about the role of oval conidia in the disease cycle. Crouch and Beirn (2009) reported the oval conidia as having an unknown role in the disease cycle but both types of conidia were able to induce anthracnose symptoms in sorghum plants (Souza-Paccola et al., 2003). Besides, the oval conidia were observed in different host tissues (Panaccione et al., 1989; Venard and Vaillancourt, 2007) suggesting the possibility that they may initiate anthracnose like the falcate conidia. Acervuli are produced both on host tissues and culture media, and appear as dark brown in color and oval to cylindrical in shape (Warren, 1986). On host tissues, masses of conidia are produced in acervuli that contain black hair like projections called setae (Fig. 9). The falcate conidia of *C. sublineolum* are hyaline, aseptate, and cylindrical to obclavate in shape and become sickle shaped with age (Warren, 1986; Personal observation; Fig 9). On culture media, conidial production by *C. sublineolum* is favored by light (fluorescent light-50 $\mu E/m^2/s$) and temperatures of 22-30^oC (Warren, 1986; Souza-Paccola et al., 2003). The spores of *Colletotrichum* spp. are surrounded by a mucilaginous substance called extracellular spore matrix (Hutchison et al., 2002), which serve as means of protection from environmental stress including desiccation (Nicholson & Moraes, 1980); and facilitate adhesion of spores and the infection processes (Mercure et al., 1994; 1995; O'Connell et al., 2000).

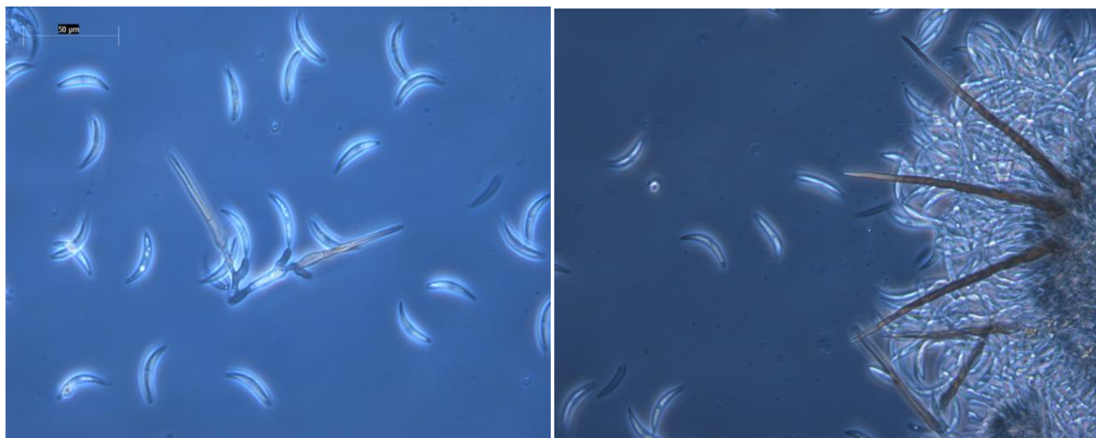

Fig. 9. Conidia and setae of *C. sublineolum* in a light microscope at 400x magnification. Photos: A. Chala and J. Razzaghian.

Survival

C. sublineolum survives/overseasons as mycelium or conidia in crop debris, infected seeds and alternate hosts (Tarr, 1962; Warren, 1986; Cardwell et al., 1989; Casela and Frederiksen, 1993; Somda et al., 2007). Among these, crop residues left on the ground could be of the

major sources of inoculum that can initiate infection on healthy newly growing crops. The conidia can survive for as long as 18 months in crop residues which are not buried in the soil (Warren, 1986). This may be the source of severe infections in areas where reduced or no tillage is practiced as a means of reducing soil erosion. However, decomposition of plant residues substantially affects the survival of *C. sublineolum* as it is known to cause lyses of spores and mycelium due to competition from other soil inhabiting fungi (Vizvary and Warren, 1982). In infected seeds, the pathogen can survive for as long as 20 months (Gwary et al., 2006) although survival in seeds may vary depending on storage conditions.

The infection process

Infection of sorghum by *C. sublineolum* can occur at any stage during plant growth and development. Generally, anthracnose is initiated when conidia dispersed by rain splash land on susceptible plant parts like leaves (Fig. 10). Once the inoculum has landed on susceptible plant parts, the conidia will adhere firmly to the surface. Adhesion of *Colletotrichum* spores to host surfaces is known to be facilitated by the presence of the extracellular matrix (Mercure et al., 1994; 1995; O'Connell et al., 2000) that contains mannose and glycoprotein, which allow adhesion on hydrophobic surfaces (Sugui et al., 1998).

Within 24 hours after contact, the conidia germinate, give rise to one or two germ tubes from any side of the conidium and produce globose, melanized appressoria (Tarr, 1962; Wharton et al., 2001). The appressoria are filled with mitochondria, lipid globules, glycogen granules, polyribosomes, multivesicular bodies, and vacuoles, surrounded by extracellular matrix material, and contain two distinct layers i.e. i) an outer, highly electron-opaque layer and ii) an inner, moderately electron-opaque layer (Wharton et al., 2001). The appressoria will then give rise to penetration pegs that penetrate directly into the epidermal cells through the cuticle by exerting mechanical force and with the assistance of cell wall degrading substances (Tarr, 1962; Amador et al., 1969; Nicholson and Moraes, 1980; Wharton et al., 2001). According to Wharton et al. (2001), a third electron-lucent layer surrounds the penetration peg, forming a thickened ring around the penetration pore. The same authors also indicated that the penetration peg emerges through the pore to directly penetrate the host cuticle and epidermal cell wall. The development of turgor pressure in appressoria due to melanisation, permeability barrier and osmolytes like glycerol is an important pre-requisite for direct host penetration by *Colletotrichum* spp. using mechanical force (Latunde-Dada, 2001). Penetration of sorghum plants by *C. sublineolum* occurs within 42 hours after inoculation (Wharton et al., 2001)

indicating a more prolonged process compared to the *C. graminicola-Zea mays* pathosystem (Mims and Vaillancourt, 2002). Penetration though openings including wounds is reported for *C. graminicola* (Bergstrom and Nicholson, 1999; Venard and Vaillancourt, 2007) and hence might occur with *C. sublineolum* too even if it is has not been substantiated thus far.

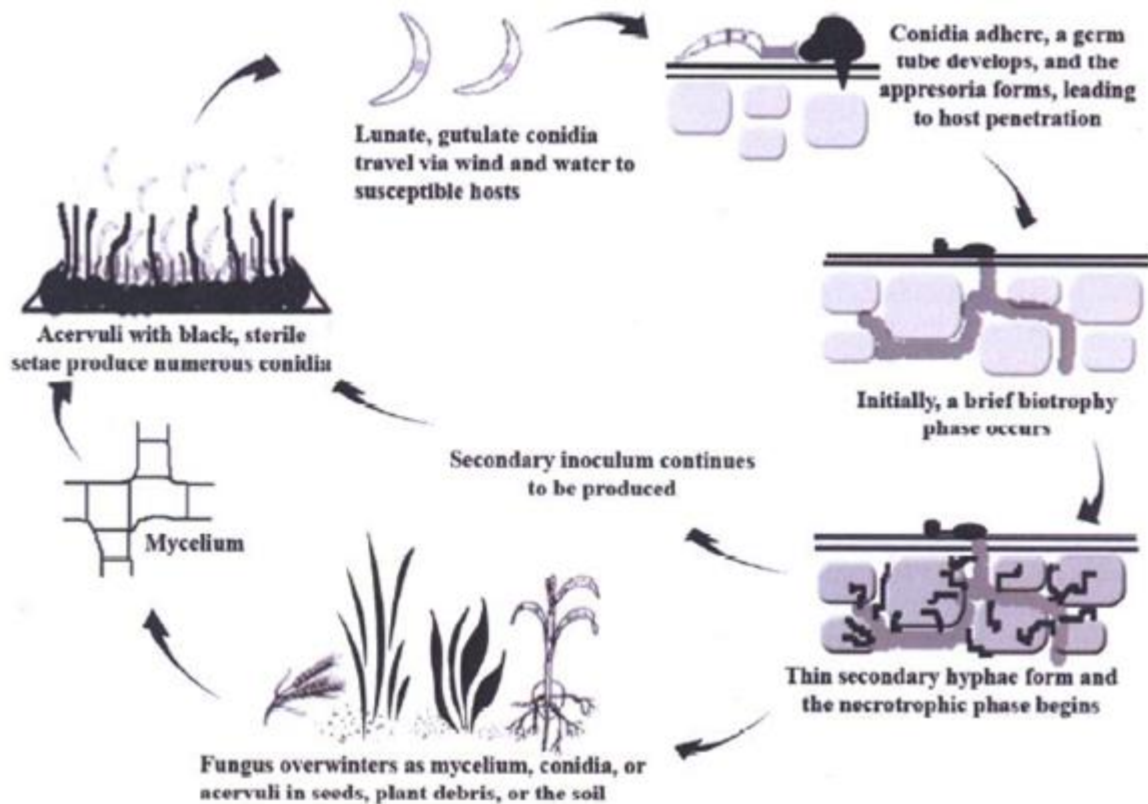

Fig. 10. Life cycle of *C. sublineolum*. (Adapted from Crouch and Beirn, 2009).

After penetration, *C. sublineolum* assumes a hemibiotrophic phase characterized by a short and early biotrophic phase lasting for 24 hours and a necrotrophic phase that follows it (Wharton and Julian, 1996; Wharton et al., 2001). During the biotrophic infection phase, intracellular infection vesicles and primary hyphae colonize epidermal and sclerenchyma cells but not mesophyll cells (Wharton et al., 2001; Fig. 10). This will be followed by the proliferation of thinner secondary hyphae that grow throughout the epidermis and into underlying mesophyll cells, ramifying through host tissue both intercellular and intracellular, leading to death of host cells (the necrotrophic infection phase) by 66 hours after penetration (Wharton and Julian, 1996; Wharton et al., 2001). This leads to necrosis of host tissues, production of acervuli from dense hyphal stroma that give rise to conidiophores and setae, and subsequent rupture of the host cuticle, and release of conidia (Wharton and Julian, 1996; Wharton et al., 2001).

Dispersal

The primary inoculum of *C. sublineolum* are conidia, which are carried by rain splash or air current from residues and wild sorghum species (Warren, 1986). After invasion of sorghum plants, acervuli are formed from necrotic tissues and give rise to numerous conidia that serve as secondary inoculum (Crouch and Beirn, 2009). Infected sorghum seeds are known to harbour *C. sublineolum* (Gwary et al., 2006; Somda et al., 2007) and hence may help in the long distance dispersal of the pathogen.

1.3.2. Symptoms

Sorghum anthracnose can affect all parts of the plant, including stems, leaves, peduncle, inflorescence and seeds (Thakur and Mathur, 2000; Casela et al., 2001b). Foliar infections are the most common types of infection with characteristic symptoms of circular to elliptical red spots of 0.5-6 mm diameter with few to numerous acervuli on the lamina (Tarr, 1962; Marley et al., 2004). Figure 11 shows the various symptoms of anthracnose on sorghum. Severe anthracnose infestation may lead to defoliation and even pre-mature death of susceptible cultivars. However, symptoms of the disease may vary depending on the host-pathogen interaction, the physiological state of the host and environmental conditions (Pastor-Corrales and Frederiksen, 1980).

Mid rib infection Anthracnose on sorghum leaves

Fig.11. Symptoms of foliar anthracnose of sorghum.

Sources: Thakur and Mathur, 2000; Souza- Paccola et al., 2003.

Anthracnose symptoms can also appear on other plant parts, including reddish or purplish lesions on the mid rib, stalks, peduncle and panicle or head (Tarr, 1962; Harris et al., 1964; Amador et al., 1969 as quoted by Dejene, 1988; King 1972; Bergquist, 1973). *C. sublineolum*

is also known to cause grain/head mould (Luttrell, 1950) and root and crown infection (Bergquist, 1973).

1.3.3. Epidemiology

Like any other infectious plant disease, anthracnose epidemics is largely dependent on the pathogen genotypes and its life cycle, host genotypes planted in the field and their growth stages, environmental conditions and human factors (crop cultivation practices).

Several research findings have revealed substantial differences among *C. sublineolum* isolates in terms of competitive ability, growth rate, susceptibility to fungicides and pathogenicity (Casela et al., 2001a; Kaboré et al., 2001; Souza-Paccola et al., 2003; Paper II of this thesis). Results of these studies, though most are based on in-vitro tests, have a good contribution in understanding the epidemics of sorghum anthracnose in the field. Better competitive ability and higher growth rate can be linked to increased chances in causing the disease and resulting in more severe symptoms by those strains that possess such characters. Highly pathogenic strains are also known to favor and intensify the disease (Pande et al., 1991).

Infected crop debris can serve as over seasoning sites for *C. sublineolum* (Casela and Frederiksen, 1993). The primary inoculum could also originate either from volunteer plants (alternate hosts) or infected seeds (Basu and Mathur, 1979; Cardwell et al., 1989) confirming the role of human cultivation practices on anthracnose development. Rain-splashed spores and dispersal of dry conidial masses are also reported as contributing factors to anthracnose epidemics (Nicholson and Moraes, 1980). However, the impact of wind as a long distance dispersing agent for *C. sublineolum* is either limited or not well established.

Sorghum anthracnose is a disease favored by high rainfall, high relative humidity, and moderate temperatures (Warren, 1986). To date controversies exist as to which environmental conditions best favor anthracnose development in the field. While the impact of high rainfall in creating favorable conditions for the pathogen and thereby increasing disease intensity is a universally agreed fact, the exact effect of temperature seems to be debated. Reports from various laboratories have revealed temperatures varying between 20 and 28ºC as most conducive for the growth of *C. sublineolum* (Kaboré et al., 2001; Souza-Paccola et al., 2003). Furthermore, reports by Ali and Warren (1987), and Ali et al. (1987), suggested higher temperature as most conducive to anthracnose. But Erpelding and Wang (2007) found low

temperature (15-28ºC) as responsible in increasing anthracnose severity in a field variety screening trial. Recent research suggested no significant relationship between temperature and anthracnose severity (Papers I and III of this thesis). Over all, it may be concluded that periods of high and prolonged rainfall and high relative humidity, which result in prolonged periods of leaf wetness, are the most important weather factors influencing the occurrence and further development of anthracnose in the field. This was further evidenced by Frederiksen (1984), which cited frequent rainfall as a common feature of all sorghum producing regions in South America, where anthracnose is a serious threat to grain sorghum production. Nevertheless, the impact of temperature on anthracnose severity under different agro-ecological conditions should be investigated further in multi location trials to come up with conclusive results.

Most sorghum genotypes under production are not immune to anthracnose. However, varieties differ in their susceptibility to the disease (Néya and Le Normand, 1998; Casela et al., 2001b; Hess et al., 2002; Papers III and IV of this thesis). Casela et al., (1993) have reported dilatory resistance to anthracnose among sorghum genotypes. We also have witnessed a significant arrest in the temporal progress of sorghum anthracnose by an Ethiopian sorghum accession to a level comparable to synthetic fungicides (Paper III of this thesis). Hence, planting resistant varieties can serve as a very efficient and environmentally friendly way of slowing down anthracnose epidemics in the field.

Sorghum varieties appear to have differential resistance to anthracnose at different growth stages with early and late growing stages found to be the most susceptible ones. Owing to this fact, shift in planting dates was also found to affect anthracnose development (Marley et al., 2004) and can thus be considered as another human related factor influencing the occurrence and further development of sorghum anthracnose.

C. sublineolum survives in crop residues and disperses mainly with the help of rain splash, and hence crop rotation and intercropping may also impact anthracnose development in the field. Nevertheless, the impact of each cultural practice under different agro-ecological conditions and farming systems needs further investigation. In addition, care must be taken in identifying the proper rotation scheme (crops to be rotated and time of rotation), and the selection of efficient and appropriate intercropping partners should also be given due attention.

1.3.4. Importance

Sorghum anthracnose is one of the most important sorghum diseases limiting grain production in most sorghum growing regions of the globe (Hulluka and Esele, 1992; Mukuru, 1993; King and Mukuru, 1994; Chala et al., 2007; Paper I of this thesis). The disease was first reported in 1902 from Togo (Sutton, 1980), and has now spread to all sorghum growing regions of the globe. If kept unchecked, anthracnose of sorghum can reach epidemic status especially in places where susceptible sorghum land races and high yielding improved varieties are grown (Marley et al., 2002).

Once it occurs in the field, the disease may cause a yield loss of 50% or even more (Harris et al., 1964; Powell et al., 1977; Thomas et al., 1996). Yield loss to sorghum anthracnose can be attributed to reduction in grain weight and seed density, and early abortion of seeds (Marley et al., 2004). As a foliar disease, anthracnose can also cause significant yield loss by reducing the photosynthetic area of the plant due to leaf drying. Losses to anthracnose are of particular significance in the tropics and subtropics due to suitability of the environment for disease development and shortage of effective management strategies.

1.3.5. Management

The ultimate aim of studying plant diseases is to devise proper management practices that contribute to prevent them from occurring or effectively reduce their further spread once they occur. In light of this, the management of sorghum anthracnose assumes two major options: i) strategies that aim to prevent the disease from being introduced in new areas and ii) measures that help effectively reduce further development and spread of the disease.

1.3.5.1. Cultural practices

Cultural practices like the choice of appropriate planting time, removal of crop residues and alternate hosts, and the use of clean planting material free from the pathogen are usually aimed at preventing diseases from being introduced into a field or a new area. The fact that *C. sublineolum*, the cause of sorghum anthracnose, overseasons on crop residues enables the use of rotation with non host plants as another cultural anthracnose management practice. These methods are cheap, easy to apply and environmentally friendly, and have played a role in the management of different plant diseases worldwide (Agrios, 2005). They are also being utilized in management of sorghum anthracnose (King, 1972; Pastor-corrales and Frederiksen, 1980; Marley, 2004). However, such methods must be applied at a large scale and in a

coordinated manner so that they can effectively serve as important means of preventing disease establishment in an area.

1.3.5.2. Resistant varieties

While complete immunity to anthracnose is not very common among many gene pools, there is clear evidence that sorghum genotypes may have a dilatory resistance (Casela et al., 1993; Papers III and IV of this thesis), which could be exploited in breeding for anthracnose resistance. Variation in susceptibility to anthracnose is known to exist in sorghum cultivars for many years (Dejene, 1988; Casela et al., 2001b; Erpelding and Prom 2004; 2006; da Costa et al., 2005; Papers III and IV of this thesis). However, this variation is not fully exploited mainly due to poor understanding of the genetic basis of resistance. Lack of consistent cultivar reaction under different environmental conditions also contributes a lot to the difficulties in breeding for resistance to sorghum anthracnose. Those cultivars, which have some degree of resistance, may not show the same reaction across different regions and years as anthracnose resistance is highly influenced by pathogen variability. Prevailing weather conditions may also affect the dynamics of pathogen population and host response further contributing to the lack of consistent cultivar reaction. This makes the continuous search for potential sources of resistance to anthracnose of sorghum a very important task.

Resistant genotypes of sorghum can be recognised by hypersensitive reaction (Nicholson and Warren, 1976; Thakur and Mathur, 2000) and presence of small sized lesions with little or no acervuli (Casela et al., 2001 a & b). On the other hand, susceptible cultivars develop typical symptoms of circular to elliptical spots of up to 6 mm in diameter (Dejene, 1988). Such larger lesions also harbour acervuli that in turn contain large amounts of conidia.

Mechanisms of Resistance

Sorghum employs both mechanical and biochemical defence mechanisms to either resist or escape infection by *C. sublineolum*.

Taller plants are always in advantageous position compared to shorter once as the top most photosynthetically active leaves can escape infection as they are farther from the soil and lower leaves that might have been infected initially. In addition, plant characteristics such as thick wax and epidermal layers, erect leaves, and closure of spikelet glumes during anthesis play a role in protecting sorghum from *C. sublineolum* (Bergquist et al., 1974; Frederiksen

and Franklin, 1980). The same authors have also revealed an association of tan plant colour and dark coloured seeds with anthracnose resistance.

Biochemical mechanisms involved in the defence of sorghum against *C. sublineolum* include phenols, toxic metabolites, and hydrogen cyanide (Harris and Sowell, 1968; Ferreira and Warren, 1982). A group of phytoalexins that are classified as 3-deoxyanthocyanidins are reported to be induced at the site of infection by *Colletotrichum sublineolum* (Ibraheem et al., 2010) are also known to take part in active defense of sorghum against this important pathogen. Phytoalexins are found to be accumulated in inclusions, in sorghum epidermal cells around the site of penetration, and released upon bursting of the inclusions to result in the death of the fungus and the cell that produces them (Snyder and Nicholson, 1990; Snyder et al., 1991). Other works that reported the role of phytoalexins in the defense of sorghum against *C. sublineolum* include (Nicholson et al., 1987; Lo et al., 1999a; Du et al., 2010).

A recent study has also reported inhibition of conidial germination and appressorium formation prior to penetration; and inducible defence responses including decrease in formation of appressoria, papilla formation, cell wall cross linking, and accumulation of H_2O_2 and phytoalexins as defence mechanisms of sorghum against *C. sublineolum* (Basavaraju et al., 2009).

As yet another potential mechanism of resistance, a pathogen-inducible stilbene synthase gene was demonstrated to encode an enzyme with stilbene synthase activity in sorghum infected with *C. sublineolum* (Yu et al., 2005) and further study revealed accumulation of trans-piceid as the major stilbene metabolite together with an unknown resveratrol derivative although its role in defending sorghum appeared to be secondary, at least at the seedling stage (Yu et al., 2008). Various pathogenesis related proteins (PR-proteins) are reported as part of plant defense against pathogens (Jwa et al., 2006). Lo et al. (1999b) demonstrated elevated and sustained levels of PR-10 proteins in sorghum plants 36 hours after inoculation with *C. sublineolum*. These proteins are known to activate ribonuclease (van Loon and van Strien, 1999) and hence have antifungal activity. Additional classes of antifungal proteins discovered in sorghum include chitinase, glucanase, alpha-amylase and proteinase inhibitors, and thionins (Chandrashekar and Satyanarayana, 2006).

Although lots of efforts have been made in the past to characterize sorghum for resistance to anthracnose, a lot more has yet to be done in further investigating sorghum genotypes to

uncover additional mechanisms of resistance that may exist in diverse gene pools so as to strengthen future breeding programs.

The genetics of resistance and breeding for resistance to anthracnose

Resistance of sorghum to anthracnose is found to be controlled by one or few genes. However, reports from different sorghum lines and cultivars suggested variation in the number of genes involved and their inheritance. Coleman and Stokes (1954) demonstrated anthracnose resistance in the sorghum line Sart to be controlled by two closely linked dominant genes. On the other hand, Erpelding (2007) reported that foliar anthracnose resistance in sorghum cultivar Redlan is controlled by a single dominant gene while resistance to midrib infection is controlled by a single recessive gene, which is not linked to the other gene conferring resistance to the foliar phase of the disease. Tenkouano (1993), quoted by Perumal et al. (2009), revealed a single genetic locus with multiple allelic forms conferring resistance to anthracnose in SC326-6. Boora et al. (1998) reported that resistance to anthracnose in SC326-6a is encoded by single recessive gene while Mehta et al. (2005) suggested a single dominant gene conferring resistance to anthracnose in sorghum line SC748-5.

Much of the efforts thus far in breeding sorghum for resistance to anthracnose largely depend on the selection of resistant genotypes from germplasms available in different countries (Dejene, 1988; Casela et al., 2001b; Erpelding and Prom, 2004; 2006; Erpelding and Wang, 2007; Paper IV of this thesis). However, the high genetic variability of the pathogen population, impact of environmental conditions and lack of complete understanding on the host-pathogen interaction have made it very difficult to achieve stable resistance (Casela et al., 1993; Rosewich et al., 1998; Mehta et al., 2005; Paper II of this thesis). Nevertheless, attempts have been made to come up with stable sources resistant genotypes by crossing sorghum genotypes with varying levels of resistance to anthracnose (Mehta et al., 2005). Recently progress has been made in identifying molecular markers linked to genes that confer resistance to anthracnose. Katile (2007) identified three AFLP markers (X*txa*607, X*txa*3181 and X*txa*4327) and three SSRs (X*txp*3, X*txp*55 and X*txp*72). Additional four AFLP markers X*txa6227,* X*txa3137*, X*txa2303* and X*txa3588*, and an SSR marker- X*txp549*- were also identified by Perumal et al. (2009). Boora et al. (1998; 1999) and Singh et al. (2006) have also reported markers associated with resistance genes. Identification of molecular markers associated with resistance genes is an important pre-requisite for marker assisted selection

that facilitates the selection of sources of anthracnose resistance from among diverse gene pools and help in pyramiding genes for anthracnose resistance. Thus it should continue in the future as an important tool in breeding programs.

1.3.5.3. Fungicides

Different fungicides are found to reduce the growth rate of *C. sublineolum* and arrest anthracnose development in the field, and are thus considered as effective strategies to control sorghum anthracnose. While Kaboré et al. (2001) demonstrated differential sensitivity of the anthracnose pathogen isolates to the fungicide benomyl, Marley (2004) reported a considerable reduction in anthracnose severity as a result of seed treatment and foliar sprays with fungicides. Significant reduction in anthracnose incidence and severity was also achieved by combining seed dressing with subsequent foliar application of fungicides (Gwary and Asala, 2006). Some of the fungicides reported to be effective in controlling anthracnose include benomyl, carbendazim, mancozeb, metalaxyl and thiram. Nonetheless, it should be stressed that the use of fungicides in the management of sorghum anthracnose is not a popular practice especially among the subsistence farmers that produce the bulk of sorghum worldwide, mainly due to associated costs. Improper use of fungicides is also known to affect the environment and threaten non target organisms.

2. The Thesis

This thesis is composed of four papers resulting from field surveys, laboratory experiments and field trials. The study objectives, methods followed, results obtained, major conclusions drawn and future prospects are described shortly in the sections below.

2.1. Project justification

Although sorghum is an important crop especially in areas with moisture stress, its production is hampered by several biotic and abiotic factors among which sorghum anthracnose is one. Much of the research work done in Ethiopia in relation to sorghum diseases is largely concentrated on identifying the causes of the diseases, measuring yield losses to some extent, pathogen characterization based on morphological features, searching for means of inoculation for study purpose and screening sorghum genotypes for disease resistance. However, basic knowledge on the disease, the pathogen and its interaction with the host and environment is lacking.

Sorghum is a plant native to Ethiopia, and the country is known to be the major center of sorghum diversity. The crop has been cultivated in a wide range of agro-ecological zones with different environmental conditions and farming systems for hundreds or thousands of years. This suggests the presence of diverse sorghum land races, and it might have also lead to variable pathogen populations. But it is not still possible to exploit the variation in the sorghum land races because information on the genetic diversity of the plant and the pathogen is very limited under Ethiopian conditions. Even if the pathogen causing sorghum anthracnose is well studied in other parts of the world, both the molecular and epidemiological aspects of the pathosystem are not fully understood worldwide in general or under Ethiopian conditions in particular. This makes such studies crucial not only to devise appropriate control measures but also to contribute to the scientific knowledge of the pathogen and its impact on the host.

Thus, the current project was undertaken with the following objectives:

2.2. Study objectives

Objectives of the current study were:

1. To determine the geographic distribution, incidence and severity of sorghum anthracnose in Ethiopia.

2. To characterize *C. sublineolum* isolates from different sorghum growing regions of Ethiopia using phenotypic and molecular markers.
3. To elucidate the impact of host genotypes and weather variables on the severity and temporal dynamics of sorghum anthracnose in Ethiopia.
4. To assess the level of resistance possessed by Ethiopian sorghum accessions and select sorghum accessions with good level of resistance to anthracnose.

2.3. Study Methodology

The major focus of the present work was on contributing towards the development of effective, safe and sustainable management practices to anthracnose, an important disease hampering sorghum productivity worldwide. To achieve the objectives, field surveys were carried out in different sorghum growing regions of Ethiopia in two cropping seasons (2005 and 2007); phenotypic and molecular characterisations of *C. sublineolum* isolates collected from different sorghum producing regions of Ethiopia were conducted in laboratory experiments; the impacts of weather and host genotypes on anthracnose development were assessed in a field experiment; and sorghum accessions were screened for resistance to anthracnose using different evaluation parameters in another field experiment.

2.3.1. Survey (Paper I)

Field surveys were carried out in the 2005 and 2007 cropping seasons on 487 randomly selected sorghum fields in 49 districts representing different geographic and weather conditions. During each survey visual inspections of fields were made to assess the incidence (percentage of sorghum plants in a field showing visible symptoms) and severity (average leaf area covered by symptoms) of anthracnose. A total of 50 and 30 sorghum plants were randomly selected to evaluate the incidence and severity of anthracnose in each field, respectively. Data obtained from sorghum fields were categorised into administrative districts, geographic regions, altitude groups and climatic zones. Incidence and severity data were subjected to statistical analysis using SAS, version 9.1 (SAS, 2003). In addition, correlation analysis was carried out to determine the relationship between weather conditions (rainfall and temperature) and disease parameters (incidence and severity).

2.3.2. Phenotypic and molecular characterisation of *C. sublineolum* isolates (Paper II)

2.3.2.1. Sample collection, isolation and cultivation of isolates

Sorghum leaves showing visible symptoms of anthracnose were collected from five different sites in four geographic regions of Ethiopia (Fig. 1 of Paper II). For each sampling site, sample collections were made from five to seven fields located within one km distance.

2.3.2.2. Phenotypic characterisation

A total of 50 randomly selected isolates (10 isolates per sampling site) were grown on PDA at 25ºC with four replications, and growth rate of the isolates was measured at 24 hour intervals for seven days. Colony colour and margin of the colony were recorded at the seventh day of growth.

2.3.2.3. Molecular characterisation

The molecular diversity of 102 *C. sublinoelum* isolates was studied based on AFLP analysis. DNA was extracted using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instruction, and AFLP analysis was conducted following the method developed by Vos et al. (1995) with some modifications, including i) the use of fluorescent labelled primers instead of radioactive labelled primers during selective amplification, ii) separation of selective amplification products in an ABI3730 DNA analyzer using GeneScan-1200 LIZ size standard (Applied Biosystems), and iii) scoring of peaks with the help of Gene Mapper version 4.0 (Applied Biosystems).

Peaks were scored as present or absent and the resulting binary matrix was used to calculate Dice similarity coefficients (Dice, 1945) using NTSYS-pc software, version 2.0 (Exeter Biological Software, Setauket, NY). For cluster analysis, a genetic similarity tree was constructed using the unweighted pair-group method with arithmetic average (UPGMA) based on Dice similarity coefficients. As an additional method of understanding the genetic separation of isolates, principal coordinate analysis was conducted using GenAlEx6 (Peakall & Smouse, 2006), and genetic distances between the group of isolates from each site were estimated using a pair-wise genetic distance method (Excoffier and Smouse, 1994). Furthermore, analysis of molecular variance was performed to separate variance components between and within sampling sites using Arlequine version 3.0 (Excoffier et al., 2005).

Average gene diversity (Nei, 1987), percent polymorphic peaks, genetic differentiation (F_{ST}) and gene flow were calculated to further elucidate the genetic variation among the isolates.

2.3.3. Field experiments (Papers III and IV)

Two different experiments were conducted in southern Ethiopia to i) assess the impact of host genotypes and weather variables on anthracnose development in the field, and ii) screen Ethiopian sorghum accessions for resistance to anthracnose. Both experiments were conducted in the same area (in fields close to one another), in two consecutive seasons (2007 and 2008). Natural infection from residues left on the soil was used as sources of inoculum for both experiments.

2.3.3.1. Study location

The study area was located in South Ethiopia (between 6º59.098´ N latitude and 37º52.645´ E longitude, more than 300 km south of the capital, Addis Ababa) at an altitude of 1947 masl. It is a high rainfall area (more than 1000 mm per annum based on 10 years data) with a moderate temperature (varying between 18 and 23ºC). The area is known to be conducive for anthracnose development (Chala et al., 2007).

2.3.3.2. Effect of host genotypes and weather variables on the severity and temporal dynamics of sorghum anthracnose in Ethiopia **(Paper III)**

Four sorghum genotypes were planted in plots of 7.5m^2 in a randomised complete block design with four replications. Anthracnose severity recordings were made at 10 days interval since the onset of symptoms on at least two genotypes. Initial, mean and final anthracnose severities and area under the disease progress curves were used as evaluation criteria. Correlation analysis was performed to determine the relationship between the different evaluation criteria, and correlation and regression analyses were conducted to study the impact of rainfall and temperature on anthracnose development.

2.3.3.3. Evaluation of Ethiopian sorghum accessions for resistance to anthracnose caused by *Colletotrichum sublineolum* **(Paper IV)**

A total of 56 Ethiopian sorghum accessions and two susceptible checks (AL70 and BTx623) were planted in single row plots in a randomised complete block design with three replications. Anthracnose severity was recorded at 10 day intervals and severity records were used to calculate area under the disease progress curves, which were then converted into

relative area under disease progress curves. Disease progress rates were also calculated for each accession. All the data were subjected to statistical analysis and the different evaluation parameters were correlated with each other. Finally, the accessions were classified into resistance groups based on final anthracnose severity records made at 140 days post planting (dpp).

2.4. Main results and discussion

Sorghum anthracnose was found to be an important disease spreading in most sorghum producing regions of Ethiopia (**Paper I**). It was detected in 84% of the surveyed districts. Anthracnose incidence ranged from 0 to 77%, and severity of the disease varied between 0 and 59% averaged for the two years. Both disease incidence and severity varied significantly among survey districts, geographic regions, altitude groups and climatic zones. Generally the disease was most severe in the Southwest region while most areas in the North and West regions of the country had lower anthracnose incidence (< 20% on average) and severity (< 10% on average). Areas with low to intermediate altitude had significantly higher anthracnose levels compared to highlands, and areas with high rainfall and moderate temperature had the highest anthracnose severity (approximately 40%). The disease was significantly correlated with amount of rainfall, while temperature had no significant impact on anthracnose development.

Results of the second experiment revealed considerable phenotypic and molecular variations among *C. sublineolum* isolates collected from different sorghum producing regions of Ethiopia (**Paper II**). Isolates differed significantly in terms of growth rate (1.7-5.8 mm/day, with a mean of 3.3 mm/day, $P = 0.0023$). These results are in agreement with previous studies that reported morphological/cultural variations among isolates of the same pathogen (Kaboré et al., 2001; Souza-Paccola et al., 2003). Isolates from Southwest Ethiopia had the fastest growth rate (ca. 4 mm/day on average) while isolates from North and East regions grew slowly (mean growth rate of 2.3 mm/day). In contrast to previous reports (Vaillancourt and Hanau, 1992; Marley et al., 2001), colony color and margin did not differ very much among the tested isolates. Generally, isolates included in the current study had grey to dark grey color and smooth margin except the Northern isolates, which had brownish orange color on the reverse side of the colony and undulated colony margins. Colony color was also found to change with the age of the colony, confirming the results by Browning et al. (1999); Crouch et al. (2006) and Rivera-Vargas et al. (2006), which reported unstable colony characters that

change over time. Some of the tested isolates were found to produce perithecia but the pertichecia failed to mature within 50 days after production.

The AFLP analysis revealed high levels of genetic variation among the *C. sublineolum* isolates, with Dice similarity coefficients varying between 0.32 and 0.96 (**Paper II**). Cluster and principal coordinate analyses differentiated the isolates into various groups largely according to their geographic origins. Isolates from South and East Ethiopia were most separated genetically from the other groups. Genetic variations were high both between (42%) and within (58%) sampling regions. Further, level of polymorphism and average gene diversity were highest (68% and 0.23, respectively) within the Southwest 2 group, indicating that this is the most genetically divergent group. On the other hand, isolates from the Southwest 1 group had the lowest level of polymorphism and average gene diversity (44% and 0.14, respectively), and hence the group was considered as the least diverse. Over all the *C. sublineolum* isolates included in our study were characterized by a high level of genetic differentiation (F_{ST} = 0.42) and limited gene flow (Nm = 0.34). This was in agreement with previous studies that reported high genetic variability within the sorghum anthracnose pathogen (Rosewich et al., 1998; Latha et al., 2003). However, the genetic variation between the *C. sublineolum* isolates for which no viable sexual stage has yet been proven was higher than those recorded for *Mycosphaerella fijiensis* (Zandjanakou-Tachin et al., 2009), and *Fusarium pseudograminearum* (Bentley et al., 2008 & 2009), two sexually reproducing fungi. Such results, if confirmed by future works, may indicate i) the lack of direct relationship between mode of reproduction and genetic diversity in plant pathogenic fungi; or ii) the need to search for the sexual stages of *C. sublineolum*, especially in areas of high diversity. Of course, the role of non sexual means of reproduction in creating and maintaining genetic diversity within *C. sublineolum* should be investigated further.

The severity and temporal dynamics of sorghum anthracnose were found to be significantly influenced by host genotype and weather conditions (**Paper III**) as suggested by Hess et al. (2002). The disease appeared earlier and progressed rapidly on susceptible sorghum genotypes while it progressed slowly on resistant genotypes. The initial, final, and mean anthracnose severities ranged from 0.9 to 16.1 %, 7.6 to 78.4 %, and 3.6 to 46 %, respectively, across the two experimental years (2007 and 2008). Area under the disease progress curves, averaged for the two years ranged from 221 to 2952. The Ethiopian sorghum genotype 2001 PWColl No. 022 had the lowest disease level regardless of the evaluation

criteria and experimental year. Anthracnose appeared late on this genotype and progressed slowly. The slowing of anthracnose progress by the resistant genotype was comparable to the one achieved by spraying fungicides (Gwary and Asala, 2006), and this may suggest the potential novel sources of resistance may have in controlling anthracnose. On the other hand, disease pressure was highest on the susceptible exotic cultivar BTx623, with initial, mean and final anthracnose severities and area under the disease progress curves being significantly higher on this cultivar than for any of the other three genotypes. The different evaluation criteria used in this study had a high correlation ($P<0.0001$), and hence results were consistent with one another. Correlation and regression analyses revealed that anthracnose development in the field was significantly affected by rainfall, which was in agreement with previous studies that reported a positive relationship between anthracnose severity and rainfall (Frederiksen, 1984; Hess et al., 2002; Ngugi et al., 2002). On the other hand, the effect of temperature was not significant and this was in conformity with results of the survey work (Paper I) but contrary to the report by Ali et al. (1987).

Ethiopian sorghum accessions exhibited differential reaction to anthracnose (**Paper IV**). This was in line with previous works that reported variations in anthracnose resistance among sorghum genotypes (Dejene, 1988; Néya and Le Normand, 1998; Casela et al., 2001b; da Costa et al., 2005; Erpelding and Prom, 2006). None of the tested accessions appeared immune; however, accessions differed significantly in terms of anthracnose severity ($P < 0.0001$), relative area under disease progress curve ($P < 0.0001$), and disease progress rate ($P = 0.0014$). Compared to the international susceptible check, BTx623, all the Ethiopian accessions had a significantly lower disease level, regardless of the evaluation parameter and experimental year. Fifteen and 18 accessions were resistant to anthracnose in 2007 and 2008, respectively, of which six sorghum accessions were consistently resistant in both years. Twelve accessions were moderately resistant to anthracnose over the years. Additional 19 accessions switched between resistant and moderately resistant groups across the years. In general, disease pressure was significantly affected by accessions, experimental year and interaction between accessions and experimental year. Many sorghum accessions appeared to lack consistent reaction to the disease over years, and this could have been caused by the influence of weather on the host's response, as suggested by Hess et al. (2002) and Ngugi et al. (2002), in addition to pathogen variability over time (Mehta et al., 2005). Anthracnose severity was low at the first and final assessments, and the disease progressed quite slowly on resistant accessions, while high disease severity and rapid progress were recorded for the

highly susceptible cultivar (BTx623) (**Fig. 5 of Paper IV**). These results were in agreement with those reported in Paper III.

The three parameters used to evaluate the sorghum accessions had a highly significant correlation, and hence a single anthracnose severity measurement was suggested as a time efficient method to assess the resistance of sorghum to anthracnose.

2.5. Conclusions and future perspectives

Ethiopia is located in the Northeast Africa, a region reported to be the first site of sorghum domestication. Besides, the country has diverse agro-ecological conditions, some of which are known to provide perfect conditions for the occurrence and further development of anthracnose in sorghum fields. Anthracnose was recorded in most sorghum growing regions of the country but at varying levels. The significant variations in terms of anthracnose incidence and severity across different sorghum producing regions of the country was attributed to weather conditions, particularly rainfall, and sorghum genotypes grown. In the future, survey works should continue at regular intervals to provide a more complete picture of the disease dynamics in Ethiopia over time. In addition, efforts should be made to understand the impact of climate change on the survival and competitive ability of the pathogen, and anthracnose development.

The current work on the diversity of *C. sublineolum* has demonstrated the presence of a very variable pathogen affected by little gene flow in Ethiopia. This has created quite distinct groups of *C. sublineolum* in different sorghum producing regions of the country, which must be given due consideration in future breeding programs. Additional methods like gene sequencing and pathogenicity tests may also be employed in future studies to better tap the diversity of this important pathogen. A possible presence of perithecia producing isolates in the Ethiopian *C. sublineolum* population may indicate the need to carefully search for a sexual stage of the pathogen especially in areas of high diversity. Crossing of perithecia producing isolates in culture may yield an important knowledge as to whether the pathogen is homothallic or heterothallic in case the production of perithecia is substantiated by further investigations. Such knowledge will also be crucial to understand the exact mechanisms behind the high genetic diversity of this economically important pathogen.

Both field experiments included in the current thesis enabled the identification of good sources of resistance against anthracnose. The fact that almost all of the Ethiopian sorghum

accession from this small number of collections had significantly lower anthracnose levels compared to the susceptible checks showed the great potential Ethiopian sorghum germplasm may have in serving as sources of resistance to anthracnose. This might not be very surprising considering the great sorghum diversity present in the country. In addition, sorghum production has a very long history in Ethiopia and, this coupled with suitable environmental conditions, might have led to the presence of *C. sublineolum* in sorghum fields, which in turn creates a perfect condition for host-pathogen co-evolution, a mechanism that can lead to the development of resistance. Nevertheless, consistent genotype reaction to anthracnose remains an issue even if some of the tested accessions showed a stable resistant reaction to the disease. This would be of great concern in breeding for anthracnose resistance, especially due to the high variability of the pathogen. So genotypes should be further tested in multi-location trials under different environmental conditions, and artificial inoculation with isolates of known virulence should also be used to further enhance the screening techniques and come up with more stable sources of resistance. More than 8,000 sorghum accessions are collected and conserved at the Institute of Biodiversity Conservation in Ethiopia. However, the reaction of these accessions to major sorghum diseases, including anthracnose, remains to be investigated. Hence, further efforts should be made to screen this huge potential as sources of resistance using modern methods like marker assisted selection, followed by field and greenhouse screening.

The high genetic variability of the pathogen and lack of consistent reaction by many cultivars call for an improved breeding strategy, including the pyramiding of resistant genes. Single plant selection can also help in identifying additional sources of resistance from among the less uniform local accessions.

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Paper I

Plant Pathology Journal 9 (1): 23-30, 2010
ISSN 1812-5387
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Incidence and Severity of Sorghum Anthracnose in Ethiopia

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Abstract: A two year survey was conducted to determine incidence and severity of sorghum anthracnose in different sorghum growing regions in Ethiopia. A total of 487 fields in 49 districts were surveyed in each of the 2005 and 2007 production season. Incidence of sorghum anthracnose was assessed as the percentage of plants with visible symptoms in a field and anthracnose severity was evaluated as the percentage of leaf area with symptoms. Also, the relationship of the incidence and severity of the disease to the altitude of the fields and weather conditions were determined. Results from the 2 years survey revealed that sorghum anthracnose is present in most (84%) of the survey districts. However, both incidence and severity of the disease varied significantly (p<0.0001) among the survey areas. Anthracnose incidence ranged from 0 to 77% and severity of the disease varied between 0 and 59% on average for the two years. The two year average anthracnose severity classes ranged from trace (<5%) to severe (up to 59%) and the disease was generally more severe in the Southwest and South regions. However, some districts in the East and North Ethiopia also had fields with severe anthracnose infection. It was also found out that the prevailing weather conditions especially rainfall has a significant impact on both anthracnose incidence and severity.

Key words: *Colletotrichum sublineolum*, epidemics, *Sorghum bicolar*, spot

INTRODUCTION

Sorghum (*Sorghum bicolar* L. Moench) is one of the most important cereal crops supporting the lives of millions of people across the globe and particularly in the developing world. Sorghum is known for withstanding harsh environmental conditions including high temperature, moisture deficit and water stagnation but it is susceptible to chilling (Sleper and Poehlman, 2006).

Sorghum production in the world including in Ethiopia is affected by different biotic and abiotic constraints among which sorghum anthracnose, caused by *Colletotrichum sublineolum*, is an important one, which is now considered as one of the most destructive diseases of sorghum in most sorghum growing regions of the globe. The disease can affect all aboveground parts of the plant including stems, leaves, peduncle, inflorescence and seeds (Thakur and Mathur, 2000; Casela *et al*., 2001). Foliar infections are the most common type of infection with characteristic symptoms of circular to elliptical red spots with few to numerous acervuli on lamina. However, symptoms of the disease vary depending on the host-pathogen interaction, the physiological state of the host and environmental conditions (Pastor-Corrales and Frederiksen, 1980).

Earlier reports have shown the importance of sorghum diseases including anthracnose in the world at large and in Africa in particular (Thomas, 1992; Thakur and Mathur, 2000; Ngugi *et al*., 2002). It has been reported as a disease of primary importance in West and Central Africa (Pande *et al*., 1993; Marley *et al*., 2001). The disease has also been identified as one of the most important diseases infecting sorghum in East African countries including Ethiopia and Kenya (Ngugi *et al*., 2002; Chala *et al*., 2009). However, most of the studies in Africa and particularly in Ethiopia, fall in short of providing a quantitative measurement of anthracnose incidence and severity. Nevertheless, such information is of paramount importance as it can be related to yield loss and hence economic impact of the disease (Teng, 1983; Jeger, 1990; Ngugi *et al*., 2002).

Assessment of the incidence and severity of plant diseases is important to determine the geographic distribution and status of the disease throughout a region

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in order to prioritize research. To get an accurate picture on the status of any disease, such studies, should give due consideration to the impact of geophysical and associated climatic and edaphic variations between regions. Ngugi *et al.* (2002) have determined the prevalence and severity of sorghum anthracnose along with other foliar diseases of sorghum in West Kenya. The objective of this survey work was to determine the geographic distribution, incidence and severity of sorghum anthracnose in different parts of Ethiopia.

MATERIALS AND METHODS

Survey area: Field surveys were conducted in Northern, North-Western, Southern, South-Western, Western and Eastern parts of Ethiopia in 2005 and 2007 production seasons. Survey areas were selected based on their accessibility and as they represent the bulk (more than 80%) of sorghum producing areas in Ethiopia. Surveyed areas include a wide range of administrative districts and agro-ecological zones, which lie between 5°40' and 14°18' North latitudes and between 35°44' and 42°06' East longitudes. The areas also varied in terms of weather conditions (Table 1) and altitude (1054 to 2337 m). The survey route followed major roads to towns and localities in 49 districts of five regional states. Survey areas were categorised into 6 different geographic regions.

Ethiopia is divided into five agro-climatic zones based on altitude, annual rainfall and temperature (USDA, 2002). Taking this classification scheme into consideration, we classified 20 of the 49 surveyed districts, for which temperature and rainfall data are available, into three climatic zones by combining the prevailing rainfall and temperature conditions. Zone 1 includes areas with moderate rainfall (800-1200 mm) and intermediate temperature (16-30°C), zone 2 has areas with moderate rainfall and high temperature (>30°C) and zone 3 represents areas with high rainfall (>1200 mm) and intermediate temperature.

Table 1: Ten year weather data of selected areas included in the survey program

Locality	Annual rainfall (mm)	Mean temperature (°C) (Minimum-Maximum)
Alaba	610-1241	13.5-27.6
Alamata	675-1063	14.6-30.3
Bita	1801-2200	15.1-22.5
Sekachekorsa	1142-2516	NA[1]
Chena	1801-2000	17.6-25
Chiro	560-1265	14.6-28
Damotgale	1001-1400	17.6-22.5
Dedo	1172-2844	11.4-23.1
Gimbo	1401-2000	15.1-22.5
Goroogutu	1078-1450	NA
Harbu	836-1124	13.3-31
Haramaya	607-1103	10.1-22.7
Hararzuria	493-1088	12.8-25.5
Kaloo	823-1640	13.8-30.9
Kombolcha	764-1319	12.4-26.8
Konta	1401-1800	15.1-27.5
Loma	1401-1800	15.1-25
Meeso	463-993	15.3-31.1
Mereka	1401-1800	15.1-25
Qarsa	1087-1735	NA
Quni	773-1322	12.4-27.8
Tehuledere	871-1441	10.8-26.4
Tulo	862-1088	10.3-27.8

NA: Data not available

Anthracnose assessment: The survey program covered the most important sorghum growing regions in Ethiopia with frequent stopping at different intervals depending on the variability of fields in terms of altitude, cropping system and sorghum types grown (based on crop morphology). Size of the district and availability and accessibility of sorghum fields were also given due consideration in deciding where to stop on the survey route. Three to five fields were assessed at each stop and hence up to 17 fields were assessed per district. The total number of surveyed fields was 487 in each year. The sorghum crop was between milk and hard dough stage during the survey in most of the fields although there were some fields with sorghum at maturity stage.

Disease assessment was made in 5 to 10 randomly selected spots in each field. Anthracnose incidence was assessed as the percentage of sorghum plants in a field showing visible symptoms out of 50 randomly selected plants whereas severity was determined as average leaf area covered by symptoms for 30 randomly selected diseased plants per field. In addition, data were recorded on altitude, latitude and longitude and weather data were obtained from the National Meteorological Agency.

Data analysis: The mean incidence and severity data were calculated for each district. Mean anthracnose incidence and severity of each district were used to make quantitative comparison between the survey districts.

The survey areas were also categorized into three altitude groups and anthracnose incidence and severity were obtained for each group by averaging the records from fields within the different groups. The same process was followed to determine anthracnose incidence and severity for the different geographic regions and agro-climatic zones.

All the statistical analysis were carried out using SAS Institute Inc. (9.1, 2003) and the Least Significant Difference (LSD) test was used for mean comparisons.

RESULTS AND DISCUSSION

Anthracnose incidence and severity across geographic regions: The survey program covered a wide range of

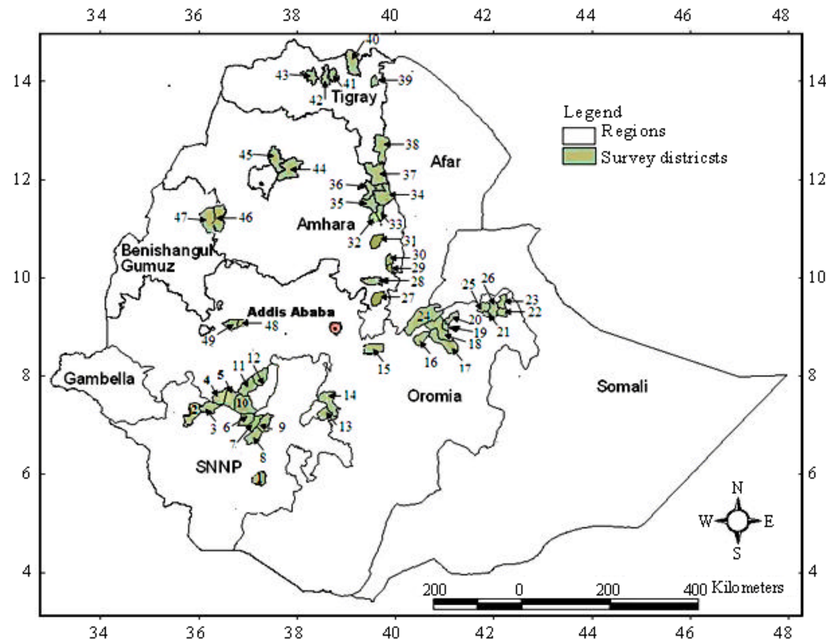

Fig. 1: Map of Ethiopia showing districts included in the survey program. 1: Gidole; 2: Chena; 3: Bita; 4: Gimbo; 5: Shebesombo; 6: Konta; 7: Loma; 8: Damotegale; 9: Mereka; 10: Dedo; 11: Qarsa; 12: Sekachekorsa; 13: Alaba; 14: Arsinegelle; 15: Welenchiti; 16: Meeso; 17: Quni; 18: Gemechis; 19: Tulo; 20: Gorrogutu; 21: Meta; 22: Hararzuria; 23: Kombolcha; 24: Chiro; 25: Kersa; 26: Haramaya; 27: Kewet; 28: Efratanagidem; 29: Jiletumuga; 30: Dewachefa; 31: Kaloo; 32: Tehuledere; 33: Ambassel; 34: Kobo; 35: Habroo; 36: Gubalafto; 37: Alamata; 38: Rayaazebo; 39: Kiliteawlalo; 40: Gantaafeshum; 41: Medebayzana; 42: Laelaymachew; 43: Taetayquoraro; 44: Libokemkem; 45: Gondarzuria; 46: Mandura; 47: Pawe; 48: Cheliya; 49: Bako. South: 1, 6, 7, 8, 9, 13, 14; Southwest: 2, 3, 4, 5, 10, 11, 12; East: 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 25; North: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45; Northwest: 46, 47; West: 48, 49

areas located in different parts of Ethiopia (Fig. 1). A total of 6 geographic regions (North, Northwest, South, Southwest, West and East) were included in the survey program and sorghum anthracnose was prevalent in all the surveyed regions but with varying intensity. Incidence of anthracnose in the different regions varied from 19 to 74% in 2005 and it ranged from 7 to 70% in 2007 (Fig. 2a), with the greatest incidence in the Northwest and Southwest. Over all, sorghum anthracnose was most severe in the Southwest (47% and 37% in 2005 and 2007, respectively) (Fig. 2b) and least severe in West and North Ethiopia.

Disease incidence and severity across districts: Although, sorghum anthracnose appeared to be prevalent in many areas of Ethiopia, both incidence and severity of the disease varied significantly across the districts (Table 2). Of the 49 districts surveyed in two years, sorghum anthracnose was recorded in 41 districts in at least one of the two years, thus anthracnose was present in ca. 84% of the survey districts. The disease was observed in 31 districts in both years and six districts in only one of the two survey years. Eight districts showed no apparent anthracnose infection in either survey year. Five districts were included in the survey program only in 2007 and four of them were found to have fields affected by anthracnose. Anthracnose incidence varied across districts from 0 to 80% and 0 to 74% in 2005 and 2007, respectively, while severity of the disease was in the range of 0 to 78% and 0 to 40% in 2005 and 2007, respectively.

Average disease incidence across the whole country was almost the same for the two years being 31.6 and 31.4% in 2005 and 2007, respectively. However, average anthracnose severity across the whole country was lower in 2007 (13%) than in 2005 (20.7%). The two year average disease incidence was highest (77%) in districts of Dedo and Shebesombo followed by Bita, Dewachefa and Gimbo, which had 71 to 72% anthracnose incidence. These areas are all located in the Southwest and South Ethiopia except Dewachefa, which is located in the North. Average anthracnose severity was the highest in Bita (59%)

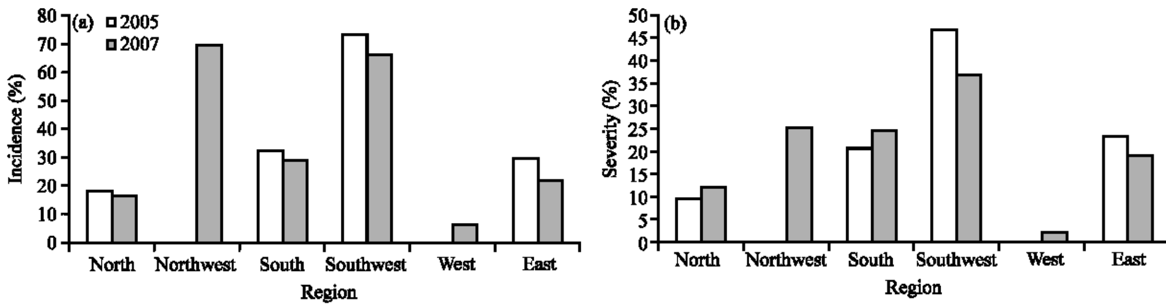

Fig. 2: (a) Incidence of sorghum anthracnose in different geographic regions of Ethiopia and (b) severity of sorghum anthracnose in different geographic regions of Ethiopia. The Northwest and West regions were not surveyed in 2005

Table 2: Incidence and severity of sorghum anthracnose in different districts of Ethiopia

				Incidence			Severity		
Region	District	No. of farms surveyed	Altitude (m)	2005	2007	Mean[1]	2005	2007	Mean
South	Alaba	5	1795	25.8	22.5	24.1ijk	31.7	9.5	20.6f-k
	Arsinegelle	5	1944	25.5	35	30.2hi	16.2	13	14.6i-q
	Damotgale	8	1947-1952	56.8	53.8	55.3e	42.2	25.5	33.9cde
	Gidole	15	1297-1590	-	69.4	-	-	13.8	-
	Konta	10	1154-1640	43.3	36	39.7gh	21.2	16.4	18.8g-l
	Loma	10	1247-1945	50	53	51.5ef	34.4	18	26.2e-i
	Mereka	7	1240-1550	61.2	57.5	59.4cde	31.9	28	29.9d-g
Southwest	Bita	12	1837-1953	75	66.7	70.8ab	77.8	40	58.9a
	Chena	15	1794-2014	70.2	67.5	68.9ab	45.7	27	36.4cde
	Dedo	15	1934-2283	78.3	75	76.7a	54.7	34.3	44.5bc
	Gimbo	15	1762-1938	72.6	70	71.3ab	62.5	42.5	52.5ab
	Qarsa	10	1728-1807	72.1	66	69.1abc	54.7	27	40.8bcd
	Sekachekorsa	8	1797-1940	68.9	62.5	65.7bcd	37	27.5	32.3c-f
	Shebesombo	6	1522-1738	80.2	73.3	76.8a	34.1	23.3	28.7d-h
East	Chiro	15	1764-2310	20.4	16.7	18.5jkl	14.3	4.5	9.4l-q
	Gemechis	5	1925-1926	4.8	2.5	3.7opq	2.9	1	1.9opq
	Goroogutu	10	2113-2275	10.2	5	7.6m-q	2.2	2.5	2.3opq
	Haramaya	10	2027-2077	40.1	36.7	38.4gh	22.5	18.3	20.4g-k
	Hararzuria	5	1940	10.7	10	10.3l-p	13.3	3	8.2l-q
	Kersa	10	2045-2134	55.4	51	53.2ef	18	15	16.5h-n
	Kombolcha	5	2225	55.3	35	45.2fg	38.4	20	29.2d-h
	Meeso	15	1360-1397	8.2	0	4.1opq	14.3	0	7.2l-q
	Meta	8	2265-2334	0	0	0q	0	0	0q
	Quni	10	1744-1764	18.9	21	19.9jkl	16.7	20	18.3g-l
	Tulo	10	2169-2337	18	15	16.5klm	14.3	3.2	8.7k-q
	Welenchiti	10	1460-1474	0	0	0q	0	0	0q
North	Alamata	15	1476-1516	45.1	66.7	55.9e	21.8	14	17.9g-m
	Ambassel	12	1546-1734	10.2	0	5.1 opq	8.2	0	4.1n-q
	Dewachefa	15	1433-1488	68.1	74.4	71.2ab	35.8	25.6	30.7d-g
	Efratanagidem	10	1477-1564	20.1	40	30.1hi	13.5	10	11.7j-q
	Gantaafeshum	5	2035	2.1	0	1pq	1.6	0	0.8q
	Gondarzuria	8	1938-2088	5	0	2.5pq	2.3	0	1.1pq
	Gubalafto	12	1496-1828	12.2	8.3	10.3l-p	5	2	3.5opq
	Harbu	17	1578-1870	0	0	0q	0	0	0q
	Jiletumuga	15	1158-1492	53.3	60.6	57de	29	28.8	28.9d-h
	Kaloo	10	1569-1684	21	17.5	19.3jkl	6	4	5m-q
	Kewet	5	1426	12.1	0	6.1n-q	8.3	0	4.2n-q
	Kiliteawlalo	5	2229	7	1	4opq	1	1.5	1.3pq
	Kobo	15	1490-1730	25.1	30.6	27.9ij	37.6	11.5	24.5e-j
	Laelaymachew	8	2055-2166	0	0	0q	0	0	0q
	Libokemkem	5	1881	0	0	0q	0	0	0q
	Medebayzana	5	2216	2.3	0	1.2pq	1.6	0	0.8q
	Rayaazebo	10	1670-1768	0	0	0q	0	0	0q
	Taetayquoraro	5	1968-1972	0	0	0q	0	0	0q
	Tehuledere	15	1669-1978	20.1	10	15k-n	11.2	8.2	9.7k-q
Northwest	Mandura	15	1061-1465	-	66.4	-	-	20.7	-
	Pawe	15	1054-1180	-	73.3	-	-	30	-
West	Bako	6	1654-1704	-	13.3	-	-	5	-
	Cheliya	5	2073-2160	-	0	-	-	0	-

Means in a column followed by the same letters are not significantly different according to LSD at 5% probability level. Areas not surveyed in 2005 p<0.0001 for both incidence and severity

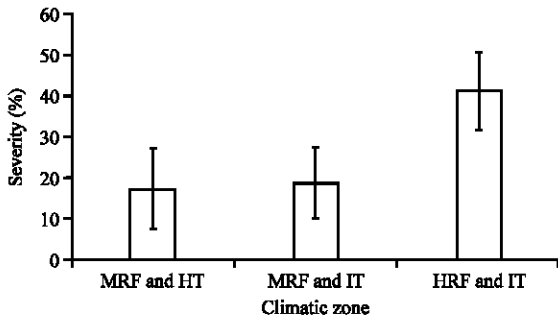

Fig. 3: Anthracnose severity in different climatic zones of Ethiopia. MRF: Moderate rainfall (800-1200 mm), HRF: High rainfall (>1200 mm) IT: Intermediate temperature (16-30°C), HT: High temperature (>30°C)

Table 3: Incidence and severity of sorghum anthracnose in different altitude groups averaged for the two survey years

		Incidence (%)		Severity (%)	
Groups	Elevation (m)	Range	Mean	Range	Mean
I	<1500	0-73	43.4a	0-35	16.8a
II	1500-2000	0-77	31.8a	0-59	19.8a
III	>2000	0-53	15.2b	0-29	7.3b

Means in a column followed by the same letter are not significantly different according to LSD at 5% probability level

followed by Gimbo (53%) and Dedo (45%). Across the survey areas, where the disease was apparent, the lowest average anthracnose incidence (1-3%) was recorded in Gantaafeshum, Gondarzuria, Kiliteawlalo and Medebayzana. The same areas, which are all located in the North, also had the lowest anthracnose severity (ca. 1%).

Incidence and severity of sorghum anthracnose across altitude groups: The survey areas were categorized in to three altitude groups based on their elevation (Table 3). Group I consists of areas, which are located at altitudes below 1500 m and hence considered as lowlands. Group II, areas with altitude ranging between 1500-2000 m, were considered as intermediate altitudes while the Group III areas, with altitude of > 2000 m, were considered as highlands. Both anthracnose incidence and severity were significantly higher ($p<0.0001$ for incidence and $p = 0.0018$ for severity) in the low and intermediate altitudes. Anthracnose severity was up to 35 and 59% while incidence reached up to 73 and 77% for the lowlands and the intermediate altitude areas, respectively. On the other hand, the highlands had a lower level of anthracnose with a maximum of 53 and 29% disease incidence and severity, respectively, indicating that fields with severe anthracnose infection are consistently located in areas below 2000 m.a.s.l.

Severity of sorghum anthracnose in different climatic zones: Complete rainfall and temperature data were available for 20 of the 49 survey districts and hence only these areas could be classified into different climatic zones. Fifty one, 70 and 100 fields were included in zone 1 (areas with moderate rainfall and intermediate temperature), zone 2 (moderate rainfall and high temperature) and zone 3 (high rainfall and intermediate temperature conditions), respectively. None of the survey areas have low rainfall and low temperature and combination of high rainfall and high temperature was also absent among the survey areas. Average results of the two year survey revealed that sorghum anthracnose was less severe in areas with moderate rainfall and intermediate temperature and in areas with moderate rainfall and high temperature (Fig. 3). The disease was severe (41% average severity) in areas with high rainfall and intermediate temperature.

Correlation between geo-climatic factors and anthracnose occurrence: Anthracnose incidence and severity had negative and significant correlation with altitude ($r = -0.35$ and $r = -0.21$, respectively). There was strong positive correlation between 10 year mean rainfall and mean anthracnose incidence and severity ($r = 0.66$ and 0.72, respectively) (Table 4). Rainfall during the actual sorghum production season (April to October) of 2005 and 2007 correlated also strongly and significantly with both anthracnose incidence and severity. On the other hand, temperature had insignificant correlation with both disease incidence and severity. This indicated the strong influence of weather conditions particularly that of rainfall on anthracnose development. A strong positive and highly significant correlation ($r = 0.86$ to 0.92) was also found between disease incidence and severity.

Sorghum is grown over a large area in many parts of Ethiopia. The current survey in different parts of Ethiopia showed great variations in anthracnose ranging from mild to severe infections. About 37% of the surveyed districts had moderate to severe anthracnose infection indicating the potential of the disease in hampering sorghum productivity. Ngugi *et al.* (2002) reported that anthracnose with severities of 20% or more, can impact sorghum yields. Given this prediction, it is possible to assume that anthracnose is likely to cause significant yield loss in 18 (37%) of the 49 surveyed districts unless sorghum plants in these areas are tolerant to the disease or other management practices are undertaken.

Variations in incidence and severity of plant diseases in general can be attributed to differences in cultural practices, which include soil cultivation and removal of

Table 4: Pearson correlation between weather conditions and anthracnose incidence and severity

	Incidence			Severity			Rainfall		
	2005	2007	Mean	2005	2007	Mean	2005	2007	10M
Incidence									
2005	-	0.97****	-	0.87****	-	0.92****	0.56*	-	0.70***
2007	-	-	-	-	0.89****	0.88****	0.45^{NS}	0.49*	0.60**
Mean	-	-	-	0.86****	0.92****	0.91****	0.51*	0.55*	0.66**
Severity									
2005	-	-	-	-	0.90****	-	0.52*	-	0.65**
2007	-	-	-	-	-	-	0.65**	0.68**	0.76***
Mean	-	-	-	-	-	-	0.59**	0.60**	0.72***
Temperature									
2005	-0.30^{NS}	-0.20^{NS}	-0.25^{NS}	-0.25^{NS}	-0.35^{NS}	-0.30^{NS}	-	-	-
2007	-	-0.25^{NS}	-0.29^{NS}	-	-0.36^{NS}	-0.36^{NS}	-	-	-
10M[1]	-0.07^{NS}	-0.02^{NS}	-0.05^{NS}	-0.14^{NS}	-0.18^{NS}	-0.16^{NS}	-	-	-

10M: Mean of 10 year data. Rainfall and temperature conditions for individual year represent the actual weather during sorghum production season (April-October). NS: Statistically not significant, *Significant at $p<0.05$, **Significant at $p<0.01$, ***Significant at $p<0.001$, ****Significant at $p<0.0001$

crop residues, host genotypes, planting time and the growing environment (Néya and Normand, 1998; Marley, 2004). Cropping systems (mono- vs. inter-cropping and use of variety mixtures) are also known to contribute to disease pressure in positive or negative ways (Agrios, 2005). It was obvious from the present study that farmers in Ethiopia do not apply any specific management practice to combat anthracnose at least consciously. In most of the surveyed areas, sorghum is grown as a sole crop but there were also fields, where it was intercropped with beans and teff (*Eragrotis teff*) in most cases and maize (*Zea mays*) in a very few cases. In our study, we did not observe any pattern between the levels of anthracnose occurrence and growing sorghum either as a sole crop or intercropped with others (data not shown). Most of the intercrops, especially beans and teff, are usually planted late in the season (end of July or later) compared to sorghum, which is usually planted in April or earlier and by that time the initial infection of sorghum by *Colletotrichum sublineolum* might have already occurred (anthracnose can be observed in the field as early as the end of June or beginning of July) as reported by Chala *et al.* (2009). Besides, most of the crops that are intercropped with sorghum are much shorter compared to the tall sorghum plants that can reach as high as 4 m, which are very common in Ethiopia and hence, may not be very effective in preventing further spread of the pathogen from one sorghum plant to the other.

Crop rotation is one important cultural practice that influence disease development through its effect on inoculum survival and carry over (Agrios, 2005). Most of the surveyed fields were continuously planted to sorghum while some were rotated with maize and this might have contributed to the high inoculums build up of *C. sublineoulm* especially in areas with conducive environmental conditions. However, the exact impact of crop rotation under Ethiopian conditions should be investigated in the future by involving different kinds of crops.

Prevailing weather is another important factor that influences the incidence and severity of plant diseases (Kranz and Rotem, 1987). In this study, most of the fields with moderate to severe anthracnose infection are located in the Southwest and South parts of the country, which usually have intermediate to high temperature and high rainfall. Information on the relative humidity of the survey areas was not available for most of the surveyed areas but it is obvious that relatively warm temperature and high rainfall may give rise to high relative humidity. Association of severe sorghum anthracnose infection with low and intermediate altitude areas is most probably attributed to the prevailing weather conditions as most areas with intermediate to high temperature and high rainfall are situated in these altitude groups. These results are in agreement with previous findings that associate a more intense sorghum anthracnose with high temperature and relative humidity (Ali and Warren, 1987; Hess *et al.*, 2002; Thomas, 1992). Exceptions to this trend were observed in three districts, where sorghum fields had low to moderate anthracnose infection despite the environmental conditions being conducive for the disease. This deviation from earlier reports associating anthracnose with warm and humid weather could be linked to the wide distribution of local land races, which might possess certain levels of resistance to the disease. Farmers in these areas have grown sorghum for several decades or even centuries and hence the land races might have developed resistance to the disease. Dogget (1980) also indicated host-pathogen co-evolution as a possible mechanism of establishing an equilibrium that in the end leads to low infection levels.

Ethiopia has 5 climatic zones depending on rainfall and temperature conditions (USDA, 2002). However, it was not possible to categorise all the surveyed areas into these climatic zones due to the lack of weather data for some of the districts included in our survey program. Combining the prevailing temperature and rainfall conditions from 23 of the 49 districts led to the

classification of these areas into 3 distinct zones. Variations in anthracnose intensity were noticeable across these zones with the highest disease severity recorded in warmer areas that received higher annual rainfall (>1200 mm). While high anthracnose incidence and severity are always associated with high rainfall, some survey areas with higher temperature had less severe anthracnose and in general the correlation between temperature and anthracnose severity was weak and insignificant (Table 4). In addition, Erpelding and Wang (2007) reported low temperature as conducive for anthracnose development in the field and Chala *et al.* (2009) suggested temperature as having a less important role on anthracnose development compared to rainfall. Given the fact that many of the surveyed areas included in the present study have more or less similar temperature conditions, we believe rainfall was the most important factor behind much of the variations in anthracnose intensity. This was also supported by the strong positive and highly significant correlations that existed between anthracnose incidence and severity and amount of rainfall (Table 4). It was observed that the 10 year average rainfall, which represents the long term and year round climatic conditions of the survey areas, had a stronger correlation with anthracnose incidence and severity compared to the actual rainfall in the growing season of each survey years. This might be because the former has an additional impact on the survival, build up and spread of the inoculum.

This is the first comprehensive study on the geographic distribution, incidence and severity of sorghum anthracnose in Ethiopia. Results of this survey revealed the geographic distribution and intensity of the disease. Occurrence of anthracnose at a severe level in some areas justifies the need for further studies to determine associated yield loss and thereby the economic importance of the disease at least across areas with moderate and high level of infection. Variations in anthracnose intensity among the survey areas indicate not only the effect environmental conditions may have on the disease but also the role local land races may play in managing the disease. Thus, we suggest future studies especially those related to yield loss assessment and disease management should be conducted across areas with different environmental conditions and should give due attention to local land races that may serve as good sources of resistance. Future studies should also give due attention to the impact of cropping practices including cultivar or species mixture on disease development.

We collected sorghum leaves from surveyed areas showing symptoms of anthracnose and the pathogen *Colletotrichum sublineolum* was isolated from the samples. Currently work is underway to characterise the isolates. Besides, field experiments are being conducted to evaluate Ethiopian sorghum germplasm for resistance to anthracnose.

CONCLUSIONS

Sorghum anthracnose was found to prevail in most sorghum growing regions of Ethiopia. However, both disease incidence and severity varied significantly across survey districts, altitude groups and climatic zones. Over all, the Southwest region had the highest anthracnose level and it was followed by the South, Northwest, East, West and North regions of the country. The disease was more severe in areas of intermediate and low altitude than high altitude areas and areas that receive higher annual rainfall had higher anthracnose level as compared to areas with low rainfall. Results of the current survey showed that anthracnose could cause a significant yield reduction in at least 37% of the survey districts and hence, management strategies should be sought to control the disease in an effective, affordable and sustainable manner.

It was found out that anthracnose development in the field is affected by weather and particularly rainfall had a significantly positive impact on the disease. Although, both long term rainfall conditions and actual rainfall during the cropping season affected the incidence and severity of anthracnose in sorghum fields, the former had more pronounced effect and this might be because of its impact on pathogen survival and inoculums build up. Nevertheless, some areas with conducive environment to anthracnose were found to have low anthracnose severity confirming role of host-pathogen co-evolution as one mechanism towards disease resistance. Further research is needed to elucidate the impact of different weather variables on this disease of global importance.

ACKNOWLEDGMENTS

Funding for this survey work was obtained from the Research and Extension Office of Hawassa University and Norwegian Agency for Development and Cooperation. The first author was also financial supported by the Lånkassen, Norway. We are grateful to Professor Ruth Dill-Macky for critically reviewing the manuscript. We thank Alemayehu Getachew, Simon Atsbaha and Tesfaye Shiferaw for their help during the field surveys and Demeke Nigusse and Mihret Denanto for creating the map. We also acknowledge Drs. Firdu Azerefegne and Tameru Alemu for their advice and kind provision of logistic support. Weather data were kindly provided by the National Meteorological Agency.

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Paper II

AFLP analysis revealed a high level of genetic differentiation and low gene flow in *Colletotrichum sublineolum* in Ethiopia, the center of origin and diversity of sorghum

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Abstract

Isolates of *C. sublineolum* were collected from different sorghum producing regions of Ethiopia and divided into five groups based on their geographic origin. The growth rate of fifty isolates showed considerable variation: 1.7-5.8 mm/day, mean 3.3 mm/day, P = 0.0023. However, the isolates displayed little variation in colony color and colony margin except for isolates from the North that were different from the others. Amplified fragment length polymorphism analysis of 102 isolates revealed much greater variations among the different groups. The Dice similarity coefficients ranged from 0.32 to 0.96 (mean 0.78). Cluster analysis and principal coordinate analysis revealed a differentiation of the isolates according to their geographic origin, and both methods clearly indicated a genetic separation between the Southern, the Eastern and the other isolates. Analysis of molecular variance (AMOVA) indicated a high level of genetic variation both among (42%) and within (58%) the *C. sublineolum* sampling sites from Ethiopia. The AMOVA also indicated a high level of genetic differentiation (F_{ST} = 0.42) and limited gene flow (Nm = 0.343). The results of this study confirmed the presence of a highly diverse pathogen, which is in agreement with the existence of diverse host genotypes and wide ranging environmental conditions in sorghum producing regions of the country. Such diversity should be taken into account in future breeding programs to achieve an effective and sustainable disease management strategy.

Key words: genetic variation, genetic differentiation, *C. sublineolum*, sorghum anthracnose

Introduction

Sorghum is an important cereal crop cultivated on more than 46 million hectares of land all over the world (FAO, 2010). It is the fifth most important cereal in the world (Sleper & Poehlman, 2006) and one of the five leading cereals in terms of production and productivity in Ethiopia (CSA, 2009). Sorghum is cultivated on more than 1 million hectares of land in Ethiopia making the country the ninth largest producer of sorghum in the world (FAO, 2010). Ethiopia is regarded as one of the centers of origin and the major center of diversity for sorghum (Sleper and Poehlman, 2006). Sorghum is known for its tolerance to adverse conditions, but its productivity is hampered by several biotic constraints, which are responsible for more than 70% yield reduction annually (Berenji and Dahlberg, 2004). One of the factors limiting sorghum production is the disease anthracnose (Hulluka & Esele, 1992; Mukuru, 1993; King & Mukuru, 1994), which is caused by *Colletotrichum sublineolum.* Sorghum anthracnose occurs in all sorghum growing regions around the world, where it prevails especially under hot and humid conditions (Tarr, 1962; Ali & Warren, 1987). In Ethiopia it is considered to be one of the major factors limiting sorghum production (Hulluka & Gebrekidan, 1980; Dejene, 1988; Chala *et al*., 2007; 2010). Anthracnose on maize and sorghum was believed to be caused by two different pathotypes of *Colletotrichum graminicola*. Nowadays, it is generally regarded that maize anthracnose is caused by *C. graminicola* while *C. sublineolum* is causing anthracnose on sorghum (Holliday, 1980; Vaillancourt & Hanau, 1992; Sheriff *et al*., 1995). Nevertheless, several reports still use *C. graminicola* to refer to isolates causing anthracnose on sorghum. However, in this paper, we use the species name *C. sublineolum* as the causative agent of sorghum anthracnose.

The genus *Colletotrichum* is very diverse and is responsible for causing a large array of diseases affecting many crops from cereals to trees. *Colletotrichum* species are genetically very variable and this has probably enabled adaptation to a wide range of hosts and

environmental conditions (Freeman *et al*., 1998; Freeman *et al*., 2001; Abang *et al*., 2002; Afandor-Kafuri *et al*., 2003). The diversity within the species *C. sublineolum* is also known to be large as revealed by molecular markers and morphological characters, as well as in physiology, competitive ability and pathogenicity (Browning *et al*., 1999; Casela *et al*., 2001; Kaboré *et al*., 2001; Latha *et al*., 2003; Souza-Paccola *et al*., 2003). This suggests the possibilities of large intra-species genetic diversity, which in turn make the development of resistant varieties more complicated.

In Ethiopia, sorghum is produced by subsistence farmers in different parts of the country that experience a wide range of environmental conditions. Furthermore, farmers maintain mixtures of sorghum land races paving the way for the existence of diverse races of *C. sublineolum*. As the management of sorghum anthracnose is largely dependent on the deployment of resistant varieties, searching for possible sources of resistance and breeding for resistance to anthracnose is an important task and this makes it crucial to understand the pathogen diversity. Diversity studies of isolates sampled at different times and sites generate important knowledge, including tracing the origin of disease outbreaks locally and globally (Cooke & Lees, 2004). Such knowledge could in principle enable us to understand the epidemiology of plant diseases. It may also suggest the role of different evolutionary forces in the population structure (Burdon & Silk, 1997) and predict the sustainability of management practices (McDonald & Linde, 2002).

Different methods have been used to study the diversity of *Colletotrichum* sp. and DNA based molecular techniques have proved to be very useful. The methods applied include random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), arbitrarily primed-PCR, A+T-rich analysis, and restriction analysis of PCR amplified intergenic spacer regions of rDNA (Freeman *et al*., 1993; Browning *et al*., 1999; Latha *et al*., 2003). Given their ability

to yield many loci per primer combination, AFLPs are reported to be one of the most powerful molecular markers (Milbourne *et al*., 1997). In addition to their robustness, AFLPs are neutral markers, making them readily useful for genetic diversity studies. An additional advantage is the lack of need for prior knowledge on the genome of the organism to be studied (Cooke *et al*., 2003; Flier *et al*., 2003).

The current study was initiated to abridge the lack of information on the diversity of *C. sublineolum* populations that exist in different sorghum producing regions of Ethiopia. *C. sublineolum* isolates from different sorghum growing regions of Ethiopia were characterized using phenotypic characters i.e. growth rate and colony morphology, and genetic markers based on AFLP analysis.

Materials and methods

Sample collection

Sorghum leaves showing visible symptoms of anthracnose were collected from sorghum fields in five localities in four geographic regions of Ethiopia (Figure 1). The sample collection sites represented different geographic locations at varying elevations and with different climatic conditions (Table 1). Five to seven fields located within one km distance were sampled per locality and three to five leaves were collected from each field. The leaf samples were stored in paper bags at room temperature until isolation of *C. sublineolum*.

Isolation and cultivation of C. sublineolum

Infected leaves collected from the different geographic regions were cut into pieces, surface sterilized using 0.5% sodium hypochlorite (NaOCl) solution for 90 seconds, and rinsed three times in sterile distilled water. The surface sterilized pieces of leaves were placed on oatmeal agar (OMA) and incubated at 25°C under continuous fluorescent light for seven days. After a

period of five to seven days, mycelium with typical *C. sublineolum* conidia were observed on the OMA plates. Pure cultures of more than 160 single spore isolates representing the different geographic regions were obtained and the cultures were maintained on potato dextrose agar (PDA), and stored at 4°C as stock cultures. Isolates collected from the same site were categorized into a single group so that there were five *C. sublineolum* groups included in the current study.

Phenotypic characterization of C. sublineolum isolates

To study the phenotypic characters (growth rate and colony morphology) of the *C. sublineolum* isolates, a total of 50 single spore isolates representing the five isolate collection sites (10 isolates per site) were randomly selected from the stock cultures and cultivated on PDA at 25°C in the dark. After three to four days of incubation, 3 mm mycelia plugs were taken from the actively growing edge of each isolate, transferred to the centre of four replicate PDA plates, and incubated in the dark at 25°C.

For each isolate, radial growth was recorded at 24h intervals for seven days. Additionally, colony color and margin were registered. The growth rate of each isolate (mm per day) was calculated and the data were subjected to statistical analysis using SAS, version 9.1 (SAS Institute, Carry NC, USA). Mean separation was carried out using the least significant difference (LSD) test at P = 0.05.

DNA extraction

Approximately 100 mg of fresh mycelium per isolate was crushed in liquid nitrogen using mortar and pestle. The fine powder of mycelium was transferred to a 2 ml microcentrifuge tube and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The quality of the extracted DNA

was controlled on 0.8% agarose gels and the DNA was stored at -20°C prior to the AFLP analysis.

AFLP analysis

AFLP analysis was conducted following the method developed by Vos *et al*. (1995) with modifications that included the use of fluorescently labeled primers instead of radioactive labeling.

Approximately 200 ng of DNA from each isolate was digested with 5 units (U) each of *Eco*RI and *Mse*I for two to three hours at 37°C in 40 µl reaction volume containing 8 µl of 5x restriction-ligation (RL) buffer (50 mM Tris-HAc (pH 7.5), 50 mM Mg- acetate, 250 mM K-acetate, 25 mM DTT, and 250 ng/µl BSA). The digested DNA samples were ligated to 5 pmol *Eco*RI adapter (made from 5´-CTCGTAGACTGCGTACC-3´ and 3´-CATCTGACGCATGGTTAA-5´) and 50 pmol *Mse*I adapter (made from 5´-GACGATGAGTCCTGAG-3´ and 3´-TACTCAGGACTCAT-5´) overnight at room temperature in a 40 µl reaction volume that contained 0.5 µl ATP (10 mM), and 0.33 µl of T4 DNA ligase (1 U/µl) (Fermentas, Glen Burnie, Maryland) in 1 µl 5x RL buffer.

Preamplification PCR was carried out with the non-selective primers *Eco*RI$_0$ (5´-GACTGCGTACCAATTC-3´) and *Mse*I$_0$ (5´-GATGAGTCCTGAGTAA-3´) in a 25 µl total reaction volume containing 5 µl of 5-fold diluted ligation mix, 2.5 µl of each primer (50 ng/µl each), 2.5 µl of 10x PCR buffer (100 mM Tris-HCl, 500 mM KCl, and 15 mM $MgCl_2$), 0.24 µl dNTP (2.5 mM) , and 0.2 µl Taq DNA polymerase (5 U/µl). The PCR program was set as follows: 94°C for 2 min for initial denaturation; 45 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 90 s; and a final cycle of 72°C for 10 min.

Six combinations of *Mse*I and *Eco*RI primers were used for selective amplification: E12 (5´-GACTGCGTACCAATTCAC-3´) X M16 (5´-GATGAGTCCTGAGTAACC-3´);

E19 (5´-GACTGCGTACCAATTCGA-3´) X M15 (5´-GATGAGTCCTGAGTAACA-3´); E19 X M16; E20 (5´-GACTGCGTACCAATTCGC-3´) X M17 (5´-GATGAGTCCTGAGTAACG-3´); E21 (5´-GACTGCGTACCAATTCGG-3´) X M16; and E21 X M17. The primers differ by two selective nucleotides at their 3´ ends and the *Eco*RI primers were labeled with the fluorescent dye 6-FAM (6-carboxyfluorescein). The selective amplification reaction mix contained 1.6 µl dNTP (2.5 mM), 2 µl of 10x PCR buffer, 0.08 µl of Taq DNA polymerase (5 U/µl), 5 µl *Mse*I-primer (6 ng/µl) and 1 µl *Eco*RI-primer (1 pmol) to which 5 µl 10 fold diluted preamplification PCR product was added as a template. The PCR amplification conditions were as follows: 1 cycle of 94°C for 30 s, 65°C for 30 s and 72°C for 60 s; 12 cycles where the annealing temperature was lowered by 0.7°C for each cycle; 23 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s; finally 72°C for 7 min.

Data scoring and analysis

Amplification products were separated in an ABI3730 DNA analyzer (Applied Biosystems Inc., Foster City, California) following the manufacturer's protocol and using GeneScan-1200 LIZ size standard (Applied Biosystems). The presence (1) and absence (0) of peaks were scored using Gene-Mapper software version 4.0 (Applied Biosystems Inc.), checked manually, and only clear and unambiguous peaks with fluorescence greater than or equal to 100 arbitrary units were entered into a binary data matrix for further analysis. The accuracy of the analysis was checked by running four randomly selected samples in duplicates.

The binary matrix was used to calculate genetic similarities between the isolates based on the Dice similarity coefficient (Dice, 1945), and the unweighted pair-group method with arithmetic average (UPGMA) was used to construct a genetic similarity tree with the help of the NTSYS-pc software, version 2.0 (Exeter Biological Software, Setauket, NY). To further elucidate the genetic relationship among the tested isolates, principal coordinate analysis was

conducted using the software GenAlEx6 (Peakall & Smouse, 2006). To separate variance components among and within sample collection sites, analysis of molecular variance (AMOVA) was carried out using Arlequine version 3.0 (Excoffier *et al*., 2005). The percentage of polymorphic peaks was calculated for each sampling site as a ratio between the number of polymorphic peaks and the total number of peaks generated for each site, and genetic distances between the group of isolates from each site were estimated using a pair-wise genetic distance method (Excoffier and Smouse, 1994). Genetic differentiation (F_{ST}) was determined from the AMOVA and its significance tested based on 10100 permutations (Schneider *et al*., 2000). The F_{ST} value was, in turn, used to estimate gene flow (Nm) as $Nm = (1/F_{ST} - 1)/4$ (Whitlock & McCauley, 1999).

Results

Phenotypic characterization

Mycelial growth rate varied considerably among isolates from the different sampling sites. Isolates representing the sites Southwest 2, South and Southwest 1 grew at average rates of 4.05, 3.91 and 3.89 mm/day respectively, while isolates from the East and North regions had significantly slower growth ($P = 0.0023$) with average growth rates of 2.31 and 2.30 mm/day, respectively (Figure 2). However, growth rate was found to vary also between isolates from the same sampling site.

Not much variation was observed among the tested isolates in terms of colony-color and -margin. The tested isolates generally appeared to have grey color on the upper side of the colony at the seventh day of growth, while their reverse side had grey to dark grey color for all isolates except the 10 isolates from North Ethiopia that appeared brownish orange on the reverse side. Also with respect to colony margin, the Northern isolates were different from the others showing undulated margin compared to the smooth margin observed in the others.

Genetic characterization

AFLP analysis was conducted on 102 isolates representing the five different sampling sites. The six primer combinations used in this study generated a total of 299 clearly scorable peaks out of which 296 (98.8%) were polymorphic. The number of peaks generated per primer combination ranged from 30 to 76 and was on average 49.8 while percent polymorphic peaks per primer combination varied between 97 and 100 with a mean of 99 (data not shown). The total number of peaks and the percentage of polymorphic peaks varied among the groups of isolates from the different sampling sites, with the largest proportion of polymorphic peaks (68%) detected within the Southwest 2 sampling site, while Southwest 1 had the lowest number and proportion of polymorphic peaks (44%) (Table 2).

Average gene diversity over loci ranged from 0.14 to 0.23 with a mean of 0.19 (Table 2) indicating a moderate gene diversity within each group of isolates. The Southwest 2 group had the highest gene diversity (0.23) followed by the North (0.22), South (0.19), East (0.15) and Southwest 1 (0.14).

The Dice similarity coefficient for the 102 isolates analyzed in this study varied from 0.32 to 0.96 (average 0.78). The Dice similarity coefficient was found to be of widest range (0.32-0.91) within the Southwest 2 group followed by the groups from the East (0.47-0.95), North (0.55-0.91), and South (0.60-0.96) (Table 2). The Southwest 1 group had the most closely related isolates with a Dice similarity coefficient ranging from 0.74 to 0.93 (average 0.85).

Cluster analysis based on UPGMA and Dice similarity coefficients categorized the isolates into seven distinct groups and 12 sub-groups (Figure 3). The first group consisted of eight isolates, all representing isolates from the sampling site North. Group 2 was made up of the largest number of isolates (45), but was divided into five sub-groups. Eleven isolates in group 2 originated from the North while the remaining 34 isolates were from the Southwest 1

and Southwest 2, confirming the close relationship among isolates of the two sampling sites in the Southwest region. Group 3 consisted of 17 isolates in two sub-clusters and all were from the East. Group 4 contained 26 isolates, all from South, and it was further divided into three sub-clusters. Groups 5, 6 and 7 consisted of one isolate from Southwest 2, three isolates from Southwest 2, and two isolates from East, respectively. Generally isolates from the same collection site clustered together with the following three exceptions: 1) The first sub-group of Group 2 consisted of only eight isolates from the North, and these clustered together with isolates from both Southwest regions indicating their common gene pool. 2) Nine isolates, from the North (13, 16, 17); Southwest 2 (68, 77, 78); and East (79, 93, 94), clustered with or closer to groups of isolates from other regions, indicating the presence of gene flow though to limited extent. 3) Isolates from Southwest 1 and 2 clustered together indicating a closer genetic relationship between them, which is in line with their geographic origin. Overall, none of the isolates appeared to be identical, two isolates (26 and 29) had, however, about 96% similarity in their peak profiles (Figure 3).

Principal coordinate analysis (PCO) also revealed the intra-species subdivision among the *C. sublineolum* isolates from the different parts of Ethiopia (Figure 4). The first three principal coordinates (PCO1-3) accounted for 34.5, 29.2, and 15.2 % of the total variation, respectively. Results of the PCO analysis were more or less in line with those of the UPGMA clustering, but the PCO analysis clearly separated the isolates from South and East from the others while reducing the overall number of groups. Isolates from the South spread over quadrants I and II of the PCO graph, while the isolates representing the Eastern region grouped together in quadrant II with one isolate a bit separated from the others. Isolates from the two Southwest sites and the North grouped together scattered over the third and fourth quadrants.

The analysis of molecular variance (AMOVA) indicated a high level of genetic variation both between isolates from different sampling sites (42%) as well as isolates within the sampling sites (58%) (Table 3). Results from this analysis revealed a significantly high ($P < 0.0001$) F_{ST} value (0.4216), indicating a high level of genetic differentiation and limited gene flow ($Nm = 0.343$).

Pair-wise genetic differences among the different groups were also high, further indicating sub-division of *C. sublineolum* according to region. Generally, the group of isolates from South Ethiopia, which had an average pair-wise difference of 0.48 to groups of isolates from other regions, was found to be the genetically most divergent group even though it is not geographically the most isolated region (Table 4). The highest pair-wise difference (0.51) was recorded between isolates from the South and the East indicating that they were the most separated in terms of genetic distance. In contrast, the two groups of isolates from Southwest Ethiopia (Southwest 1 and 2) had the lowest pair-wise difference (0.11), making them the genetically most related groups compared to the others.

Discussion

The findings reported here are from the first comprehensive study to characterize *C. sublineolum* isolates from different sorghum growing regions of Ethiopia. Isolates were collected from five different sorghum producing areas in four geographic locations and characterized using phenotypic characters i.e. growth rate, colony color and margin, as well as AFLP fingerprinting.

In the current study, phenotypic characterizations of *C. sublineolum* isolates representing different regions revealed considerable variations in growth rate, but very little difference in colony morphology (pigmentation and margin of the colonies). This is in contrast to previous studies from other parts of the world that have reported a significant

variation in colony morphology among *C. sublineolum* isolates (Vaillancourt & Hanau, 1992; Marley *et al*., 2001). Morphological characterizations may provide a basis for both inter- and intra-species diversity studies. However, such characters are often unstable and rather changeable with the age of the colonies (Browning *et al*., 1999; Crouch *et al*., 2006; Rivera-Vargas *et al*., 2006) making them less reliable unless supplemented with other features. We also witnessed a temporal change in the color of the tested isolates from white to grey and dark grey as the colony grew older on the PDA plates. We also observed the production of perithecia in culture by some isolates (data not shown) suggesting the possibility of sexual reproduction of *C. sublineolum*. However, the perithecia failed to develop to maturity within the experimental period (50 days). Perithecia have never been reported for this pathogen in nature.

AFLP analysis of 102 isolates revealed a large variation among the isolates, both between and within the different geographic groups. A relatively high F_{ST} value (0.42) and a low *Nm* value (0.34) were obtained from the AMOVA, indicating a high level of genetic differentiation and a low gene flow in *C. sublineolum* in Ethiopia. F_{ST} values above 0.25 indicate a large genetic differentiation in populations (Wright, 1978). Inter population divergence was also high with pair-wise differences among the geographic groups ranging from 0.11 to 0.52. The Southern and Eastern groups were found to be the most divergent ones as shown by both the AMOVA and PCO. These two groups of isolates also formed distinct groups on the UPGMA tree. Gene diversities were moderately high (0.14 to 0.23, mean 0.19) among the *C. sublineolum* isolates included in our study. The results were comparable to those reported previously for the sorghum anthracnose pathogen (0.18-0.23, average 0.22) (Rosewich *et al*., 1998). Notably the gene diversities were higher than what have been reported for populations some sexually reproducing fungi such as of *Mycosphaerella fijiensis* (Zandjanakou-Tachin *et al.*, 2009) and *Fusarium pseudograminearum* (Bentley *et al*., 2008 &

2009). This may indicate the lack of direct relationship between the reproduction strategy of a fungus and its genetic diversity as suggested by Rosewich *et al*. (1998). On the other hand, these results may also suggest the need to search for the sexual stages of *C. sublineolum* in nature, especially in areas of high diversity.

C. sublineolum is mainly spread through rain splash over very short distances (Thakur & Mathur, 2000) and this might have contributed to the inter population divergence and limited gene flow among the different sorghum growing regions of Ethiopia. Long distance dispersal of *C. sublineolum* depends on the movement of infected seeds (Thakur & Mathur, 2000; Somda *et al*., 2007). Institutes engaged in sorghum research are located in the North, East and Southwest of Ethiopia, and they might be involved in the movement and distribution of sorghum seeds including those infected with *C. sublineolum*. However, the high genetic differentiation and low gene flow detected in our studies make the possible contribution of seeds in the long distance dispersal of *C. sublineolum,* not very likely in Ethiopia. We rather suggest geographic separation, the production of sorghum under different environmental conditions in various parts of the country, and the presence of highly diverse sorghum genotypes as the possible driving forces behind the evolution and diversity of *C. sublineolum* in Ethiopia.

In this first attempt to characterize Ethiopian *C. sublineolum* isolates using molecular markers, we discovered a high genetic diversity using AFLP analysis, which should be given due consideration in future sorghum breeding programs to achieve effective and long lasting resistance to anthracnose. We suggest that the study of genetic diversity in *C. sublineolum* should continue and include those areas not covered in the current study. Such studies should also be conducted at different time intervals to get comprehensive information on the temporal and spatial dynamics of the pathogen.

Ethiopia lies within the area considered to be the centre of origin of sorghum and sorghum diversity is high within the country (Sleper & Poehlman, 2006). Moreover, Ethiopian farmers maintain mixtures of sorghum land races within each field and these might have contributed to the existence of high genetic variation within *C. sublineolum* populations. It is therefore also important to study the virulence spectrum of *C. sublineolum* isolates from different sorghum growing regions of the country to get a complete picture of the diversity of this important pathogen.

Acknowledgment

This project was financed by the Norwegian Agency for Development and Cooperation, and the Development Innovation Fund of Hawassa University, as well as Lånekassen, Norway. Thanks are due to members of the crop protection section of Hawassa University for their help during survey work and sample collection, and to Arne Roseth and Abdelhameed Elameen for practical help with the AFLP analysis. Biosciences for eastern and central Africa and the Gates foundation are acknowledged for allowing AC to take part in a research paper writing workshop.

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Table 1. Description of sample collection sites

Sampling site	Geographic location[1]	Altitude (m)	Rainfall (mm)[2]		Temperature (°C)[2]	
			Range	Mean	Maximum	Minimum
N	North	1476-1516	675-1063	813	30.3	14.6
E	East	2027-2077	607-1103	800	22.7	10.1
S	South	1947-1952	948-1526	1239	24.7	13.4
SW1	Southwest1	1797-1940	1142-2516	1737	NA[3]	NA
SW2	Southwest2	1728-1807	1145-2068	1590	26.8	12.2

[1]Refere to Figure 1 for latitude and longitude of the locations.

[2]The weather data are annual rainfall and temperature over a 10 year period (1997-2007). The data were obtained from meteorological stations located within 15 km radius of the sample/isolate collection sites.

[3]NA: Data not available. However, the Southwest region is generally a high rainfall area with high humidity and warm climate.

Table 2. Percent polymorphic peaks, Dice similarity coefficient and average gene diversity over all loci (Ĥ) of *C. sublineolum* isolates from different regions of Ethiopia

Sampling site	**No. of isolates**	**Polymorphic peaks**		**DSC[1]**		**Ĥ[2]**
		No.	**%**	**Range**	**Mean**	
North	19	178	61.38	0.55-0.91	0.77	**0.221**
South	26	165	56.51	0.60-0.96	0.78	**0.193**
Southwest1	22	131	44.41	0.74-0.93	0.85	**0.144**
Southwest2	16	197	67.70	0.32-0.91	0.70	**0.225**
East	19	160	58.18	0.47-0.95	0.81	**0.151**
Total	**102**	-	**-**	0.32-0.96	**-**	**-**
Mean	**-**	**166.2**	**57.64**	**-**	**0.78**	**0.187**

[1]DSC: Dice similarity coefficient

[2]Ĥ: Gene diversity measured according to Nei (1987)

Table 3. Analysis of molecular variance

Source of variation among isolates	df[1]	Sum of squares	Variance component	Percentage of variation
Between sampling sites	4	1731.075	20.00613	42.16
Within sampling sites	97	2662.503	27.44849	57.84
Total	101	4393.578	47.45462	

[1]df: degrees of freedom.

Table 4. Pair-wise genetic distance among *C. sublineolum* isolates from different sorghum producing regions of Ethiopia

Region/ Region	**North**	**South**	**Southwest1**	**Southwest2**
South	0.47	-	-	**-**
Southwest1	0.28	0.50	-	**-**
Southwest2	0.20	0.42	0.11	**-**
East	0.45	0.51	0.52	0.41
Mean	**0.35**	**0.48**	**0.32**	

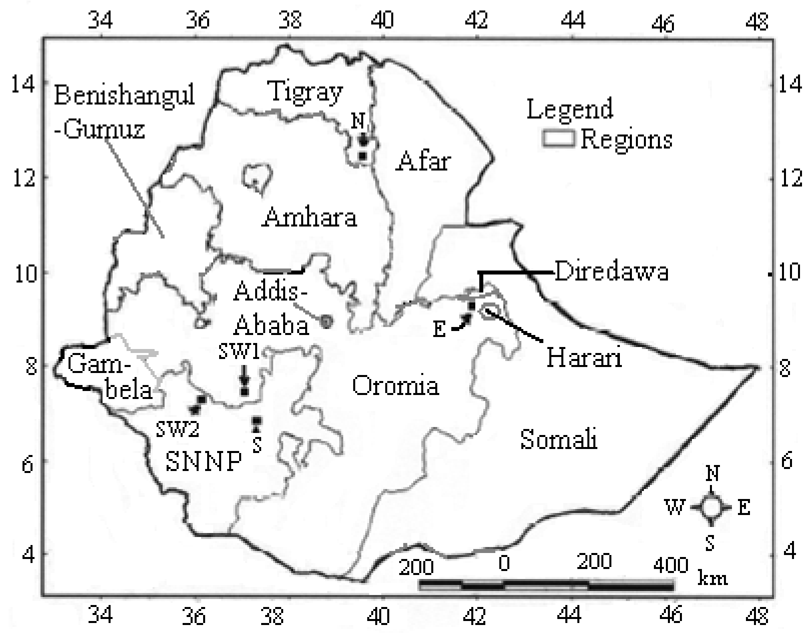

Figure 1. Map of Ethiopia showing sample collection sites. E- East; N- North; S-South; SW1- Sothwest 1; SW2 – Southwest 2. Latitudes and longitudes are indicated at the sides.

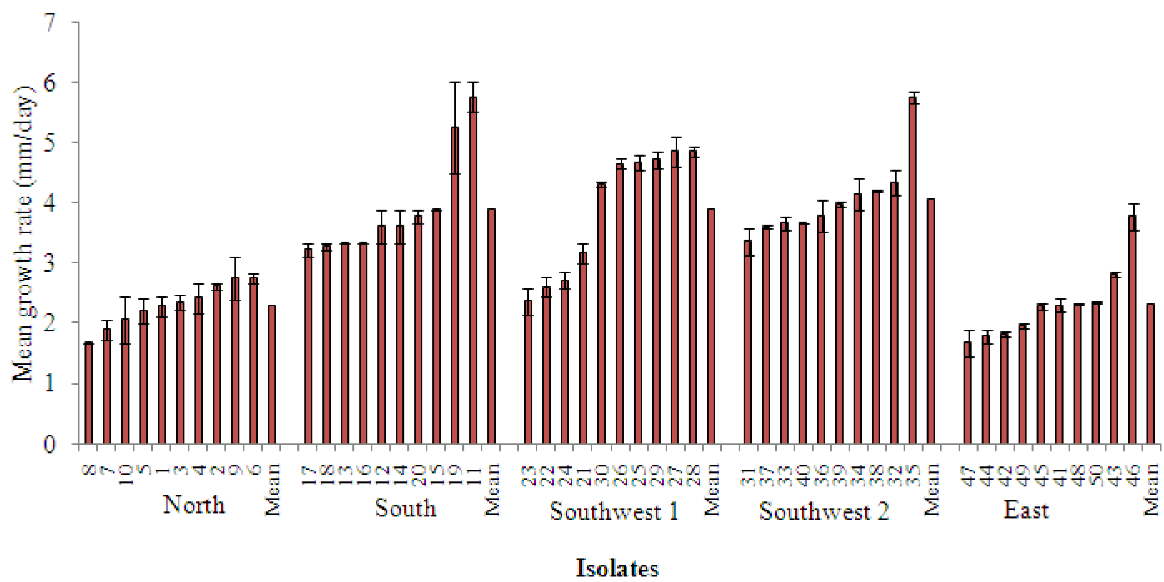

Figure 2. Average growth rates of *C. sublineolum* isolates from different regions of Ethiopia. Each value is the average of 28 readings made on four individual plates per isolate for seven consecutive days at 25ºC.

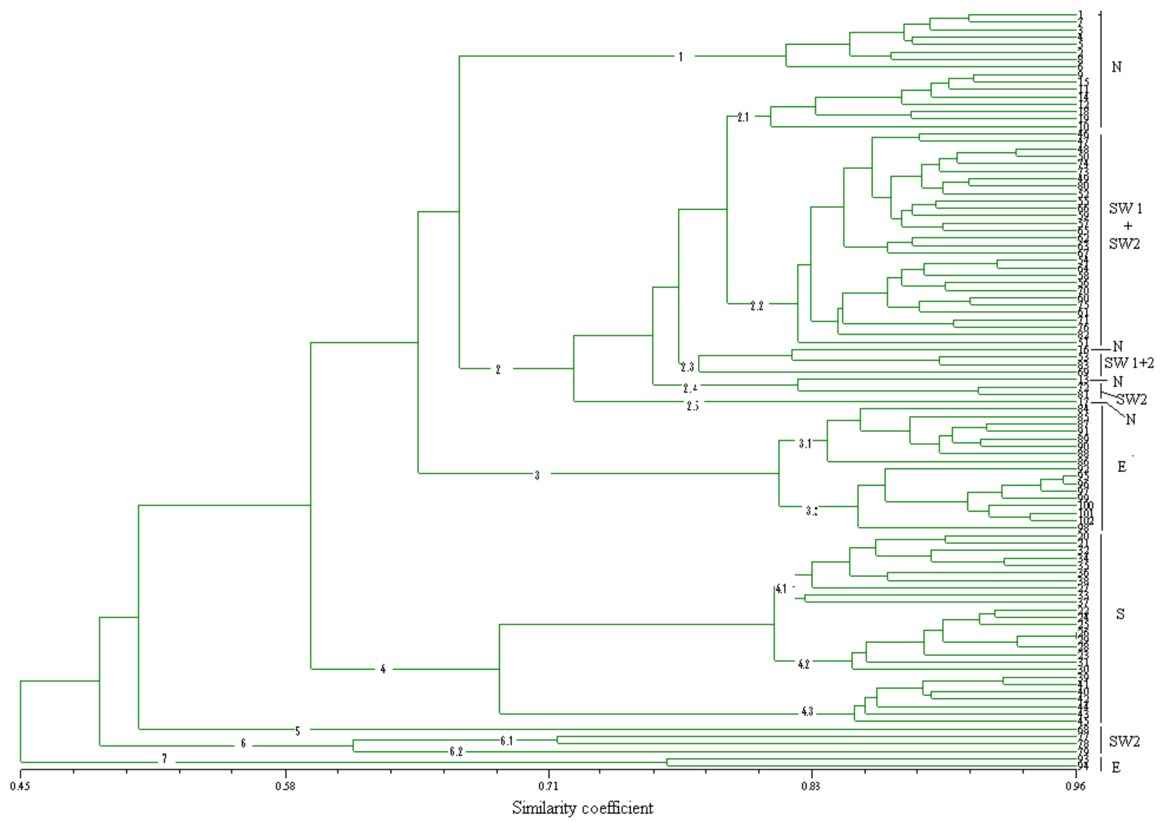

Figure 3. UPGMA tree of 102 *C. sublineolum* isolates from different parts of Ethiopia.

N – North; S – South; SW1 – Southwest 1; SW2 – Southwest 2; E – East.

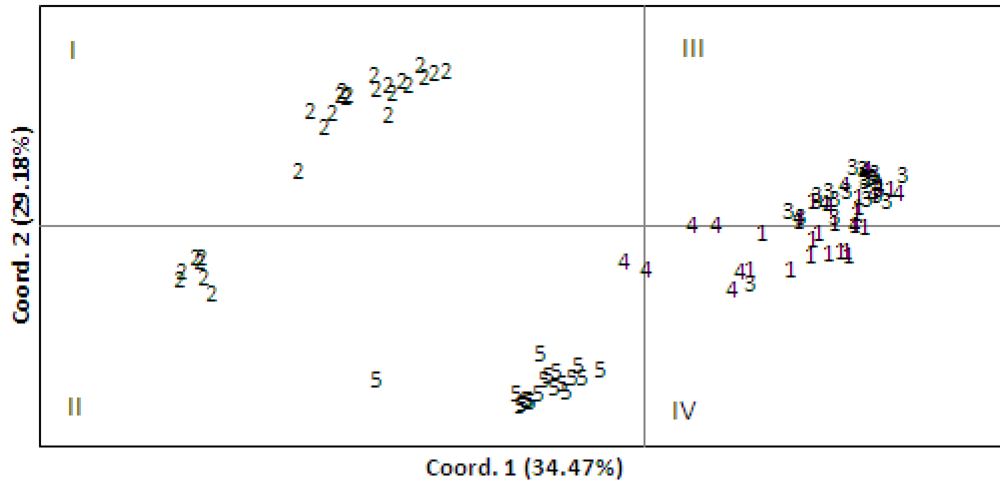

Figure 4. Principal coordinate analysis of 102 *C. sublineolum* isolates from different parts of Ethiopia.

Numbers indicate isolate groups: 1 – North; 2 – South; 3 – Southwest 1; 4 – Southwest 2; 5 – East

Paper III

Plant Pathology Journal 9 (1): 39-46, 2010
ISSN 1812-5387
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Effect of Host Genotypes and Weather Variables on the Severity and Temporal Dynamics of Sorghum Anthracnose in Ethiopia

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Abstract: The severity and temporal dynamics of anthracnose on susceptible (BTx623 and AL70) and resistant lines (2001 PWColl No. 022 and 2001 HararghieColl No. 12) were studied in field plots during the 2007 and 2008 growing seasons in southern Ethiopia. The initial, final and mean anthracnose severities and area under disease progress curves were used as criteria to evaluate the response of the genotypes. Over the two years, the initial, final and mean anthracnose severities ranged from 0.88 to 16.13%, 7.56 to 78.38% and 3.57 to 46%, respectively, while area under disease progress curve averaged for the two years ranged from 221.31 to 2951.88. All the evaluation criteria showed highly significant variations ($p<0.0001$) among the genotypes and the Ethiopian genotype 2001 PWColl No. 022 consistently had the lowest disease levels regardless of the evaluation criteria and growing season. The disease appeared rather late and progressed slowly on this genotype. On the other hand, the exotic cultivar, BTx623, showed the most sever anthracnose infection. Initial anthracnose severity was significantly higher and the disease developed rapidly on BTx623 than on the other three genotypes. The other two genotypes showed intermediate response and progression of the disease. Correlation and regression analyses revealed a significantly strong association between rainfall and anthracnose severity but temperature appeared to have little/no impact on the development of anthracnose in the field. The present study confirmed the effect of both host genotypes and weather, particularly rain on anthracnose development. The Ethiopian sorghum genotype 2001 PWColl No. 022 was recommended as stable source of resistance against this important disease.

Key words: Area under disease progress curve, *C. sublineolum*, environment, resistance, susceptible

INTRODUCTION

Sorghum is a major staple food crop for millions of people especially in the developing world (FAO, 2009). Ethiopia ranks eighth in total production of the crop, which amounts to 2.8 million tons per annum on about 1.6 million ha of cultivated land (FAO, 2009; CSA, 2009). This makes sorghum the fourth most important crop next to maize (*Zea mays*), teff (*Eragrotis teff*) and wheat (*Triticum* species) in terms of both area planted and total production (CSA, 2009). Sorghum in Ethiopia is mainly grown by subsistence farmers either as a sole crop or intercropped with other field crops including maize, teff and beans and to some extent with chat (*Chata edulis*). Due to the diverse nature of the farming systems and climatic conditions under which sorghum is grown, the production of sorghum in Ethiopia is adversely affected by several biotic and abiotic constraints among which the disease anthracnose is the major one (Hulluka and Esele, 1992; Chala *et al*., 2007). Sorghum anthracnose is caused by a fungal pathogen *Colletotrichum sublineolum* and since its report in 1960 (Sutton, 1980), the disease has been identified as one of the major factors constraining sorghum production worldwide (Pande *et al*., 1993; Thomas *et al*., 1996; Thakur and Mathur, 2000). On infected sorghum in the field, anthracnose can cause yield losses of 50% or more (Harris *et al*., 1964; Ferreira and Warren, 1982). The development of anthracnose in sorghum fields largely depends on host susceptibility and prevailing weather conditions (Pastor-Corrales and

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Frederiksen, 1980; Ali and Warren, 1987; Néya and Normand, 1998; Marley *et al.*, 2001; Hess *et al.*, 2002; Erpelding and Prom, 2004, 2006; Erpelding and Wang, 2007). Cultural practices including residue and weed management, planting disease free seeds, crop rotation are also known to impact anthracnose development in the field (Warren, 1986; Cardwell *et al.*, 1989; Casela and Frederiksen, 1993; Somda *et al.*, 2007).

Currently the management of sorghum anthracnose largely depends on the deployment of resistant varieties. In addition, the choice of planting dates is also found to significantly impact the development of the disease (Ngugi *et al.*, 2000; Marley, 2004) and hence could play a role in reducing anthracnose severity. Furthermore, understanding the temporal dynamics of the disease is an essential task to identify the proper planting time, which in turn contributes towards developing effective, affordable, safe and sustainable management strategies. Studies on the temporal progress of plant diseases have yielded substantial knowledge on their epidemiology, which is essential to design proper management practices (Olatinwo *et al.*, 1999; Benson *et al.*, 2006; Mouen-Badimo *et al.*, 2007). Thus, the current project was designed to study the severity and temporal dynamics of anthracnose on different sorghum genotypes and additionally, to determine the impact of weather variables on anthracnose development.

MATERIALS AND METHODS

Experimental site: The experiment was conducted in 2007 and 2008 sorghum growing seasons in Southern Ethiopia. The area is characterized by high rainfall (>1200 mm annum^{-1}) and moderate temperature (18.5°C) based on 11 year data. The research sited is located between 6°59.098′ N latitude and 37°52.645′ E longitude and has an altitude of 1947 m.a.s.l. The area is known to be suitable for anthracnose development based on existing weather conditions and previous study (Chala *et al.*, 2007).

Planting, experimental design and inoculation: Three sorghum genotypes of Ethiopian origin and a universally susceptible cultivar, BTx623, were evaluated for their reaction to anthracnose in 2007 and 2008 cropping seasons. Planting was conducted in April and May of 2007 and 2008, respectively, in plots of 2.5×3 m at spacing of 75×15 cm. The experiment was laid out in randomized complete block design with four replications. The 2008 plating time was delayed due to late onset of rainfall. All the management practices including hand weeding were carried out as needed and an insecticide, Endosulfan 35% EC, was applied at a rate of 21 t ha^{-1} at 4 and 6 weeks after germination to control of stalk borers. Disease assessments were conducted on the naturally-infected plants in both years.

Data collection and analysis: Monthly weather data of 11 consecutive years (1997-2008) of the area were obtained from the National Meteorological Agency.

Data were collected on anthracnose severity as percentage of leaf area covered by the symptom at 10 days interval for eight and seven consecutive times in 2007 and 2008, respectively, starting from the onset of clear symptoms on at least two of the sorghum genotypes. All the plants within the inner two rows of each plot were used for data collection. Area Under Disease Progress Curve (AUDPC) was calculated from the severity data following the method proposed by Madden *et al.* (2008) with some modifications as:

$$\text{AUPDC} = \sum_{i=1}^{n-1} [(X_i + X_{i+1}/2)]\,(t_{i+1} - t_i)$$

Where:
X = Disease severity
t_i = Time in days of the ith assessment from the first assessment date
n = Total number of assessments.

Initial Anthracnose Severity (IAS) (the first severity record at 60 days post planting), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and AUDPC were analyzed statistically using SAS computer package, version 9.11 (SAS Institute Inc, 2003). LSD test at the 0.05 probability level was used for mean comparisons. Correlation analysis was performed using PROC CORR procedure of the SAS computer package to determine the relationship among the different disease parameters and weather variables. A similar regression analysis procedure used by Tarekegn *et al.* (2006) was applied to further elucidate the effect of rainfall and temperature on the severity of anthracnose. The regression analysis was performed between rainfall and temperature conditions of each month and average anthracnose severity record of the same month.

RESULTS

Weather conditions: The research area generally received a high but variable rainfall during the 2007 and 2008 experimental seasons. Overall total rainfall was higher during the 2007 experimental season than in 2008 and the 11 year average rainfall of the area. The total monthly

Table 1: Weather parameters of the research site during the 2007 and 2008 growing seasons

	Rainfall (mm)			Temperature (°C) Maximum			Minimum			Average		
Months	2007	2008	11 year	2007	2008	11 year	2007	2008	11 year	2007	2008	11 year
April	158.10	74.10	174.1	25.2	26.7	25.7	14.4	14.4	14.3	19.8	20.6	20.0
May	213.70	108.8	169.4	25.1	24.2	24.6	14.5	13.9	14.2	19.8	19.0	19.4
June	198.80	100.1	117.1	22.1	22.4	22.6	13.7	17.7	13.5	17.9	20.1	18.1
July	207.50	146.1	171.8	21.1	20.7	21.2	12.9	13.0	13.2	17.0	16.8	17.2
August	229.00	183.9	152.3	21.1	21.4	21.7	13.6	13.3	13.2	17.4	17.3	17.5
September	269.30	129.7	116.8	22.6	23.3	23.4	13.7	14.0	13.4	18.1	18.6	18.4
October	21.100	159.9	112.9	24.7	24.4	24.4	12.8	13.1	13.2	18.8	18.8	18.8
Total	1297.5	902.6	1262	-[NA]	-	-	-	-	-	-	-	-
Mean	185.40	128.9	144.9	23.1	23.3	23.4	13.6	14.2	13.6	18.4	18.7	18.5

-[NA]: Not applicable

Table 2: Combined analysis of variance for Initial Anthracnose Severity (IAS), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and Area Under Disease Progress Curve (AUDPC) of four sorghum lines tested in 2007 and 2008 cropping seasons

Parameters	Source	df	Sum square	Mean Squar	F-value	Pr>F
IAS	Year	1	227.111328	227.111328	81.37	<0.0001
	Genotype	3	1162.474609	387.491536	138.84	<0.0001
	Year*Genotype	3	417.771484	139.257161	49.89	<0.0001
	Rep (Year)	6	11.183594	1.863932	0.67	0.6767
FAS	Year	1	123.95251	123.95251	14.52	0.0013
	Genotype	3	23587.70254	7862.56751	920.87	<0.0001
	Year*Genotype	3	50.88754	16.96251	1.99	0.1521
	Rep (Year)	6	39.13896	6.52316	0.76	0.6075
MAS	Year	1	185.710531	185.710531	51.45	<0.0001
	Genotype	3	8304.817761	2768.272587	766.89	<0.0001
	Year*Genotype	3	91.691616	30.563872	8.47	0.0010
	Rep (Year)	6	8.396505	1.399418	0.39	0.8773
AUDPC	Year	1	79036.96	79036.96	4.74	0.0430
	Genotype	3	34533033.74	11511011.25	690.56	<0.0001
	Year*Genotype	3	40250.51	13416.84	0.80	0.5074
	Rep (Year)	6	41013.73	6835.62	0.41	0.8627

Table 3: Initial Anthracnose Severity (IAS) at 60 days post planting, Final Anthracnose Severity (FAS) at 130 days after planting, Mean Anthracnose Severity (MAS) and Area Under Disease Progress Curve (AUDPC) of sorghum genotypes

	IAS (%)			FAS (%)			MAS (%)			AUDPC		
Genotypes	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
BTx623	7.25a[1]	25.00a	16.13a	78.00a	78.75a	78.38a	40.84a	51.16a	46.00a	2841.25a	3062.5a	2951.88a
AL70	3.00b	4.06b	3.53b	53.25b	60.75b	57.00b	22.88b	27.68b	25.28b	1548.75b	1613.6b	1581.19b
2001 HarargheColl No. 12	1.50c	4.00b	2.75b	24.88c	29.75c	27.32c	10.11c	12.71c	11.41c	676.85c	720.9c	698.89c
2001 PWColl No. 022	0.88c	0.88b	0.88c	6.25d	8.88d	7.56d	2.79d	4.34d	3.57d	187.63d	255.0d	221.31d
Average	3.15	8.48	5.82	40.6	44.53	42.56	19.16	23.97	21.56	1313.62	1413.02	1363.317
p-value	<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001	

[1]Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on LSD for multiple comparisons

rainfall of the research area varied from 21 to 269 mm in 2007 and from 74 to 184 mm in 2008 growing seasons, while the 11 year monthly rainfall ranged from 112 to 174 mm (Table 1). Total rainfall was lower in most months of 2008 as compared to that of 2007 and the 11 year average. However, minimum, maximum and average temperature of the area showed little or no variability during all the experimental months except in June 2008, when the maximum temperature was much higher than that of 2007 and the 11 year average. Overall, the area was found to have a favorable climate for the development of sorghum anthracnose.

Disease assessment: None of the tested sorghum genotypes was immune from anthracnose infection in any of the experimental seasons. However, IAS and MAS were significantly affected by genotype ($p<0.01$) and genotype by year interaction ($p<0.01$) (Table 2). The final anthracnose severity also was significantly affected by genotype ($p<0.01$). Significant genotype by year interaction may indicate that the genotypes responded differently during the 2007 and 2008 growing seasons.

Disease severity: The disease severity was based on a scale of 1-100%. IAS, FAS and MAS averaged 3, 41 and 19% in 2007 and 9, 45 and 24% in 2008, respectively (Table 3), suggesting the presence of enough disease pressure in the research area that enabled for successful evaluation in both years. In 2007 experimental season, IAS, FAS and MAS ranged from 0.9 to 7.3%, 6.3 to 78%

and 2.8 to 40.8%, respectively and showed highly significant (p<0.0001) variations among the tested genotypes. These three severity records were all lowest on 2001 PWColl No. 022 followed by 2001 HararghieColl No. 12 and AL70, while the exotic cultivar, BTx623, had the highest severity records at any time of assessment.

Overall disease pressure was higher in 2008 than in 2007 with IAS, FAS and MAS varying from 0.9 to 25%, 8.9 to 78.8% and 4.3 to 51.2%, respectively. This indicated a 35-245, 0.96-14.1 and 25.7-55.6% increase in IAS, FAS and MAS, respectively. The genotypes differed significantly (p<0.0001) in all the severity records and 2001 PWColl No. 022 had the lowest IAS, FAS and MAS in 2008 followed by 2001 HararghieColl No. 12 and AL70. Again, BTx623 exhibited the highest IAS, FAS and MAS. Thus, confirming its susceptibility to anthracnose.

AUDPC: AUDPC was calculated for each genotype and found to be highly significant. The AUDPC values varied between 188 and 2841 in 2007 and from 255 to 3063 in 2008 experiment. The lowest AUDPC values in both years were obtained from the genotype 2001 PWColl No. 022 followed by 2001 HararghieColl No. 12 and AL70. The highest and most significant AUDPC values in both 2007 and 2008 experimental seasons were calculated for BTx623 and results of this analysis were in line with those obtained from the various severity records.

Overall, results of the two years experiments suggest a differential but stable reaction by the genotypes to natural infection by anthracnose.

Temporal dynamics: Both the onset and rate of progress of anthracnose varied among the tested genotypes in both experimental years. The disease started earlier (data not shown) and progressed very rapidly on BTx623, while it showed a relatively late appearance and slow development on the genotypes 2001PWColl#022 and 2001 HararghieColl No. 12. The local susceptible check, AL70, had a somewhat intermediate anthracnose development both in 2007 and 2008.

During the first assessment, the severity of anthracnose on BTx623 was around 7 and 25% in 2007 and 2008, respectively. Data collected at 10 days interval showed remarkable increase in anthracnose severity (4.5-16% in 2007 and 0.3-13.5% in 2008) on BTx623, with the highest increase in severity occurring during late developmental stages of the plants. This period coincided with the end of August and beginning of October in 2007 and 2008, respectively (Fig. 1). On the other hand, the resistant genotype 2001 PWColl No. 022 exhibited anthracnose severity of 0.9% at the first assessment in both years. The increase in anthracnose severity for this resistant line at 10 days interval was -0.2-2.3 and 0-2.4% in 2007 and 2008, respectively. The highest increase in anthracnose severity on 2001 PWColl No. 022 was recorded in September of each year. The remaining two genotypes i.e., AL70 and 2001 HararghieColl No. 12 showed an intermediate increase in anthracnose severity with the former being more susceptible and showing a more rapid disease increase than the later.

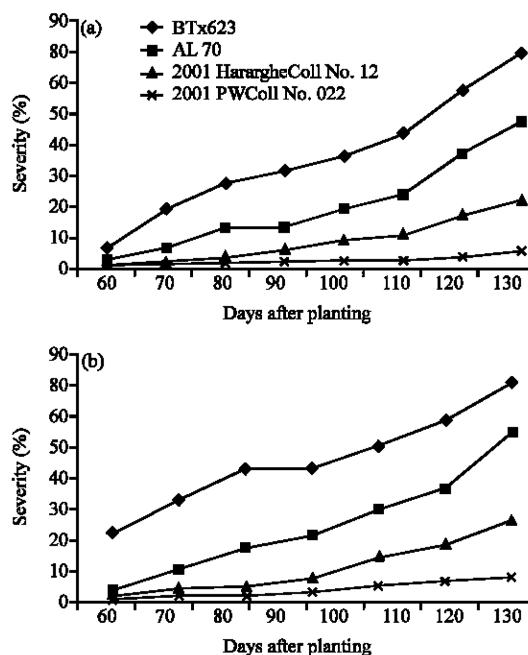

Fig. 1: Disease progress curves of anthracnose on four sorghum genotypes in (a) 2007 and (b) 2008 growing seasons

Results of this study generally suggest the time frame between August and end of September as the most important period for anthracnose development on sorghum.

Correlation and Regression analysis: Correlation coefficients between the various evaluation criteria based on the two years averaged data varied from 0.67 to 0.99, suggesting a highly significant (p<0.0001) association between the parameters (Table 4). This may indicate the possibility of using any of the parameters as evaluation criteria but care must be taken with the IAS as it might be linked with age related resistance.

Correlation and regression analyses between mean anthracnose severity and weather variables were conducted only on the 2007 data due to the very variable weather condition in 2008, which makes this year's analysis much more difficult. Results of both analyses revealed a positive and significant relationship between

Table 4: Pearson's correlation coefficients between Initial Anthracnose Severity (IAS), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and Area Under Disease Progress curve (AUDPC) over the two years

Severities	FAS	MAS	AUDPC
IAS	0.67***	0.83***	0.78***
FAS	-	0.96***	0.97***
MAS	0.96***	-	0.99***

***Significant at p<0.0001

Table 5: Pearson's correlation and regression analyses between mean anthracnose severity and weather parameters

	Genotype			
Statistical analysis	BTx623	AL70	2001 HararghieColl No. 12	2001 PWCol No. 022
r				
TRF	0.96911*	0.99299**	0.99321**	0.99740**
MinT	0.21480^NS	0.33289^NS	0.39538^NS	0.30860^NS
MaxT	0.32171^NS	0.44553^NS	0.47703^NS	0.51855^NS
AveT	0.30941^NS	0.44154^NS	0.48710^NS	0.48487^NS
R^2				
TRF	0.94	0.99	0.98	0.99
MinT	0.05	0.11	0.16	0.10
MaxT	0.10	0.20	0.23	0.27
AveT	0.10	0.20	0.24	0.24

r: Correlation coefficient; TRF: Total rainfall; MinT: Minimum temperature; MaxT: Maximum temperature; AveT: Average temperature; R^2: Coefficient of determination of the regression analysis. *Significant at p<0.05, **Significant at p<0.01. NS: Not significant

rainfall and anthracnose severity but a rather weak and none significant relationship between temperature and disease severity (Table 5). This indicates that rainfall could be a better predicator of anthracnose development than temperature.

DISCUSSION

The bulk of sorghum especially for food purpose is produced by low income subsistence farmers in the developing world, who can't afford high input agriculture like the use of fungicides. Thus, there is a constant demand for efficient and yet less costly control measures against sorghum diseases, including anthracnose which has long been known to pose a major threat to sorghum production in many parts of the world (Hulluka and Esele, 1992; Casela *et al*., 1993; Mukuru, 1993). Hence, research in identifying sources of anthracnose resistance has been and continues to be conducted in many countries. New sources of anthracnose resistant lines have been identified across the world (Casela *et al*., 2001; Hess *et al*., 2002; Erpelding and Prom, 2004, 2006; Mehta *et al*., 2005). Research results, which are yet to be published, have also confirmed the presence of wide sources of resistance to anthracnose among Ethiopian sorghum accessions. Results of the current research also gave a good indication for the existence of varying levels of resistance among the three sorghum genotypes used in this experiment as evidenced by the highly significant variations in terms of disease severity and AUDPC. The most resistant genotype (2001 PWColl No. 022) reduced anthracnose by several folds as compared to the susceptible check, BTx623, suggesting its potential as good source of resistance. This genotype was collected from Northwest Ethiopia, a region which has quite optimum environmental conditions for anthracnose and where sorghum has been under cultivation for several hundreds if not thousands of years. Sorghum genotypes in this region might have been co-evolved along with pathogens infecting sorghum and this could lead to the selection of resistant genotypes for current production. Host-pathogen co-evolution is one of the various mechanisms leading to development of resistance as suggested by previous findings including Dogget (1980). However, the use of resistant genotypes against anthracnose has its own limitations due to high variability and virulence patterns in the pathogen population (Cardwell *et al*., 1989; Browning *et al*., 1999; Latha *et al*., 2003), which may cause breakage of resistance. In this study, the most resistant genotype 2001 PWColl No. 022, retained its resistance to anthracnose despite the fact that it was collected from southern Ethiopia (more than 800 km), a hotbed for anthracnose. This suggests that there may be additional resistant sources from the region. Further research may be needed to affirm this in the future.

Other research findings have reported sowing dates as having significant impact on the severity of plant diseases, including anthracnose (Ngugi *et al*., 2000; Marley, 2004; Park *et al*., 2005). Thus, change in planting dates can serve as an alternative means of managing anthracnose in farmers' fields. However, for planting dates to be used as a viable option in disease management, one has to first determine the cycle of the disease in question and find out the optimum time when the disease reaches its peak levels. Our current findings showed the differential impact of genotypes with varying levels of resistance on the timely development/progress of anthracnose in a conducive environment. While variations in disease progress were remarkable among the genotypes with anthracnose showing a very slow progress on the genotype 2001 PWColl No. 022, the duration between August and end of September was found to be a peak time for anthracnose development in the field. This could be very much related to the environmental conditions that prevail during this time in addition to the life cycle of the pathogen and developmental stage of the crop, which both have to be studied in detail in the future. Based on our present results, it's likely that early planting may enable sorghum

to escape the high and most damaging levels of anthracnose. But further research is needed to precisely identify the best planting time to lessen the impact of the disease.

Suppressing the development of anthracnose has been considered as one useful strategy in the management of this important disease. Gwary and Asala (2006) achieved this through the application of fungicides, while Casela *et al.* (1993) and Marley *at al.* (2001) reported a slowed development of anthracnose as useful mechanism of resistance in some cultivars. Present results confirm a remarkable arrest in the temporal progress of anthracnose by the genotype 2001 PWColl No. 022, which was another evidence for this genotype to be considered as a useful source of resistance against anthracnose.

Weather conditions including rainfall and temperature are also known to influence the development of plant diseases in different pathosystems (Estrada *et al.*, 2000; Tarekegn *et al.*, 2006; Harikrishnan and del Río, 2008). The prevailing conditions during the course of the research were very favorable to the initiation and further progress of anthracnose as evidenced by the higher disease pressure of up to 79% severity and rapidly increasing disease severities especially on the susceptible genotypes. Although rainfall was generally more variable and less intense in 2008 than in 2007 and the 11 year average in present research area, it actually exceeded those recorded and reported as optimal amounts in other parts of the world (Néya and Normand, 1998; Hess *et al.*, 2002).

There exist some contradictions as to which weather variables are most suitable to anthracnose. While Ali and Warren (1987) reported warm and humid weather as most favorable to sorghum anthracnose, Erpelding and Wang (2007) found out low temperature as more conducive to the disease. Correlation and regression analyses made on our data clearly showed a strong and positive association between anthracnose severity and total rainfall. However, present results revealed no significant impact of temperature on anthracnose development. Hence, we conclude that rainfall could be the most decisive weather variable affecting anthracnose development in sorghum fields. But the temperature range on which our analysis was based was rather narrow and hence values well below and above those recorded for our research area should be considered to reach a more conclusive result in the future.

ACKNOWLEDGMENTS

Financial support to this research was obtained from the Research and Extension Office of Hawassa University and Norwegian Agency for Development and Cooperation. The first author was also financial supported by the Lånkassen, Norway. We thank Dr. Firdu Azerefegne for his valuable advice and for providing logistic support. We also acknowledge Simon Atsbha, Dawit Kassa and Paulos Dana for their help during the field work.

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Paper IV

Evaluation of Ethiopian sorghum accessions for resistance to anthracnose caused by *Colletotrichum sublineolum*

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Abstract

A field experiment was conducted in the 2007 and 2008 cropping seasons in southern Ethiopia to assess the reaction of 56 Ethiopian sorghum accessions and two susceptible checks (AL70 and BTx623) to anthracnose from naturally occurring inoculum. Final anthracnose severity (FAS), relative area under the disease progress curve (rAUDPC, where a commonly used susceptible cultivar was set to 1) and anthracnose progress rate were used as evaluation parameters. All the evaluation parameters revealed significant variation among the tested accessions. In 2007, anthracnose severity varied between 6.6 and 77.7%, and in 2008 it ranged from 9.7 to 76%. The Ethiopian sorghum accessions had rAUDPC ranging from 0.13 to 0.88 in 2007 and from 0.18 to 1.35 in 2008. Anthracnose progress rate varied between 0.02 and 0.06 and from 0.01 to 0.05 units per day in 2007 and 2008, respectively. Results from the three parameters were highly correlated (0.18-0.87), suggesting FAS would be a suitable selection parameter for germplasm screening. Fifteen accessions in 2007 and 18 in 2008 were rated as resistant, with six accessions rated as resistant in both years. All the Ethiopian accessions were more resistant to anthracnose than the susceptible controls suggesting germplasm from Ethiopia would be useful for breeding resistance to anthracnose.

Key words: *Colletotrichum sublineolum,* disease progress, *Sorghum bicolar*

Introduction

Anthracnose caused by *Colletotrichum sublineolum* is among the most destructive diseases of the sorghum plant (Thakur and Mathur, 2000; Casela et al., 2001). The disease is easily recognized by the characteristic symptoms of circular to elliptical red spots with few to numerous acervuli on leaf laminas (Tarr, 1962; Marley et al., 2004). Sorghum anthracnose occurs in all sorghum growing regions of the world (Huluka and Gebrekidan, 1980; Pande et al., 1993; Marley et al., 2001; 2002; Ngugi et al., 2002) causing yield losses as high as 50% (Harris et al., 1964; Powell et al., 1977; Thomas et al., 1996).

Anthracnose development in sorghum fields is influenced by weather factors, inoculum density, pathogenicity of the strains, host plant resistance and cultural practices (Warren, 1986; Pande et al, 1999; Chala et al., 2010a; 2010b). While infected plant debris, seeds and alternate hosts serve as sources of the primary inoculum (Warren, 1986; Cardwell, 1989; Casela and Frederiksen, 1993; Somda et al., 2007), shifts in planting dates and the choice of cultivars were found to influence the development of anthracnose in the field (Marley, 2004).

Thus, management practices like altering planting dates, removal of crop residues and alternate hosts (wild sorghum), planting disease free seeds, and crop rotation can serve as important options in controlling sorghum anthracnose. Such schemes are environmentally friendly and inexpensive, but may be ineffective, especially if they are not implemented across a large region. They could also prove ineffective at times of epidemics. It is also possible to use fungicides to control anthracnose. However, this option is usually too costly for subsistence farmers, who produce the bulk of sorghum worldwide.

The use of resistant cultivars is considered the most cost effective and efficient option in combating sorghum anthracnose. Hence, searching for possible sources of resistance and breeding for disease resistance are important tasks for researchers engaged in finding effective and sustainable means of controlling anthracnose. Sorghum is a diverse crop, and this has given

ample room to look for sources of resistance. To-date considerable variations in resistance to anthracnose are reported among sorghum genotypes in different parts of the world (Néya and Le Normand, 1998; Casela et al., 2001; Erpelding and Prom, 2004; 2006; Chala et al., 2010b). Nonetheless, the high variability of the pathogen (Rosewich et al., 1998; Kaboré et al., 2001; Souza-Paccola et al., 2003) and differential reaction of sorghum genotypes under different environmental conditions (Hess et al., 2002) call for a greater effort to continuously look for potential sources of resistance from diverse gene pools.

Sorghum has its origin in Africa and the greatest genetic diversity in native sorghum is found in Ethiopia (Sleper and Poehlman, 2006). Hence, this centre of origin could also serve as a centre of diversity for host plant resistance to anthracnose. The present study was therefore conducted to assess the level of resistance possessed by sorghum accessions collected from diverse regions of Ethiopia and to identify sorghum accessions with good level of resistance to anthracnose.

Materials and methods

Experimental site

Field evaluations of sorghum accessions for resistance to anthracnose were conducted in Wolayta, southern Ethiopia. The area is conducive to anthracnose development (Chala et al., 2007; 2010a; 2010b). The experimental field was situated at 6°59.098´ N latitude and 37°52.645´ E longitude, at an altitude of 1947 masl. It is a high rainfall area with annual rainfall of more than 1000 mm and a moderate mean annual temperature (around 20°C).

Planting, experimental design and field management

Fifty six sorghum accessions were obtained from the germplasm collections of three different research stations in Ethiopia (Table 1) and were evaluated for resistance to anthracnose in 2007 and 2008 cropping seasons. The accessions were originally collected from different parts of Ethiopia (Table 1). Sorghum cultivars AL70 from Ethiopia and BTx623 from Texas, USA, were also included as susceptible checks. The accessions were sown manually on April 20, 2007 and May 25, 2008. A delay in the onset of rainfall forced the 2008 planting time to be postponed until May. The accessions were planted in single row plots of 2.5 m length with a spacing of 75 cm between rows and 15 cm between hills within a row, in a randomized complete block design with three replications. Two seeds were planted per hill and plants were thinned to one plant per hill 18 days after planting. Thus, there were about 16 sorghum plants in each row. All management practices, including hand weeding, were carried out as needed. Stalk borers were controlled by applying an insecticide, Endosulfan 35% EC, at a rate of 2 liters ha^{-1} at four and six weeks after germination.

Source of inoculum

The experimental field was planted to maize and sorghum during and prior to 2006, and residues were left on the soil to serve as sources of inoculum for the actual experimental years. The 2008 planting was made in the same field with plant debris from the 2007 experiment as the source of primary inoculum. Availability of inoculum was further enhanced during both experimental years by planting the susceptible local cultivar (AL70) along the peripheries of the field.

Data collection and analysis

Disease severity (percentage of leaf area covered by anthracnose) was recorded for eight consecutive times at 10 day intervals starting from the onset of disease (clearly visible symptoms) on 50% of the accessions. All plants in each row except the two plants at the ends were used for data collection (i.e. assessments were made on 14 plants).

The recorded data (mean anthracnose severity per plant) were entered into Microsoft Excel table function (version, 2007) to calculate mean severity values for each plot. The area under the disease progress curve (AUDPC) was calculated for each sorghum accession based on Madden et al. (2008) as follows:

$$AUDPC = \sum_{i=1}^{n-1} [(X_i + X_{i+1})/2](t_{i+1} - t_i)$$

Where X = percent leaf area covered by anthracnose, t_i = time in days of the ith assessment from the first assessment date and n = total number of assessments.

AUDPC values were then converted into relative AUDPC (rAUDPC) as a ratio of the actual AUDPC of the sorghum accession to that of the local susceptible check (AL70). Disease progress rate (R) was also calculated for each sorghum accession as the slope of the regression line for disease severity against time after transformation of the severity records with the logistic model ln[y/(1-y)] (Campbell and Madden, 1990) as explained by Sahile et al. (2008). Final anthracnose severity (FAS) readings at 140 days post planting (dpp) were converted to a 1 to 5 scale by modifying earlier suggestions made by Harris and Sowell (1970), and Mehta et al. (2005): where 1 = anthracnose affecting 1 to 15% leaf area of sorghum plants, 2 = 16 to 30%, 3 = 31 to 45%, 4 = 46 to 60%, 5 = > 60% of leaf area affected. Severity scales were categorized into resistance groups as follows: severity scale of 1 was considered as resistant

while scales 2, 3, 4 and 5 were considered as moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively.

Correlation analysis was conducted to determine the relationship between the different anthracnose evaluation parameters i.e. FAS, rAUDPC and R. The statistical analyses were carried out using the General Linear Model (GLM) of SAS computer package, version 9.11 (SAS, 2003). LSD test at the 0.05 probability level was used for mean comparisons. FAS, rAUDPC and R data from each year were analysed separately due to the presence of genotype x year interaction.

Results

Weather conditions

The research area received a total rainfall of 1298 and 903 mm during the sorghum production seasons (April to October) of 2007 and 2008, respectively, while the temperature of the area averaged at 18°C during the same period of each experimental year (Fig. 1). Rainfall varied between years and even between months of the same year while the temperature of the area fluctuated very little especially between the different years. The lowest (21 mm) and the highest (269 mm) monthly rainfall were recorded in October and September of 2007, respectively.

Anthracnose severity

All the sorghum accessions showed disease symptoms. However, the accessions varied significantly in terms of FAS, rAUDPC and disease progress rate (Table 2). The 58 sorghum accessions included in the screening trial were classified into different severity classes based on the FAS records at 140 dpp. Figure 2 shows the frequency distribution of the sorghum

accessions with respect to FAS. In 2007 26, 60, 10, 1.7 and 1.7 % of the tested accessions had anthracnose severity of 1-15, 16-30, 31-45, 46-60 and >60 %, respectively, in 2007. In 2008 31, 38, 28, 1.7 and 1.7 % of the accessions had anthracnose severity of 1-15, 16-30, 31-45, 46-60 and >60 %, respectively.

All the local accessions had significantly lower anthracnose severity ($P < 0.0001$) compared to the susceptible checks (AL70 and BTx623) (Table 2). Nevertheless, both experimental year and accession by year (G x E) interaction also had a significant impact ($P < 0.0001$) on anthracnose development (data not shown). The overall average severity of anthracnose was slightly higher (26%) in 2008 than that in 2007 (24%).

rAUDPC

rAUDPC for the Ethiopian accessions varied between 0.13 and 0.88 in 2007, and the values ranged from 0.18 to 1.35 in 2008. The highest rAUDPC values (1.73 and 1.52 in 2007 and 2008, respectively) were recorded for the susceptible check from Texas, BTx623 (Table 2). In comparison to the local susceptible check (AL70), all the Ethiopian accessions had a significantly lower ($P<0.0001$) anthracnose level in both experimental years except four accessions that showed slightly higher disease level than AL70 in 2008.

Frequency distribution of the tested accessions into different rAUDPC classes is given in Fig. 3. In 2007 34.5, 50, 8.6, 3.5, 1.7 and 1.7 % of the tested accessions had rAUDPC of 0.10-0.29, 0.30-0.49, 0.50-0.69, 0.70-0.89, 0.90-1.00 and >1.00, respectively. In 2008 32.8, 34.5, 12.1, 10.3, 1.7 and 8.6 % of the accessions had rAUDPC of 0.10-0.29, 0.30-0.49, 0.50-0.69, 0.70-0.89, 0.90-1.00 and >1.00, respectively.

Disease progress rate

Almost all the tested sorghum accessions showed at least some level of anthracnose by the end of June to mid July (data not shown) and the disease progressed at an average rate of 0.04 units per day (Table 2). Sorghum anthracnose progressed at a rate of 0.02-0.06 units per day in 2007 and 0.01-0.05 units per day in 2008, and the rate of disease progress differed significantly ($P < 0.0014$) among the tested accessions. The highest anthracnose progress rates were recorded for BTx623.

The tested sorghum accessions were grouped into six classes of disease progress rate (Fig. 4). In 2007 0, 5.2, 22.4, 44.8, 24.1 and 3.5 % of the tested accessions had anthracnose progress rate of 0.010-0.019, 0.020-0.029, 0.030-0.039, 0.040-0.049, 0.050-0.059 and 0.060-0.069 units per day, respectively. In 2008 1.7, 1.7, 37.9, 46.6, 12.1 and 0 % of the accessions had anthracnose progress rate of 0.010-0.019, 0.020-0.029, 0.030-0.039, 0.040-0.049, 0.050-0.059 and 0.060-0.069 units per day, respectively.

Genotype evaluation

The sorghum accessions differed in their reaction to anthracnose (Table 2). In 2007, 15 (25.9%) sorghum accessions were rated as resistant while 18 accessions (31%) were identified as resistant in 2008. Six accessions: 2001PWColl#022 (53), 2001PWColl#012 (44), 2001PWColl#023 (45), ETS-2752 (39), 2001 HarargheColl#32 (2) and ACC#293 (42) were consistently rated as resistant in both experimental years. Additionally, 19 accessions were either resistant or moderately resistant over the years, while 12 accessions were rated as moderately resistant in both years.

Figure 5 shows the temporal progress of sorghum anthracnose for the different resistance groups between the initial assessment (70 dpp) and final disease assessment (140 dpp). Initial anthracnose severity (IAS) for the resistant group of accessions averaged on 1.5 and 1.2 % in

2007 and 2008, respectively. The disease progressed slowly on this group and reached only at 10.7 and 12.4 % severity at 140 dpp in 2007 and 2008, respectively. IAS of 11.9 and 12.8 % was recorded for the highly susceptible (HS) cultivar (BTx623) in 2007 and 2008, respectively. Anthracnose progressed rapidly on this cultivar and reached a severity of 77.7% in 2007 and 76% in 2008 during the final assessment at 140 dpp. Excluding the HS cultivar IAS, FAS and disease progress were highest on the susceptible cultivar (AL70) followed by moderately susceptible and moderately resistant groups in that order.

Correlation analysis

Three evaluation parameters were used to evaluate the response of sorghum accessions to infection by *C. sublineolum*. Correlation analysis revealed significant relations between the parameters evaluated with correlation coefficients varying from 0.18 to 0.87 (Table 3).

Discussion

The management of sorghum anthracnose largely depends on the deployment of resistant cultivars, and significant variations in the reaction of sorghum genotypes to anthracnose have been documented (Pande et al., 1994; Marley et al., 2004; Erpelding and Prom, 2004; 2006; Erpelding, 2007). The present work demonstrated considerable variation in response of 56 sorghum accessions collected from different regions of Ethiopia to natural infection by *C. sublineolum*. All the Ethiopian accessions had a significantly lower disease levels compared to the susceptible checks, indicating that germplasm from Ethiopia may be useful sources of anthracnose resistance.

Response of sorghum genotypes to anthracnose is highly influenced by environmental conditions (Hess et al., 2002) in addition to the pathogenicity and virulence of the pathogen

races present in the field (Mehta et al., 2005). There was variation between the two growing seasons for some of the sorghum accessions evaluated in the current work. Both total rainfall and mean monthly rainfall varied between the two cropping seasons (Fig. 1) and this might have been one possible cause for the variation in the response of the accessions. *C. sublineolum* is a highly variable pathogen (Rosewich et al., 1998; Browning et al., 1999; Zanette et al., 2009). This might have been also another possible reason for the differential reaction of the sorghum accessions to anthracnose. The variation in accessions' response to anthracnose was much more pronounced in some sorghum accessions that showed low disease severity, thus asserting the need to carry out several screening tests under different environmental conditions. Single plant selection may also improve future screening trials in case such variations are substantiated to be caused by lack of genetic uniformity among the tested accessions. Pyramiding of resistance genes should also be considered in future breeding activities to come up with genotypes that possess a more stable resistance to anthracnose.

High rainfall and relative humidity, and warm temperature are reported to enhance anthracnose development in the field (Ali and Warren, 1987; Hess et al., 2002). On the other hand, Erpelding and Wang (2007) attributed high disease pressure to lower temperature (15-28ºC, mean 23ºC). The evaluation parameters used in our study were relatively higher in 2008 than in 2007. These results were obtained despite a lower mean rainfall in 2008. Therefore, we suggest a higher inoculum load from the previous growing season as a possible reason for higher disease pressure in 2008 than in 2007. The variation in planting dates between the two experimental years may also have an impact on disease development as suggested by Marley (2004).

In this screening trial we employed three different parameters: FAS, rAUDPC and disease progress rate to evaluate sorghum accessions for resistance to anthracnose. All parameters

successfully revealed significant variation in the reactions of the sorghum accessions to anthracnose and were positively correlated. rAUDPC and disease progress rate take into account different time spans, while FAS is based on a single anthracnose evaluation, hence the previous two parameters are assumed to be more reliable indicators of disease development in the field. However, all parameters correlated significantly (Table 3), and therefore FAS may be recommended as a time efficient method to evaluate sorghum genotypes for resistance to anthracnose. This is in agreement with previous studies that recommend single disease scoring as an efficient evaluation criterion in other pathosystems (Wang et al., 2005; Li et al., 2006).

The current work has resulted in the identification of accessions that possess a good level of resistance to anthracnose in an area known to be a hot bed for sorghum anthracnose (Chala et al., 2010a; 2010b). This suggests the potential Ethiopian sorghum germplasm may have in serving as sources of resistance in future breeding programs. Most of the accessions from Northwest Ethiopia appeared to be either resistant or moderately resistant to anthracnose. Lower anthracnose severity was also recorded in this area despite moderately high anthracnose incidence and favourable climatic conditions for disease development (Chala et al., 2010a). These two results along with the fact that sorghum production is an ancient and dominant practice in the area suggest a long history of host-pathogen co-evolution towards the development of resistance by the host. Hence, future works should focus on this remote part of the country for germplasm collection and breeding activities. Anthracnose is a major disease affecting sorghum production worldwide (Ali and Warren, 1992; Ngugi et al., 2002; Chala et al., 2010a). Thus, farmers would benefit a lot by planting genotypes that resist this important disease as shown in the current work. In many genotypes, resistance to anthracnose is found to be controlled by one or few dominant genes (Coleman and Stokes, 1954; Mehta et al., 2005; Erpelding, 2007) making the introgression of resistance to elite breeding materials relatively easier. As a center of origin and diversity of sorghum, Ethiopia possesses a very diverse

sorghum germplam (Sleper and Poehlman, 2006) but most of the accessions remain to be tested for resistance to anthracnose. Hence, future breeding activities should give due considerations to this relatively untapped resource and marker assisted selection will undoubtedly facilitate the screening of this huge germplasm.

Acknowledgements

This research was financed by the Research and Extension Office of Hawassa University, the Norwegian Agency for Development and Cooperation, and the Development Innovation Fund at Hawassa University. The first author was also financially supported by the Lånekassen, Norway. We thank Dr. Firdu Azerefegne for kind provision of logistic support during the field work. We are grateful to Simon Atsbha, Dawit Kassa and Paulos Dana for their help during data collection. We are grateful to Dr. Arne Stensvand for his critical review of the manuscript. Thanks are also to Asfaw Adugna, Dr. Louis K. Prom, BARC, MARC and PARC for the kind provision of sorghum seeds. Weather data was kindly provided by the National Meteorological Agency, southern Branch.

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Table 1. Entry (Identification), Origin and Seed source of sorghum accessions

Entry (Identification)	**Seed source**[1]	**Origin**
1 (2001 HarargheColl#12), 2 (2001 HarargheColl#32), 3 (2001 MiesoColl#29), 4 (2001 MiesoColl#58), 5 (1999 HarargheColl#4), 6 (1999 HarargheColl#10)	MARC	East Ethiopia
7 (ETS-2416), 8 (ETS-0001), 9 (ETS-00601), 10 (ETS-3931), 11 (ETS-4335), 12 (ETS-2952), 13 (ETS-3166), 14 (ETS-3286), 15 (ETS-3469), 16 (ETS-3235), 17 (ETS-3378), 18 (ETS-4313)	MARC	North Ethiopia except 8 and 9 for which origin is not known
19 (ETS-2779)	MARC	East Ethiopia
20 (ETS-2488), 21 (ETS-3217), 22 (ETS-2441), 23 (90 AN 5049), 24 (90 AN 5052), 25 (ETS-3089), 26 (ETS-3122), 27 (ETS-3131), 28 (ETS-3476), 29 (ETS-00717), 30 (ETS-00916), 31 (ETS-4381), 32 (ETS-4370), 33 (ETS-4098), 34 (ETS-2969), 35 (ETS-4754), 36 (ETS-2982), 37 (ETS-3135), 38 (ETS-3196)	MARC	North Ethiopia except 23 and 24, which are local crosses, and 29 and 30, which are of unknown origin
39 (ETS-2752), 40 (Chiro)	MARC	East Ethiopia
41 (ACC#245), 42 (ACC#293)	BARC	West Ethiopia
43 (2001PWColl#008), 44 (2001PWColl#012), 45 (2001PWColl#023), 46 (2001PWColl#068), 47 (2001PWColl#077), 48 (2001PWColl#083), 49 (2001PWColl#039), 50 (2001PWColl#006), 51 (2001PWColl#063), 52 (2001PWColl#054), 53 (2001PWColl#022), 54 (2001PWColl#029), 55 (2001PWColl#043), 56 (2001PWColl#018)	PARC	Northwest Ethiopia
57 (AL70)	MARC	East Ethiopia
58 (BTx623)	TAM	Texas, USA

[1]Seed source: MARC = Melkassa Agricultural Research Center; BARC = Baco Agricultural Research Center; PARC = Pawe Agricultural Research Center; TAM = Texas A&M University, Texas, USA.

Table 2. Final anthracnose severity (FAS) at 140 days post planting, relative area under disease progress curve (rAUDPC), anthracnose progress rate (Rate) and resistance classes of Ethiopian sorghum germplasm after natural infection by *Colletotrichum sublineolum*. Accessions are listed according to FAS in 2007.

	FAS			rAUDPC			Rate			Resistance class[1]	
Entry	**2007**	**2008**	**07-08[2]**	**2007**	**2008**	**07-08[2]**	**2007**	**2008**	**07-08[2]**	**2007**	**2008**
53	6.64	11.11	8.87	0.14	0.23	0.18	0.02	0.04	0.03	R	R
44	7.71	13.33	10.52	0.17	0.19	0.18	0.03	0.05	0.04	R	R
45	9.02	12.22	10.62	0.14	0.20	0.17	0.03	0.05	0.04	R	R
39	11.17	10.67	10.92	0.25	0.17	0.21	0.03	0.04	0.04	R	R
2	11.39	9.70	10.55	0.23	0.19	0.21	0.03	0.01	0.02	R	R
42	14.57	12.89	13.73	0.30	0.21	0.25	0.03	0.05	0.04	R	R
54	5.67	17.78	11.72	0.15	0.33	0.24	0.02	0.04	0.03	R	MR
46	6.39	16.00	11.20	0.13	0.31	0.22	0.02	0.04	0.03	R	MR
52	8.06	18.22	13.14	0.15	0.27	0.21	0.03	0.04	0.04	R	MR
56	10.60	17.33	13.97	0.31	0.26	0.29	0.04	0.04	0.04	R	MR
32	11.33	18.89	15.11	0.21	0.50	0.35	0.03	0.03	0.03	R	MR
23	14.58	28.89	21.74	0.37	0.41	0.39	0.04	0.04	0.04	R	MR
7	15.04	26.04	20.54	0.21	0.33	0.27	0.03	0.03	0.03	R	MR
40	12.29	35.56	23.92	0.24	0.67	0.46	0.04	0.04	0.04	R	MS
36	15.33	34.44	24.89	0.27	0.83	0.55	0.04	0.03	0.03	R	MS
48	15.94	13.33	14.64	0.36	0.29	0.33	0.03	0.05	0.04	MR	R
30	16.00	13.78	14.89	0.27	0.23	0.25	0.03	0.04	0.04	MR	R
34	16.00	13.11	14.56	0.29	0.20	0.24	0.03	0.04	0.04	MR	R

Table 2. Continued from the previous page

	FAS			rAUDPC			Rate			Resistance class	
Entry	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**
51	16.44	14.89	15.67	0.30	0.21	0.25	0.04	0.04	0.04	MR	R
55	16.97	11.11	14.04	0.33	0.24	0.28	0.04	0.03	0.03	MR	R
43	18.18	10.89	14.53	0.30	0.20	0.25	0.04	0.04	0.04	MR	R
47	18.42	13.78	16.10	0.37	0.29	0.33	0.03	0.03	0.03	MR	R
31	18.50	15.56	17.03	0.31	0.33	0.32	0.04	0.03	0.03	MR	R
10	20.83	10.00	15.42	0.33	0.20	0.27	0.04	0.04	0.04	MR	R
50	21.57	14.44	18.01	0.40	0.30	0.35	0.04	0.05	0.04	MR	R
11	27.32	12.44	19.88	0.49	0.23	0.36	0.04	0.04	0.04	MR	R
24	28.89	9.33	19.11	0.43	0.18	0.31	0.05	0.03	0.04	MR	R
14	16.11	25.11	20.61	0.24	0.54	0.39	0.04	0.04	0.04	MR	MR
41	16.52	18.89	17.71	0.31	0.43	0.37	0.03	0.03	0.03	MR	MR
49	17.33	20.00	18.67	0.36	0.30	0.33	0.04	0.05	0.04	MR	MR
15	17.83	20.67	19.25	0.23	0.46	0.35	0.04	0.03	0.03	MR	MR
5	18.52	25.27	21.89	0.34	0.40	0.37	0.04	0.03	0.04	MR	MR
13	20.95	16.44	18.70	0.30	0.32	0.31	0.04	0.03	0.03	MR	MR
37	22.75	20.67	21.71	0.37	0.33	0.35	0.04	0.04	0.04	MR	MR
8	25.56	25.67	25.61	0.42	0.41	0.42	0.04	0.03	0.04	MR	MR
1	26.17	29.72	27.95	0.40	0.37	0.39	0.05	0.03	0.04	MR	MR
18	26.22	25.56	25.89	0.29	0.51	0.40	0.05	0.03	0.04	MR	MR
29	26.90	25.11	26.01	0.50	0.32	0.41	0.05	0.04	0.05	MR	MR

Table 2. Continued from the previous page

	FAS			rAUDPC			Rate			Resistance class	
Entry	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**
6	29.44	23.12	26.28	0.36	0.27	0.32	0.05	0.02	0.03	MR	MR
25	21.21	43.33	32.27	0.39	0.73	0.56	0.04	0.04	0.04	MR	MS
16	21.53	32.67	27.10	0.27	0.52	0.39	0.04	0.04	0.04	MR	MS
12	22.00	42.89	32.44	0.28	1.35	0.81	0.04	0.03	0.03	MR	MS
33	23.40	32.22	27.81	0.39	0.41	0.40	0.04	0.04	0.04	MR	MS
9	23.58	40.00	31.79	0.28	1.01	0.64	0.05	0.03	0.04	MR	MS
22	24.29	40.00	32.14	0.34	0.79	0.57	0.04	0.03	0.04	MR	MS
19	26.03	43.33	34.68	0.47	1.17	0.82	0.04	0.03	0.04	MR	MS
17	26.24	31.11	28.68	0.49	0.78	0.63	0.05	0.04	0.04	MR	MS
21	27.13	40.00	33.56	0.52	1.05	0.78	0.04	0.03	0.04	MR	MS
27	29.11	32.22	30.67	0.40	0.54	0.47	0.04	0.04	0.04	MR	MS
26	29.65	38.89	34.27	0.48	0.83	0.65	0.05	0.04	0.05	MR	MS
28	32.56	21.11	26.83	0.53	0.37	0.45	0.05	0.03	0.04	MS	MR
38	34.72	20.00	27.36	0.45	0.41	0.43	0.06	0.03	0.04	MS	MR
35	44.67	30.00	37.33	0.88	0.56	0.72	0.05	0.04	0.04	MS	MR
3	33.33	38.00	35.67	0.82	0.48	0.65	0.05	0.04	0.04	MS	MS
20	38.89	35.56	37.22	0.61	0.81	0.71	0.05	0.03	0.04	MS	MS
4	42.38	33.00	37.69	0.64	0.48	0.56	0.05	0.04	0.04	MS	MS
57	53.67	50.67	52.17	1.00	1.00	1.00	0.05	0.04	0.05	S	S
58	77.67	76.00	76.83	1.73	1.52	1.62	0.06	0.05	0.05	HS	HS

Table 2. Continued from the previous page

	FAS			rAUDPC			Rate			Resistance class	
Entry	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**	**07-08**	**2007**	**2008**
Mean	23.67	26.21	24.94	0.41	0.50	0.46	0.04	0.04	0.04		
LSD	9.52	11.20	7.31	0.16	0.22	0.13	0.01	0.01	0.01		
CV(%)	26.44	28.03	27.36	24.83	28.31	27.09	21.08	24.75	22.88		

[1]Resistance class: R = Resistant (1-15 % severity); MR = Moderately resistant (16-30 % severity); MS = Moderately susceptible (31-45 % severity); S = Susceptible (46-60 % severity); HS = Highly susceptible (> 60% severity).

[2]07-08 = Combined analysis of results from the two experimental years i.e. 2007 and 2008. $P < 0.0001$ for all variables except anthracnose progress rate, for which $P = 0.0014$. Refer to Table 1 for identification and description of entries.

Table 3. Pearson's correlation coefficients between the different parameters used to evaluate the resistance of sorghum accessions to anthracnose

	FAS[1]	rAUDPC[2]
FAS	-	0.87**
Rate[3]	0.41**	0.18*

[1]FAS= Final anthracnose severity at 140 dpp; [2]rAUDPC= relative area under the disease progress curve (with respect to the local check AL70); [3]Rate = anthracnose progress rate after logistic transformation; *Significant at $P = 0.0009$, **Significant at $P < 0.0001$.

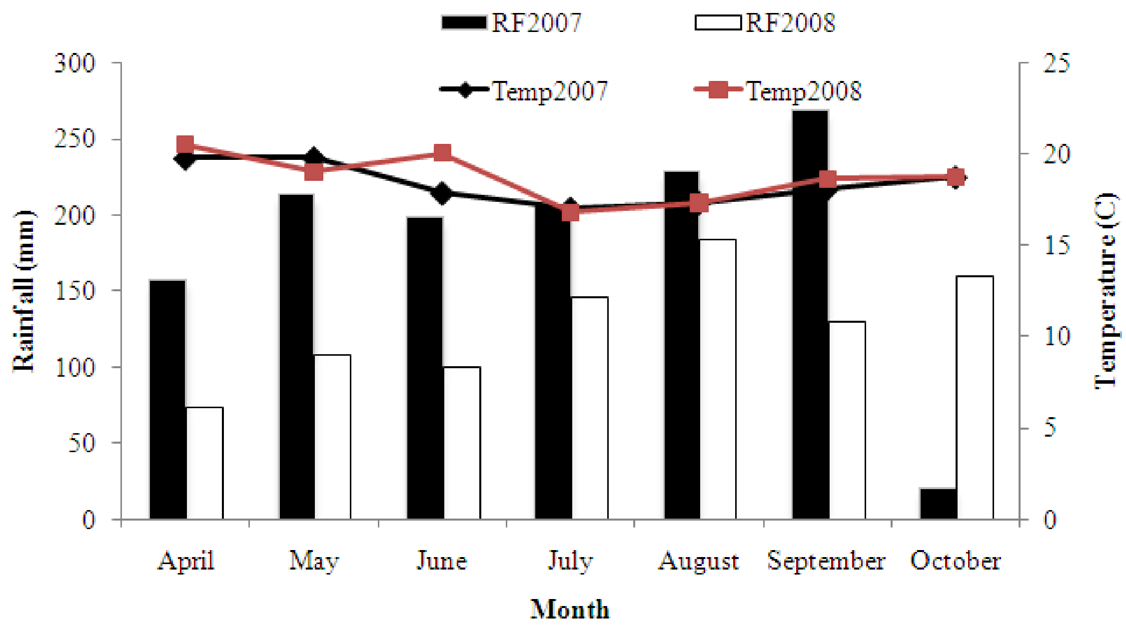

Fig. 1. Mean monthly rainfall and temperature during the 2007 and 2008 sorghum growing seasons at the research area.

RF: Rainfall; Temp: Temperature

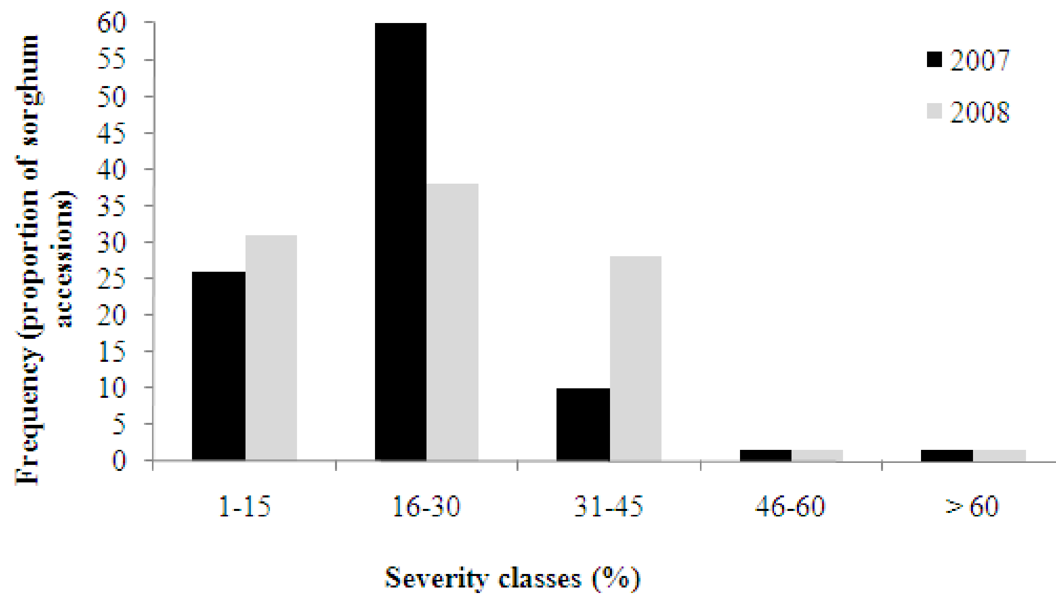

Fig. 2. Frequency distribution of sorghum accessions in five severity classes.

Severity classes: 1-15%-resistant; 16-30%-moderately resistant; 31-45%-moderately susceptible; 46-60%-susceptible; > 60%-highly susceptible.

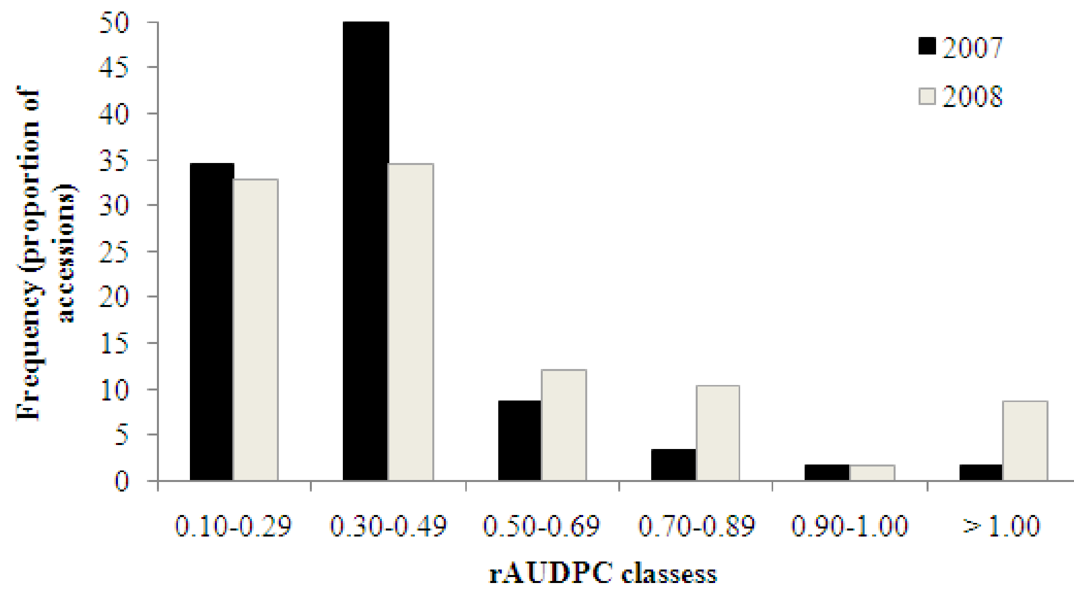

Fig. 3. Frequency distribution of sorghum accessions in different rAUDPC classes.

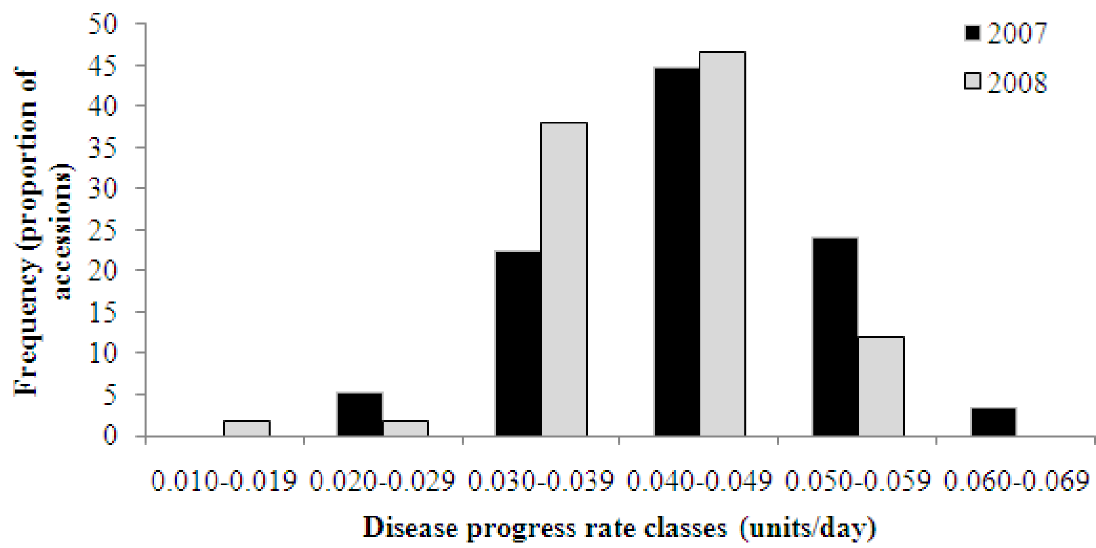

Fig. 4. Frequency distribution of sorghum accessions in different classes of anthracnose progress rate.

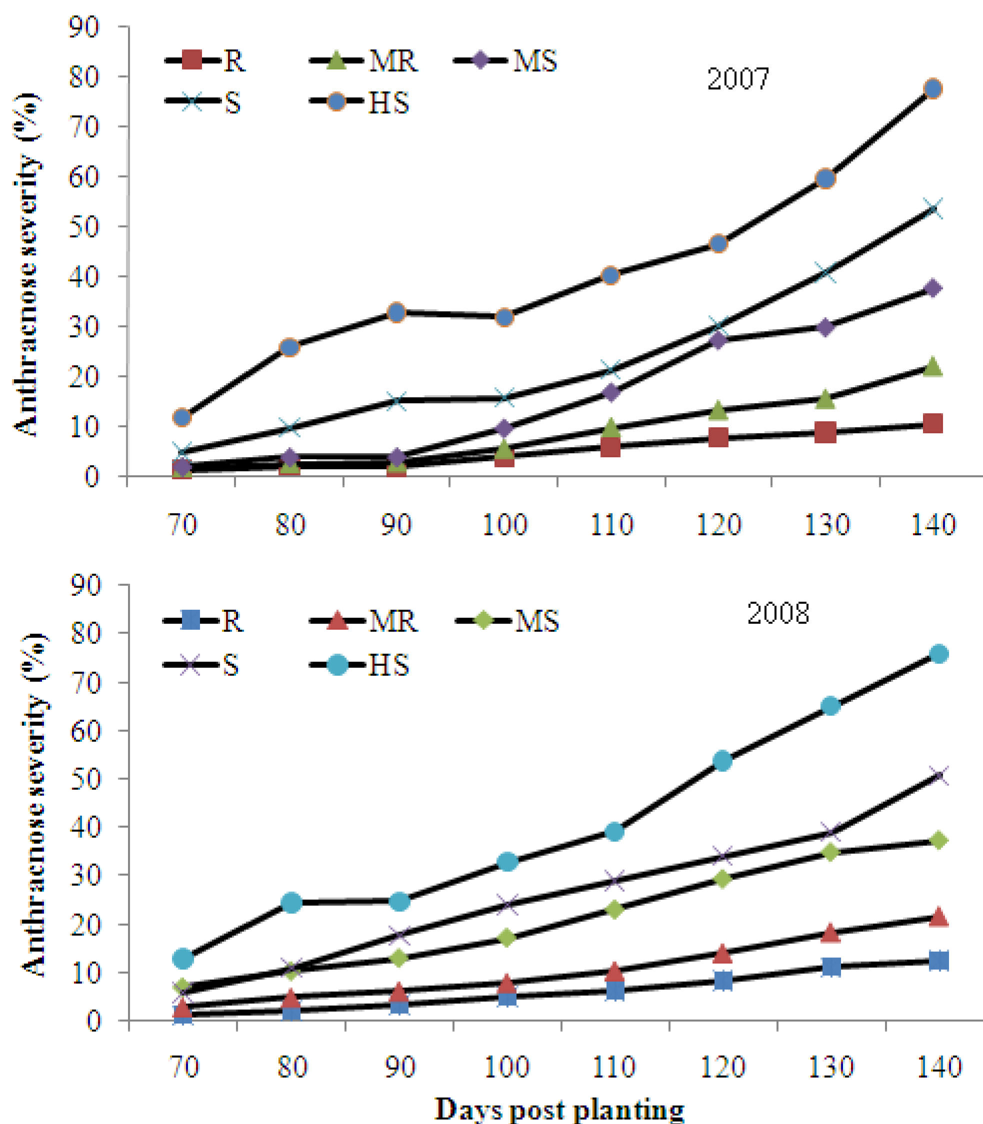

Fig. 5. Area under the disease progress curves for different resistance groups of sorghum accessions in the 2007 (above) and 2008 (below) growing seasons.

R: Resistant group (1-15% anthracnose severity); MR: Moderately resistant (16-30% severity); MS: Moderately susceptible (31-45% severity); S: Susceptible (46-60% severity); HS: Highly susceptible (>60% severity).