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Full Length Research Paper

Virulence and pathogenicity of *Colletotrichum sublineolum* and *Colletotrichum gloeosporioides* from leaf, stem panicle tissues on advanced sorghum genotypes and genetic basis of observed responses

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***Colletotrichum* species are among the destructive fungal pathogens of sorghum in Lake in Kenya. It is not clear whether all pathogenic species and sub-species infecting the three plant parts differ in their virulence and pathogenicity. Previous studies indicated that phosphorous efficient and *Striga* resistant genotypes were resistant to anthracnose while those resistant and/or susceptible to drought, aluminium toxicity and midge insect pest had mixed reactions. Sixteen genetically stable genotypes and four checks were randomized as sub-plots in plastic tubes in a split-plot arrangement under completely randomized design replicated twice in screenhouse. Morphologically identified leaf, stem and panicle isolates were randomized as whole plots. Inter-simple sequence repeat (ISSR) primer was used in genetic diversity assessment between *Striga* and phosphorous groups. Virulence and pathogenicity data were analyzed on Genstat version 12.2 while genetic diversity was assessed on NTSYS-pc version 2.1. *Striga* resistant and P-use-efficient genotypes were resistant to all isolates while the aluminium, drought and midge groups had mixed responses. *Striga* and P-use-efficient genotypes were genetically similar but differed from *Striga* susceptible and P-use-inefficient genotypes. Leaf isolates were less virulent than stem and panicle. Therefore, the level of virulence and pathogenicity of *Colletotrichum* species is influenced by hosts and pathogens genetic make-up.**

Key words: *Colletotrichum* isolates, sorghum, virulence, pathogenicity, ISSR, genetic diversity.

INTRODUCTION

Sorghum bicolor is ranked fifth as most important world cereal and also a native cereal in Africa and serves as a dietary staple of more than 500 million people in more than 30 countries in Africa (Murty and Renard, 2001; Obilana, 1995). In the recent past, the production of this crop has been decreasing thus exposes most developing countries including Kenya to a food insecurity state because over 80% of the total world area devoted to sorghum is found in developing countries (FAO, 1999). Despite being originated from Africa, only Nigeria, Sudan and Ethiopia can compete in the world market in terms of sorghum production but with very insignificant role in the market (FAO, 2005). In Kenya, sorghum production has been constrained by combined effects of drought, pests, diseases and soil fertility aspects including mineral toxicities and this was confirmed in the year 2005 ranking of the top world sorghum producers where Kenya did not appear in the top ten (Bio Earn, 2009; FAO, 2005.

Anthracnose is one of the most destructive diseases for sorghum due to rapid spread of the disease on susceptible cultivars (Erpelding, 2010a; Erpelding, 2010b). *Colletotrichum sublineolum* is the confirmed fungal pathogen causing anthracnose disease in sorghum and yield losses of up to 70% have been reported and under severe epidemics (Mathur et al., 2003). The fungus infects all the above ground tissues of sorghum plant with highly variable symptoms depending

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on the interaction between host plant, pathogen and environment, with symptoms appearing in about 40 days after seedling emergence (Marley et al., 2001) but infection occurs at any stage of plant development (Thakur and Mathur, 2000).

Most studies have indicated the presence of many physiological races of *Colletotrichum* strains infecting sorghum. These species and sub-species are both morphologically and genetically diverse (Marley et al., 2001; Meirelles et al., 2009; Moore et al., 2008; Pande et al., 1991; Rivera et al., 2006). In search of solution, many resistant cultivars have been developed by breeders but these lose resistance due to changes in the virulence patterns and pathogenicity of the *Colletotrichum* pathotypes among other reasons (Ngugi et al., 2002; Pande et al., 1991). Additional findings show that apart from *C. sublineolum*, *C. gloeosporioides* can also infect a variety of crops including *Graminea* family (O'Neill et al., 1989) but this has not been confirmed. Moreover, despite the fact that leaf, panicle and stem infections by anthracnose fungus have been assessed by different scientists, it is unclear whether these strains differ in terms of their virulence and pathogenicity on sorghum. Previous findings that phosphorous-use-efficient and *Striga* resistant genotypes are resistant to foliar anthracnose while P-use-inefficient and *Striga* susceptible genotype are susceptible (Were and Ochuodho, 2011) but this has not been proved through genetic studies.

Based on importance of sorghum, uses and losses due to anthracnose, this study was therefore set to confirm the previously observed field responses to disease under field conditions determine the genetic similarity and differences of advanced sorghum groups with similar responses to anthracnose and to assess the virulence and pathogenicity of leaf, stem and panicle isolates of anthracnose fungus.

MATERIALS AND METHODS

Planting and experimental design in screenhouse

Sixteen advanced and genetically stable sorghum genotypes were planted in 15 cm diameter plastic tubes using triple super phosphate fertilizer which was thoroughly mixed with two months solarized soils to reduce chances of infection by soil inoculum. The tubes were arranged in a split – plot manner in completely randomized design in the screenhouse. Sixty isolates of *Colletotrichum* species that is 20 from each plant part were obtained from previous studies (Were and Ochuodho, 2012) and randomized as whole plots with two replications. The sixteen sorghum genotypes were grouped as aluminium, drought, midge insect pest and *Striga* resistant/susceptible and phosphorous-use-efficient/inefficient genotypes according to the findings by an ongoing research (Bio Earn, 2009). These were randomized as sub - plots alongside two known anthracnose resistant and two known anthracnose resistant checks.

Inoculum and test host (*Sorghum bicolor*) preparation

Monoconidial isolates from leaf, stem and panicle tissues were grown on half-strength PDA plates at 25°C under alternating 12 h white fluorescent light and 12 h dark cycle for 14 days to induce sporulation (Than et al., 2008). Each plate was then flooded with 5 ml of sterile distilled water, gently swirled and scrapped with a scalpel to harvest macro conidia. The conidial suspensions were passed through a double layer of cheesecloth to retain fragments of mycelia and culture media and transferred into test tubes. The filtered spores were adjusted to a concentration of 1×10^6 spores/ ml after counting on Buker - Turk haemocytometer (Mathur et al., 1989).

The 20 genotypes were placed inside transparent plastic containers for inoculation and transferred to room temperatures at the pathology laboratory. About 3 small holes were made on the top of each inoculation container to ensure proper air circulation.

Inoculation

Leaves of 21-day-old plants in each chamber were pre-moistened with sterile distilled water and inoculated immediately with about 5 ml of a 1×10^6 spores/ ml concentration of each inoculum using a hand sprayer until runoff and covered immediately to maintain high humidity. Inoculated plants were maintained under high relative humidity (>95%) by misting after every 12 h using sterile distilled water and left at room temperature in normal light regimes (Than et al., 2008) for four consecutive days to ensure successful infections. On the fifth day, the infected plants were acclimatized and returned to the normal screen house conditions.

Pathogenicity and virulence assessments

Pathogenicity of *Colletotrichum* species on 20 sorghum genotypes was done using the 60 isolates while virulence (degree of pathogenicity) of the 60 isolates was determined using four known susceptible genotypes (N_4 and $K5_e$ - advanced genotypes from Bio-Earn project; ICSV 700 and SRN 39 - susceptible checks from ICRISAT).

The virulence was also assessed based on the 15 morphologically different isolates (Were and Ochuodho, 2012) on the 20 and responses categorized as strong, moderate and weak.

Assessments and statistical analysis

Virulence and pathogenicity assessments were done once on the 7th day after inoculation on a 1 to 5 rating scale (Erpelding and Prom, 2004). Responses to morphologically distinct isolates were also considered during similar assessment. The virulence and pathogenicity data was subjected to analysis of variance on Genstat version 12.2 and means were separated by contrast comparison.

Genetic diversity based on response to foliar anthracnose

Two *Striga* resistant (N57 and T52) and two P-use-efficient (L6 and O2) genotypes plus one *Striga* susceptible (R5) and one P-use-inefficient (K5e) genotypes were assessed for their genetic differences and similarities based on their previous responses both in the field and screen house.

Total DNA was extracted and quantified from 14 days old leaf tissue of each genotype (Li et al., 2007). Approximately 0.5 g of fresh leaf sample was weighed and ground into a fine powder using liquid nitrogen, a mortar and a pestle making sure that the material does not thaw before being added to the isolation buffer. Each powder was transferred into 15 ml of pre-warmed (60°C) isolation buffer (100 mM Tris-HCl, Ph 8.0; 20Mm EDTA; 2% CTAB) in a capped polypropylene tube and incubated at 60°C in a water bath (Citizen) for 30 minutes. The solution was cooled to room temperature, then one volume chloroform: isoamyl alcohol (24:1) was added and mixed well to ensure there is emulsification of the phases. The mixture was centrifuged using a Hermle micro centrifuge (Eppendorf) at 3000 rpm and 10°C for 25 min. The upper aqueous phase was transferred into a fresh tube and 2 ml of 10% CTAB at 65°C was mixed. A repeat of chloroform-isoamyl alcohol extraction was done by adding 20 ml chloroform- isoamyl alcohol and shaken for 20 minutes at room temperature. The final aqueous phase was transferred to a centrifuge tube using a large-bore pipette, two thirds volume isopropanol added, covered with parafilm and mixed gently but thoroughly by inverting the tube several times. The DNA suspended in the solution was then allowed to precipitate and thereafter the liquid was drained carefully. About 20 ml of 70% ethanol was added and the pellets for each sample was gently agitated for a few minutes and collected by centrifugation (10 min, 5000 rpm, 4°C). The tubes were then inverted and drained on a paper towel for about one hour, taking care that the pellets did not slip out of the glass wall. Pellets were neither allowed to contain residual ethanol nor be too dry. 100 µl volume of TE buffer (10 mM Tris- HCl, 1 mM EDTA, pH 8.0) was added and pellets dissolved at 4°C without agitation. Pellets were then drained and dissolved in 100 µl of TE buffer and the DNA samples were then stored at - 20°C.

The quantified DNA was amplified on a polymerase chain reaction machine (Eppendorf) (Skroch and Nienhuis, 1995) using inter-simple sequence repeat primer UBC 835 of the sequence AGA GAG AGA GAG AGA. The universal primer (UBC) was sourced from Bio-Earn project of crop production in acid soils by Chepkoilel University College, a constituent college of Moi University. This primer was developed in the Biotechnology Laboratory in the University of British Columbia. Sterile 20 µl PCR tubes was loaded with a mixture of 2 µl of DNA of each genotype plus 8 µl of a PCR premix (DNA polymerase 1 ul, 0.1 mM DNTPs, 0.5 unit Taq, 0.4 µM primer, 1× reaction buffer, 2 mM $MgCl_2$, 50 mM Tris, pH 8.5, 20 mM KCl). DNA amplification was set at two cycles of 60 s at 94°C (Denaturation step), 7 s at 54°C (Annealing step), and 70 s at 72°C (Extension/elongation step), and 38 cycles of 1 s at 94°C (Denaturation), 7 s at 54°C (Annealing), and 70 s at 72°C (Elongation). The final step was done at 72°C for 7 min (Final elongation) for any remaining single stranded DNA to be fully extended and a cooling step (Final hold) at 4°C.

The amplified PCR products were separated on gel electrophoresis kit (Eppendorf) (Cardle et al., 2000). The agarose powder was mixed with electrophoresis buffer TAE (Tris-acetate-EDTA, pH 8.0) to obtain 1.5% concentration then heated in a microwave oven until completely melted. After cooling to 60°C, the gel was poured into a casting tray with a sample comb then allowed to solidify at room temperature. After solidification, the sample combs was removed carefully. Each sample containing DNA was mixed with loading buffer (50% glycerol) then pipeted directly into sample wells. Electrophoresis was conducted at 90 mV for one and a half hours. The gel was stained for 20 min with ethidium bromide (0.5 µg ml^{-1}) and then destained for 10 min in deionized water. The profiles were then visualized under UV light box (transIlluminator).

Since SSR is a co-dominant marker, the separated DNA bands were scored visually as present (1) or absent (0). The genetic data was subjected to Unweighted Pair Group Method Arithmetic (UPGMA) on NTSYS-pc version 2.1 (Rohlf, 2000) to determine the genetic diversity and similarity among the selected genotypes.

RESULTS

Response of sorghum genotypes to 60 isolates of *Colletotrichum* species

The differences in response by the groups of advanced sorghum genotypes to *Colletotrichum* isolates were highly significant ($p < 0.05$) and most observations were similar to those made in the field (Were and Ochuodho, 2011). The effect of interaction between sorghum

Table 1. Reactions of sorghum genotypes with different traits to 60 isolates of *Colletotrichum* species under greenhouse conditions.

Genotype	Trait	Mean anthracnose	Category
C1	Aluminium toxicity resistant	1.9	
G2	Drought resistant	2.1	
T52	Striga resistant	2.2	Resistant
O2	Phosphorous efficient	2.4	
IS 21016	Anthracnose resistant check	2.4	
L6	Phosphorous efficient	2.7	
IS 8852	Anthracnose resistant check	2.8	
L5	Aluminium toxicity susceptible	3.1	
Wagita	Midge resistant	3.1	
C26	Drought susceptible	3.1	Tolerant
N57	Striga resistant	3.1	
AF28	Midge resistant	3.1	
N64	Phosphorous inefficient	3.2	
P5	Aluminium toxicity resistant	3.2	
R5	Striga susceptible	3.5	
N68	Midge susceptible	3.6	
ICSV 700	Anthracnose susceptible checks	4.3	Susceptible
SRN 39	Anthracnose susceptible checks	4.3	
N4	Drought resistant	4.4	
K5e	Phosphorous inefficient	4.5	

	Isolates	Genotypes	Isolate × Genotype
Grand mean	3.154	3.154	
S.E	0.0691	0.035	0.2733
S.E.D	0.0977	0.0495	0.3865
% C.V			12.20%
F. probability	< 0.001***	< 0.001***	< 0.001***

genotypes and *Colletotrichum* isolates was also significant ($p < 0.05$) (Table 1).

Resistant, tolerant and susceptible reactions were the three main categories of disease severity observed in the screenhouse with genotypes exhibiting different traits showing different disease levels (Table 1).

Purple and circular, elliptical necrotic spots on leaf lamina and midribs and brownish necrotic spots with acervuli were the common symptoms expressed by sorghum genotypes screened. This varied not only from one genotype to the other but also from one species/strain of the *Colletotrichum* isolate to the other. In particular, *C. gloeosporioides* gave brownish necrotic spots with acervuli on leaf lamina and pink elliptical lesions on the mid rib (Figure 1).

After inoculation, all susceptible checks including genotypes K5e and N4 showed anthracnose symptoms after three days. However, the tolerant and resistant genotypes expressed symptoms five days after inoculation. In addition, midrib infection was common in genotypes with tolerance to the disease while leaf lamina infection was frequently observed in genotypes with highly susceptible and hypersensitive reactions to anthracnose.

Recovery reactions to infection by *Colletotrichum* isolates

Approximately 28 days after inoculation, most genotypes recovered from foliar anthracnose infection. Disappearance of symptoms and hypersensitive reactions on upper and middle leaves were the common observations. For example, genotype T52 showed symptoms of infection 3 days after inoculation with the *Colletotrichum* species but the symptoms disappeared later with new leaves showing no symptoms of infections while the lower leaves dried due to infection by isolate 4. A similar observation was made on genotype O2 except that after 28 days, hypersensitive reactions which formed lesions without acervuli were seen on the leaves (Figure 2).

Some *C. sublineolum* isolates produced symptoms similar to those produced by *C. gloeosporioides* and

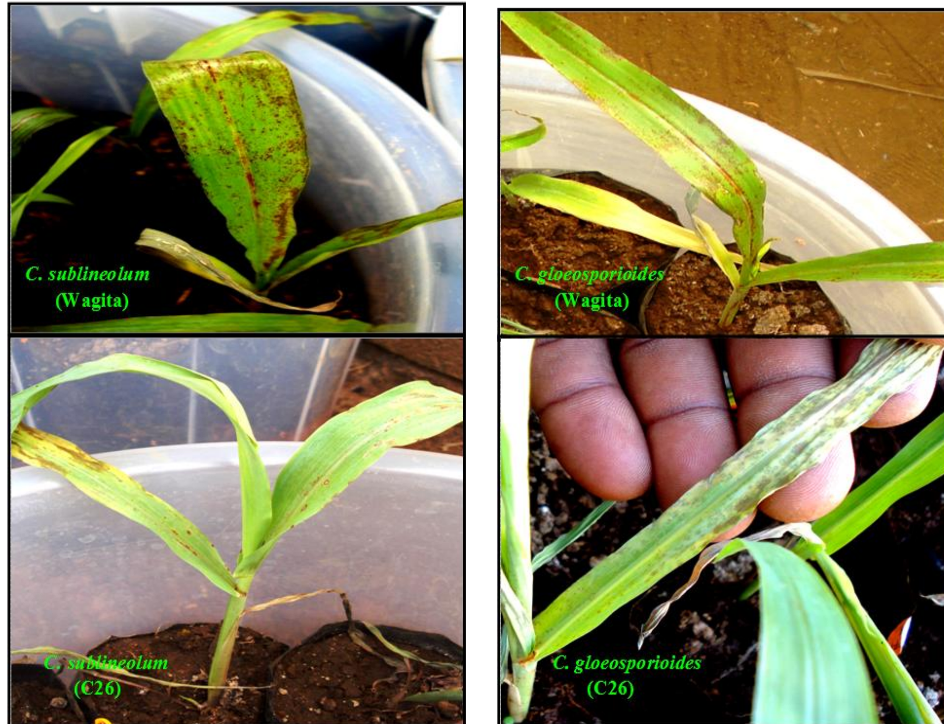

Figure 1. Symptoms of anthracnose on sorghum genotypes (Wagita and C26) infected with *C. sublineolum* and *C. gloeosporioides*. These symptoms were observed seven days after inoculation. The two genotypes were tolerant both in field and greenhouse evaluation.

Figure 2. Recovery (T52) and hypersensitive (O2) reactions of sorghum genotypes (7th and 28th days after inoculation with *Colletotrichum* species, isolate 4).

these include isolates 1, 8, 9 and 15 (Were and Ochuodho, 2012).

The virulence of morphologically diverse *Colletotrichum* species

Among the 15 morphologically different isolates, *C. sublineolum* strains were less virulent compared to the suspected ex-isolates of *C. gloeosporioides* on the 20 genotypes. Low virulence was scored on anthracnose resistant genotypes including C1, G2, O2 and T52. However, genotypes AF28, C26, L5, L6, N57, N64, N68, Wagita, P5 and R5 expressed moderate virulence to the pathotypes. In contrast, all pathotypes expressed strong virulence on K5e and N4 genotypes, indicating that the two were highly susceptible to the disease (Table 2).

All the isolates gave obvious positive symptoms on the susceptible checks (SRN39 and ICSV700) with all pathotypes expressing strong virulence. Moreover, all the resistant checks scored 2 on average for all the isolates except isolates 13 and 14 (suspected to be the ex isolates of *C. gloeosporioides*) (Table 2).

Virulence of leaf, stem and panicle isolates of *Colletotrichum* species

All the leaf, stem and panicle isolates of *Colletotrichum* species differed significantly ($p < 0.05$) in their virulence to sorghum genotypes. This shows that the isolates from these sources varied in their ability to infect sorghum genotypes under similar environmental conditions.

Based on the isolate source, those from the leaf were less virulent, followed by those obtained from the panicle. However, stem isolates were more virulent and this may be due to the fact that majority of the stem isolates were *C. gloeosporioides*. Therefore, higher severity could be due to differences between the two species (Table 3).

Pathogenicity of *C. sublineolum* and *C. gloeosporioides* on sorghum

Pathogenicity of a pathogen may be influenced by species of the pathogen as well as the prevailing environmental conditions at the time of contact between host and the pathogen. Using four genotypes with known susceptibility to foliar anthracnose, *Colletotrichum* isolates from Sega and Kibos sites differed significantly ($p < 0.05$) in their pathogenicity. Significant differences were also noted to exist between species that is *C. sublineolum* and *C. gloeosporioides* ($p < 0.05$). However, the pathogenicity of *C. sublineolum* from Sega and Kibos sites did not differ significantly and this was similar to *C. gloeosporioides* from the two sites ($p > 0.05$) (Table 4).

Genetic diversity and similarity of *Striga* resistant and P-use-efficient sorghum

Gel electrophoresis of extracted DNA from the six selected genotypes indicated that all the *Striga* resistant and the P-use-efficient genotypes had similar number of bands. In terms of PCR amplification, the genetic studies found that both genotypes O2 and L6 had similar number of bands but some genes were more expressed and amplified than the rest. For instance, genotype L6 contained two unique genes while O2 had only one unique gene as far as the genetic diversity is concerned (Figure 3).

For *Striga* resistant genotypes, T52 expressed three unique genes that were also present but not much expressed in genotype N57. However, the P-use-inefficient and *Striga* susceptible genotypes showed similar number and position of DNA bands on agarose gel, an indication of genetic similarity for the two genotypes (Figure 3).

The UPGMA cluster analysis generated two major groups of sorghum genotypes. This further confirmed that both Striga resistant and P-use-efficient sorghum genotypes were genetically similar. However, the genotypes exhibiting *Striga* susceptibility and P-use-inefficiency traits differed genetically from the first group but were similar in their genetic make-up (Figure 4).

DISCUSSION

The significant differences in reactions by the 20 sorghum genotypes to 60 isolates of *Colletotrichum* species shows that the isolates used varied in their genetic makeup as well their interactive association with the sorghum genotypes. Moreover, the existence of several physiological strains of this pathogen with different pathogenicity confirms some of the reasons for varied reactions during field screening. This may also mean that sorghum genotypes reacted differently based on the interaction between the hosts' susceptibility genes and pathogens' virulent genes.

The recovery reactions 28 days after inoculation in most genotypes in the screenhouse may be due to induced resistant mechanisms by phytoalexin (Basavaraju et al., 2009; Wharton and Nicholson, 2000) whose production is triggered in sorghum shortly after infection by the fungus. Hypersensitive reactions leading to drying of the susceptible genotypes may be due to overproduction of phytoalexins and other defense compounds used for resistant reactions and in the process of killing the pathogen, they become toxic to the plant tissues (Agrios, 2005).

The higher virulence of stem isolates, mostly constituting *C. gloeosporioides* strains is a clear indication this species is a new threat to sorghum production and the differences in virulence between *C.*

Table 2. Virulence of the 15 morphologically distinct isolates of *Colletotrichum* species on sorghum genotypes.

		Advance sorghum genotypes																	
Isolate	**Morphology**	**AF28**	**C1**	**C26**	**G2**	**K5e**	**L5**	**L6**	**N4**	**N57**	**N64**	**N68**	**O2**	**T52**	**Wagita**	**P5**	**R5**	**Av.**	**Virulence**
1	Grey, grey ring, cream center	2.5	1.8	3.0	1.8	3.8	2.8	2.3	3.0	3.0	3.0	3.3	2.0	2.0	3.0	3.5	3.5	2.8	Moderate
2	Grey, grey ring, grey center	3.5	1.0	3.0	1.8	4.3	2.0	2.0	2.5	2.5	2.5	3.3	2.0	2.0	2.5	2.8	3.3	2.6	Moderate
3	Grey, grey ring, black center	3.0	1.8	3.0	2.0	3.8	3.0	2.5	3.5	2.8	2.8	3.0	2.5	1.8	3.0	2.5	3.0	2.8	Moderate
4	Grey, pink ring, grey center	3.1	1.5	3.0	2.0	4.5	3.0	2.8	4.8	3.0	2.8	3.3	2.0	2.0	3.0	3.0	3.5	3.0	Moderate
5	Grey, pink ring, whitish center	3.0	2.0	3.1	2.0	4.8	3.3	3.0	4.6	3.0	3.8	3.5	2.8	2.0	3.0	3.3	3.6	3.2	Moderate
6	Grey, pink ring, light grey center	3.3	1.5	3.0	2.0	4.1	3.5	2.5	4.4	3.0	3.0	3.1	2.5	2.0	3.0	2.8	2.8	2.9	Moderate
7	Grey, white ring, grey center	2.5	1.3	3.0	2.0	4.3	2.4	2.8	4.1	2.5	2.8	2.8	1.5	1.8	2.8	2.6	2.5	2.6	Moderate
8	Grey, pink ring, black center	3.0	1.5	2.8	1.8	4.3	2.5	2.0	4.1	2.8	2.8	3.0	1.8	1.5	2.3	2.5	2.6	2.6	Moderate
9	Light grey, dark grey ring, black center	3.0	1.5	3.0	1.5	4.5	2.5	3.1	4.5	3.1	3.0	3.5	2.0	1.8	3.0	2.8	3.5	2.9	Moderate
10	Grey, no ring, black center	3.0	2.0	3.0	2.0	5.0	2.5	2.8	5.0	3.0	3.0	3.8	2.0	2.0	3.0	3.0	3.6	3.0	Moderate
11	Grey, pink ring, brown center	3.0	1.8	3.0	1.5	3.8	3.0	2.8	3.6	2.8	3.0	3.0	2.0	2.0	3.0	3.0	3.8	2.8	Moderate
12	Grey, no ring, cream center	3.0	2.0	3.0	2.0	4.8	2.8	2.5	4.5	3.0	3.0	3.5	2.0	2.0	3.0	3.0	3.8	3.0	Moderate
13	Pink, brown ring, grey center	3.6	2.0	3.3	2.5	5.0	3.5	3.0	5.0	3.5	3.5	4.5	3.0	3.0	3.6	3.3	4.0	3.5	Strong
14	Pink, grey ring, grey center	3.0	2.0	3.3	2.3	5.0	3.3	3.0	5.0	3.0	3.8	4.1	2.8	2.8	3.3	3.3	3.9	3.4	Moderate
15	Pinkish, no ring, black center	3.0	2.0	3.0	2.0	5.0	3.0	2.3	4.8	3.3	3.0	3.3	2.3	2.0	3.1	3.3	3.9	3.1	Moderate
	Mean	3.0	1.7	3.0	1.9	4.5	2.9	2.6	4.2	3.0	3.1	3.4	2.2	2.0	3.0	3.0	3.4	2.9	Moderate
	Reaction Category																		

Susceptible checks				Resistant checks			
SRN39	**ICSV700**	**Av.**	**Virulence**	**IS 21016**	**IS 8852**	**Av.**	**Virulence**
3.4	4.0	3.7	Strong	2.0	2.3	2.2	Weak
3.8	4.3	4.1	Strong	2.1	2.3	2.2	Weak
3.5	3.8	3.7	Strong	2.3	2.3	2.3	Weak
4.0	4.5	4.3	Strong	2.0	2.5	2.3	Weak
4.4	4.3	4.4	Strong	2.0	2.9	2.5	Moderate
4.5	4.3	4.4	Strong	2.5	2.5	2.5	Moderate
3.9	4.1	4.0	Strong	2.3	2.0	2.2	Weak
4.5	4.3	4.4	Strong	2.0	2.3	2.2	Weak
4.3	4.0	4.2	Strong	2.0	2.0	2.0	Weak
4.9	4.8	4.9	Strong	2.0	2.0	2.0	Weak
4.0	3.5	3.8	Strong	2.0	2.0	2.0	Weak
4.8	4.8	4.8	Strong	2.5	2.0	2.3	Weak
5.0	5.0	5.0	Strong	3.5	4.8	4.2	Strong
4.8	4.8	4.8	Strong	3.3	4.3	3.8	Strong
4.8	4.5	4.7	Strong	2.8	2.3	2.6	Moderate
4.3	4.3	4.3	Strong	2.4	2.6	2.5	Moderate

Table 3. Virulence of leaf, stem and panicle isolates on grouped sorghum genotypes.

	Isolate source							
Sorghum groups	**Leaf**	**Panicle**	**Stem**	**Av. Anthracnose**	**F pr.**	**S.E**	**S.E.D**	**% C.V**
Aluminium	2.4	2.7	3.0	2.7				
					< 0.001***			
Phosphorous	3.0	3.2	3.5	3.2				
					< 0.001***			
Striga	2.7	2.9	3.2	2.9				
					< 0.001***			
Drought	2.9	3.2	3.4	3.2		0.0541	0.0662	26.6
					0.239			
Midge	3.1	3.2	3.5	3.3				
Resistant checks	2.4	2.4	3.2	2.6				
					< 0.001***			
Susceptible checks	4.1	4.4	4.6	4.4				
Mean anthracnose	2.9	3.1	3.4	G = 3.2				
S.E (Overall)		0.0296						
S.E.D		0.0419						
% C.V		26.60%						
F-probability		< 0.001						
KEY: G = Grand mean								

Table 4. Pathogenicity of *C. sublineolum* and *C. gloeosporioides* isolates on four susceptible genotypes.

Susceptible genotype	Anthracnose pathotypes				Mean
	Sega site		Kibos site		
	C. sublineola	***C. gloeosporioides***	***C. sublineola***	***C.gloeosporioides***	**Anthracnose**
ICSV 700	4.2	4.9	4.1	4.7	4.5
SRN 39	4.3	4.9	4.0	4.6	4.5
K5e	4.4	4.9	4.3	4.7	4.6
N4	4.1	5.0	4.3	4.9	4.6
Mean anthracnose	4.3	4.9	4.2	4.7	Grand mean = 4.5
S.E		0.6492			
S.E.D		0.1048			
% C.V		14.80%			
F - probability		< 0.001***			

sublineolum and *C. gloeosporioides* may be due to their genetic makeup. This is because processes leading to compatible and incompatible interaction between the pathogen and host entirely depend on their genotype and ability to produce defense compounds (Agrios, 2005; Wharton and Julian, 1996; Wharton et al., 2001). These interactions may vary from one genotype to the other.

As far as anthracnose disease epidemiology is concerned, some morphologically diverse *Colletotrichum* species exhibited similar virulence and pathogenicity on sorghum genotypes. This means that despite being morphologically different, some strains of *C. sublineolum* might be genetically identical.

The genetic differences between *C. sublineolum* and *C.*

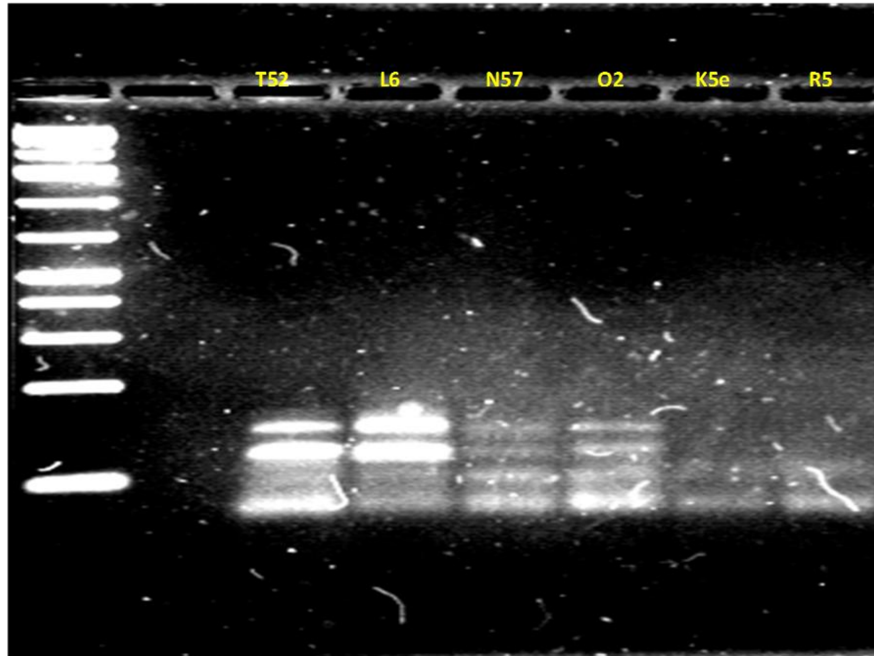

Figure 3. Bands of amplified DNA showing the genetic diversities and similarities among sorghum genotypes exhibiting different traits.

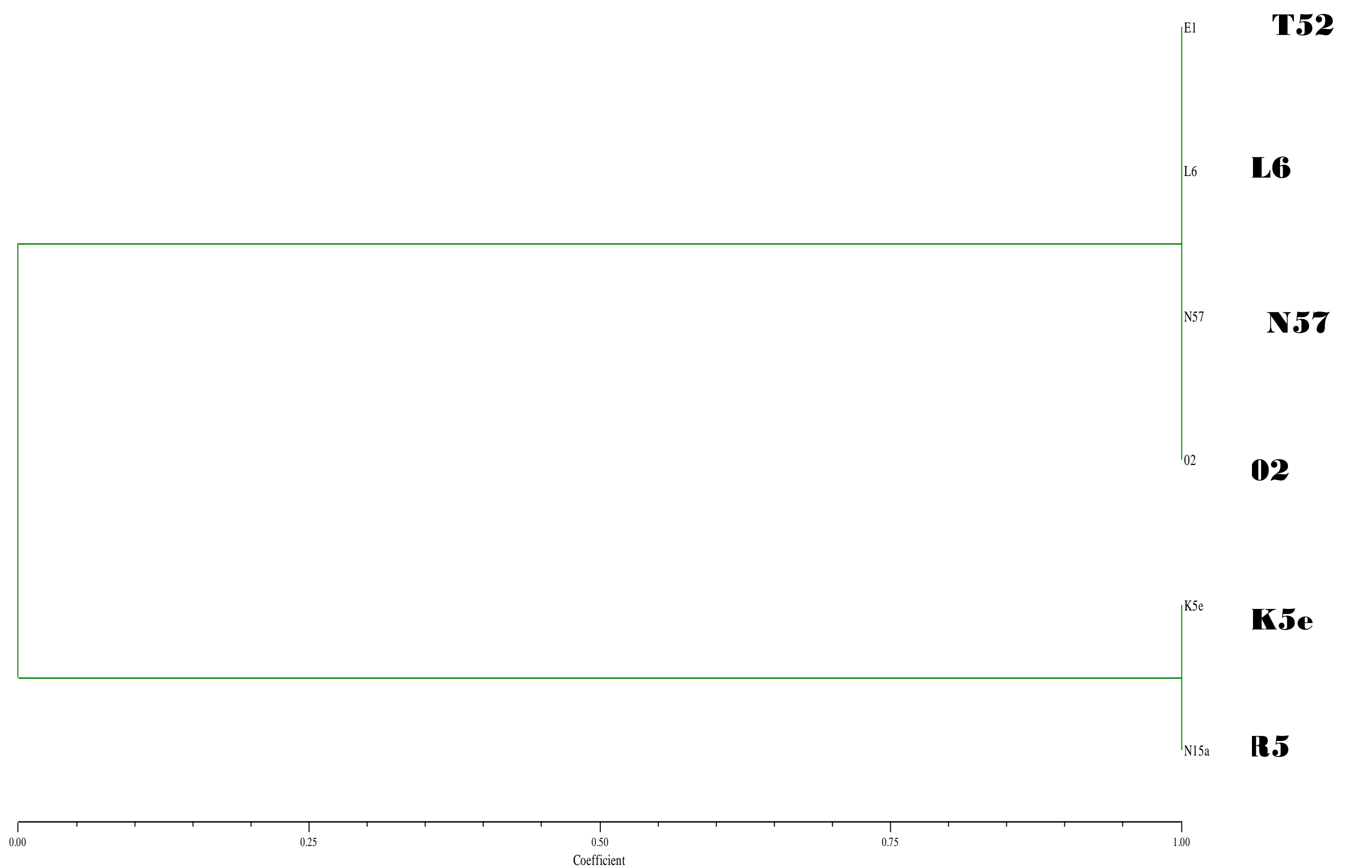

Figure 4. UPGMA cluster analysis based dendrogram showing the genetic relationship among the six sorghum lines.

gloeosporioides pathotypes may have influenced their pathogenicity and virulence. However, similarities in virulence for some species may imply that the first alteration an environment can have on the pathogen takes place on its morphology and after which, genetic differentiation may occur.

Genetic similarity observed among the P-use-efficient and *Striga* resistant sorghum genotypes implies that resistance to anthracnose by these genotypes was fully controlled by similar genes. In addition, the over-expression of some genes in sorghum genotypes than others also explains the observed differences in anthracnose severity even in sorghum genotypes with the same traits. The over-expression and under-expression of anthracnose resistant genes in the P-use-efficient and *Striga* resistant genotypes is similar to the previous findings that anthracnose resistance is a gene-for-gene type (Zambolim et al., 2011) and this involves both recessive and dominant genes.

CONCLUSIONS AND RECOMMENDATION

Resistance to *Striga* and P-use-efficiency traits in sorghum are related to anthracnose resistance. Genotypes with such traits also have similar genetic make-up with some genes being expressed more than others depending on the sorghum genotype. Additionally, susceptibility to *Striga* and P-use-inefficiency traits also confers susceptible response to foliar anthracnose in sorghum but other than environmental and host factors, the level of susceptibility depend on the species of the pathogen causing the disease. However, this study recommends the need to assess the host ranges of the *C. gloeosporioides* strains to ascertain whether sorghum can act as its alternate host. Further genetic diversity and similarity assessments on *Striga* resistant and P-use-efficient genotypes should be conducted using several primers since one primer (UBC 835) used may not give finer details as far as genetic studies are concerned. This will provide more information which is vital for sorghum breeders in Kenya and other parts of the world.

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