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## RESEARCH ARTICLE

### EFFECTS *SORGHUM BICOLOR* TRAITS ON RESISTANCE TO FOLIAR ANTHRACNOSE (*Colletotrichum sublineolum*) IN THE LAKE BASIN REGIONS OF KENYA

Javan Omondi Were<sup>1\*</sup> and Julius Onyango Ochuodho<sup>1</sup>

<sup>1</sup>Chepkoilel University College (A Constituent College of Moi University), School of Agriculture and Biotechnology, Department of Seed, Crop and Horticultural Sciences, P.O. Box, 1125 - 30100, Eldoret, Kenya

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#### ABSTRACT

*Colletotrichum sublineolum* is the most destructive sorghum disease in Kenya. This pathogen is genetically and morphologically diverse. It evolves continuously into new strains to overcome resistance. Various traits exhibited by newly bred varieties also have great bearing on the level of disease resistance. Therefore, the study aimed at determining how commonly desired traits influence resistance to sorghum anthracnose. Advanced *Sorghum bicolor* previously screened for *Striga*, drought, midge aluminium cation toxicity and phosphorous-use-efficiency were screened for anthracnose resistance under anthracnose inoculated soils. The design used was randomized complete block design with four replicates under long and short rains in two different sites. One-to-five severity scales was used to assess response between and within groups. The phosphorous-efficient and *Striga* resistant genotypes were resistant to foliar anthracnose while phosphorous-inefficient and *Striga* susceptible genotypes susceptible to the disease. Aluminium group exhibited mixed reactions to the disease while majority of the genotypes in the drought and midge groups were resistant/ tolerant to the disease. This study concludes that response to anthracnose in sorghum varies from one genotype to the other but such responses are dependent on the traits exhibited by genotypes.

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#### INTRODUCTION

Anthrachnose is one of the most destructive fungal diseases of sorghum due to its rapid spread on susceptible cultivars (Erpelding, 2010a; Erpelding, 2010b; Erpelding, 2011). *Colletotrichum sublineolum* is the fungal pathogen causing sorghum anthracnose but the control of this pathogen has been hindered by evolution of several other subspecies (Mathur *et al.*, 2003). These strains easily overcome resistance by the newly released sorghum varieties. It can cause yield losses of up to 70% under severe epidemics (Thakur and Mathur, 2000) through defoliation and tissue death. This disease also contributes to significant loss of grain quality due to incomplete grain filling as portrayed by low seed weight and density (Erpelding and Prom, 2006). The anthracnose fungus infects all the above ground tissues of sorghum plant with highly variable symptoms depending on the interaction between host plant, pathogen and environment, with symptoms appearing in about 40 days after seedling emergence (Marley *et al.*, 2001). These include: elongated lesions which coalesce as the disease progresses and covers most leaf tissue, presence of acervuli as black spots at the center of the lesion as the fungus sporulate. These symptoms eventually cause leaf senescence and premature defoliation thus retarded plant growth or death before maturity under severe foliar infection. Foliar infection is the most frequent and cause greatest reduction in yield with several studies indicating the presence

of many physiological races of *Colletotrichum* species infecting sorghum (Cardwell *et al.*, 1989; Casela *et al.*, 2001; Crouch and Beim, 2009; Pande *et al.*, 1991). However, resistant cultivars developed normally lose resistance after a short period of time due to continuous evolution of more virulent strains (Casela *et al.*, 2001) coupled with climate change. In most circumstances, aluminium toxicity, drought, phosphorous-use-inefficiency, *Striga* and midge infestation are the additional factors other than anthracnose causing lower yields especially in tropics and sub-tropics (Bio Earn, 2009). It is therefore important for sorghum breeders to take into consideration multiple stress factors approach in formulating their objectives. In the endeavor to improve food production and security, the search for anthracnose resistant genotypes is of paramount importance in Kenya. Therefore, this study was set to assess how advanced sorghum genotypes respond to *Colletotrichum* species with respect to their traits under field conditions of the Lake Basin regions of Kenya.

#### MATERIALS AND METHODS

Advanced and genetically stable sorghum lines were sourced from Moi University research program on 'Crop Production in Acid Soils'. Two known resistant and two known susceptible checks to anthracnose were obtained from the International Crop Research Institute for the Semi – Arid Tropics (ICRISAT). Five groups of genotypes were formed according to the reaction to aluminium toxicity, drought, *Striga*, phosphorous and midge insect pest. Each group consisted two

\*Corresponding author: javanomondiwere@yahoo.com

resistant and one susceptible genotype to each stress. One multistress tolerant genotype (O2) exhibiting resistance to aluminium and P-use-efficiency was used as a standard. This study was conducted at Kibos in Kisumu district and Sega in Siaya district. Both sites are within the Lake Basin Regions of Kenya where foliar anthracnose has been reported to be a major problem to sorghum production. Kibos is located 1,131m above sea level and lies between latitude 0° 05' S and longitude 34° 48' E. The site receives average annual precipitation of 1184mm whose distribution is marked by long rains (March – June) and short rains (August – November). Predominant soil type in this site is cotton black loam soils. Other than being anthracnose and midge hot-spot, this site is also *Striga* endemic (Bio Earn, 2009). Average daily temperature and rainfall for both study sites are according to the Central Bureau of Statistics (C.B.S., 2009). Sega site is located at 1,400m above sea level and lies between latitude 0° 03' N and longitude 34° 25' E. This area receives bimodal rainfall pattern with long rains starting from March to June with a peak in May, and the short rains from September to November with a peak in October but the rains is very unreliable in this area (Bio Earn, 2009). The average annual precipitation ranges from 800 to 2,000 mm whose distribution is marked by wet and dry seasons. The predominant soil type in this site is red loam. This site was chosen since the soils are highly acidic (pH = 4.73) and P is unavailable (Kifuko *et al.*, 2006; Okalebo *et al.*, 2009). The soils in this site have high aluminium cation concentration (Obura *et al.*, 2010) hence crops are prone to toxicity.

During the field screening, Kibos and Sega sites recorded average temperatures of 30.8°C and 29.4°C and mean annual rainfall of 1,921 and 1,388 mm respectively. In both seasons, Sega site received more precipitations (215.8 mm and 164.3 mm) than Kibos (158.5 mm and 101.3 mm) for short and long rains respectively. However, night temperatures at Kibos were higher (23.2°C) compared to Sega (10.3°C). In contrast, Sega was hotter (30.8°C) during the day than Kibos site (29.2°C). In both sites, experiments were planted in April, 2010 (long rains) and in early September, 2010 (short rains). Randomized Complete Block Design with single row for each genotype randomized as a plot with four replicates. Each plot was 4.5m long, 1m between row spacing and 0.3 m within rows and 15 plants in each plot were assessed. Each site was surrounded by known anthracnose susceptible genotype (ICSV 700) which was planted earlier. Triple super phosphate fertilizer was applied at planting. The two sites were previously known to be naturally inoculated with *Colletotrichum* species (Bio Earn, 2009) and therefore, screening was done against natural inoculation. Disease severity was assessed on a 1 to 5 rating scale (Erpelding and Prom, 2004) at 60, 70 and 80 days after sowing up to physiological maturity. Genotypes rated 1 and 2 were considered to be resistant, 3 were tolerant while 4 and 5 were susceptible to disease. Resistant and susceptible responses were differentiated by the presence of acervuli on the flag leaves. Severity data was subjected to analysis of variance on Genstat statistical software, version 12.3 and mean responses separated by contrast comparison to assess the response between and within the groups.

## RESULTS

The groups of the advanced sorghum genotypes gave significantly different reactions to anthracnose ( $p < 0.05$ ), indicating that five groups exhibited particular trends of

responses to foliar anthracnose (Table 1). Within trait comparisons showed significant ( $p < 0.05$ ) differences in terms of anthracnose reactions. In particular, P-use-efficient genotypes including O2 (Figure 1) were generally resistant to the disease than P-use-inefficient genotypes ( $p < 0.05$ ). In contrast, P-use-inefficient genotypes including K5e (Figure 1) and N64 were highly susceptible to foliar anthracnose. However, the degree of susceptibility between N64 and K5e genotypes to foliar anthracnose varied in both sites and seasons. Like P group of genotypes, *Striga* resistant genotypes were resistant to anthracnose disease compared to those susceptible to *Striga* and these differences were significant ( $p < 0.05$ ). For example, T52 (Figure 1) and N57 (*Striga* resistant) showed resistant and tolerant reactions respectively to the disease. However, R5 (*Striga* susceptible) exhibited mixed reaction varying from sites and seasons. Advanced genotypes exhibiting resistance or susceptibility to Al<sup>+3</sup>, drought or midge did not differ significantly from each other in their reaction to anthracnose ( $p > 0.05$ ). For instance, Al<sup>+3</sup> susceptible genotypes gave resistant response to the disease while those resistant to Al<sup>+3</sup> were resistant and/or tolerant to the disease. Genotype L5 and P5 (Al susceptible) expressed resistant and susceptible reaction to anthracnose respectively in both sites and seasons. However, genotype C1 (Figure 1) which is resistant to Al cation toxicity showed resistant reaction to the disease. Exposure to heat stress normally interferes with the physiological functions of the plants especially if such plants are sensitive to heat stress. For example, G2 (Figure 1) known to be drought resistant was resistant to foliar anthracnose. However, N4 genotype which is drought resistant was susceptible to anthracnose. Advanced sorghum genotypes with resistance and/or susceptibility to midge insect pest were all tolerant to foliar anthracnose under field conditions. For instance, genotypes AF28 and Wagita (midge resistant) were tolerant to foliar anthracnose while N68 (midge susceptible) showed resistant response to the disease. There was significant interaction between the sites, seasons and genotypes ( $p < 0.05$ ). Kibos recorded the highest disease severity while Sega gave low disease levels on average.

## DISCUSSIONS

The diversity in group response to foliar anthracnose may imply that screened genotypes were genetically diverse, just as they were grouped and selected and expression of symptoms from lower leaves towards the upper leaves as seen in all screened genotypes (tolerant and susceptible) in the field can be due to the presence of different host resistant genes that controls the site of infection (Thakur and Mathur, 2000) as the sorghum plants grow and age. Delayed anthesis in G2 and C1 genotypes may have conditioned resistance to anthracnose infection but the mechanisms behind the influence of late anthesis and sensitivity to day – length is unknown and such observations reassembled those of Erpelding and Prom, (2006) and Rivera *et al.*, (2006) where genotypes with delayed anthesis exhibited tolerance to foliar anthracnose especially during the wet growing season. Resistant reactions by early maturing T52 and O2 genotypes are similar to observations in other parts of the world (Erpelding, 2010b; Erpelding, 2011; Rivera *et al.*, 2006) Various studies have indicated that the amount phosphorous in plants influence susceptibility or resistance to certain diseases (Agrios, 2005). This is because phosphorous perform vital roles as a catalyst in biochemical reactions, trapping solar energy and converting it into ATP,

**Table 1. Reactions of advanced sorghum genotypes to foliar anthracnose under field conditions. The disease was assessed on a 1-5 rating scale with 1-2 (resistant), 3 (tolerant) and 4-5 (susceptible)**

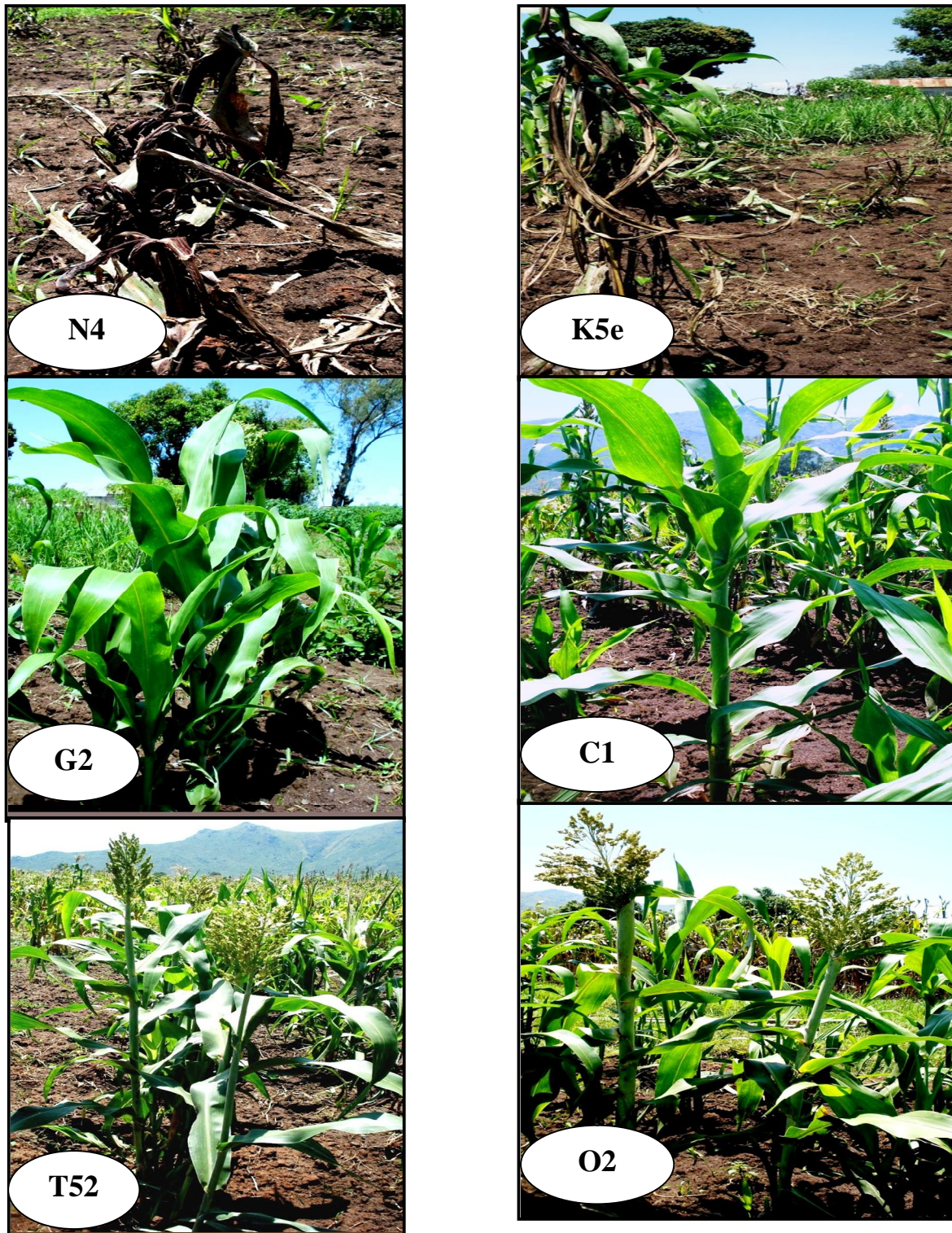
Genotype	Trait	Sega	Kibos	Av. anthracnose	Category
C1	Aluminium toxicity resistant	1.0	1.0	1.0	Resistant
G2	Drought resistant	1.0	1.0	1.0	
T52	<i>Striga</i> resistant	1.0	1.0	1.0	
O2	Phosphorous efficient	1.9	1.5	1.7	
IS 21016	Anthracnose resistant check	2.0	2.0	2.0	
L5	Aluminium toxicity susceptible	2.9	2.0	2.5	
Wagita	Midge resistant	2.8	2.6	2.7	Tolerant
N68	Midge susceptible	3.0	2.4	2.7	
L6	Phosphorous efficient	3.1	2.5	2.8	
C26	Drought susceptible	3.0	2.6	2.8	
AF28	Midge resistant	3.0	2.8	2.9	
R5	<i>Striga</i> susceptible	2.9	3.4	3.2	
N64	Phosphorous inefficient	3.9	2.3	3.1	
N57	<i>Striga</i> resistant	3.0	3.5	3.3	
IS 8852	Anthracnose resistant check	3.5	4.0	3.8	Susceptible
P5	Aluminium toxicity resistant	3.2	4.6	3.9	
SRN 39	Anthracnose susceptible check	4.4	4.6	4.5	
K5e	Phosphorous inefficient	4.8	4.3	4.6	
N4	Drought resistant	4.9	4.8	4.9	
ICSV 700	Anthracnose susceptible check	4.5	5.0	4.8	

Source of variation	Severity	S.E	S.E.D	F pr.	% C.V	
Genotypes	2.9	0.114	0.161	< 0.001***	15.5	
Seasons	Long rains	3.1	0.036	0.051		< 0.001***
	Short rains	2.8				
Sites	Sega site	2.9	0.036	0.051		0.111
	Kibos site	3.0				

component of DNA and RNA (Griffith, 1999). These past studies corresponds with the findings of this study where O2 and L6 (P-use-efficient) were resistant and tolerant respectively to foliar anthracnose. This may mean that during screening, the two absorbed more P from soil and other sources thus conferring the observed responses to anthracnose. The P-inefficient genotypes might have absorbed less P to catalyze the synthesis of more proteins (enzymes) required to enhance defense mechanisms. However, differences in degree of susceptibility to anthracnose may be due their differences in P-use-efficiency and utilization which has been shown to vary from one genotype to another (Camacho *et al.*, 2002). Apart from P, higher levels of silicon (Si) in sorghum plant has been confirmed to induce resistance to anthracnose disease while low levels increase susceptibility (Resende *et al.*, 2009). The differences expressed within the *Striga* group may be due to variation in the levels of *Striga* infestation that has been shown to determine the quantity of defense compounds (phytoalexins) produced (Showemimo and Kimbeng, 2005). Genotypes T52 and N57 may have resisted or tolerated anthracnose disease due to their genetic makeup, which has been shown to initiate the production of some resistance compounds before or soon after the initial contact with *Striga* haustoria (Reda *et al.*, 2010) while R5 genotype may not have produced these resistance compounds thus leading to susceptibility to anthracnose. The response by L5 in the aluminium group may be due to excessive absorption of  $Al^{+3}$  which is known to increase cell

wall rigidity, reduce DNA replication, modify structure and functions of plasma membrane (Mossor - Pietraszewska, 2001) and most importantly, it forms strong complexes that precipitate DNA and RNA in sorghum plant (Rout *et al.*, 2001). This alters the physical structure and hardens the leaf surfaces thereby reducing the ability of spore germlings to penetrate into the cell wall and plasma membrane hence inhibiting numerous infections by anthracnose fungus. Genotype C1 is also resistant to drought and exposure to high temperature conditions of Sega and Kibos sites may have enhanced the capacity of enzymatic antioxidant system (Takele and Farrant, 2009) thus making this genotype to synthesize superoxide dismutase (SOD) (Menezes-Benavente and Teixeira, 2004) which catalyzes the conversion of oxygen ( $O_2$ ) to hydrogen peroxide ( $H_2O_2$ ) which provides a strong defense mechanism against infectious diseases such as foliar anthracnose. The response by G2 may be due to excessive production of SOD enzyme whose production is triggered by exposure to heat stress. For N4, it is possible that its resistance to drought is through a different mechanism. The midge resistant genotypes tend to contain higher levels of tannins and proteins with adverse effect on the biology of this pest. Such genotypes also contain low levels of sugar (Sharma *et al.*, 2008) and these mechanisms of resistance may have been exhibited by Wagita and AF28 genotypes which showed tolerance to foliar anthracnose in the field. This contradicts other work where midge resistant cultivar showed (ICSV 247)





**Figure 1. Response of advanced sorghum genotypes to *Colletotrichum* species under field conditions. N4 and K5e were highly susceptible; G2 and C1 were resistant but late maturing while T52 and O2 were resistant but early maturing.**

susceptible response to both foliar and panicle anthracnose (Ajayi, 1994; Tenebe and Kamara, 2002). Therefore, despite being susceptible to midge, N68 genotype may have produced tannins and/or proteins thus conferring tolerance to the disease. Alternatively, this genotype may exhibit a different form of resistance mechanism to foliar anthracnose. The significant interaction between site, season and genotype effects indicates that responses to foliar anthracnose was influenced by the additive effects of genotypes, sites and seasonal factors and

this is similar to other studies (Erpelding, 2010a; Erpelding, 2010b; Erpelding, 2011; Hess *et al.*, 2002). Being that Kibos site is close to the lake, high night temperatures may have provided higher relative humidity in both seasons which provided favourable environmental conditions required by the *Colletotrichum* species for proper infection. This could have led to high anthracnose severity in Kibos than Segga despite the fact that Segga was hotter during the day than Kibos site. Coupled with the fact that Kibos is anthracnose hot spot, warm

night temperatures may have also prolonged the dew period which contributed to higher infection rates and severity. Since water is very vital in the infection process both at the initial stage and as the disease progresses, more precipitation during the first season in both sites provided adequate water which may have diluted mycosporine-alanine (spore germination inhibitor) (Leite and Nicholson, 1992). Consequently, much water during the long rains may have reduced the concentration of this spore germination inhibitor to a lower concentration thus enhancing not only ascospores germination but also tissue infection and colonization by millions of spores thus resulting in higher severity in long rains season. Based on these findings, the study concludes that traits exhibited by sorghum plays a very significant role in the disease resistance or susceptibility. Therefore, breeders aiming at tolerance and/or resistance to multistress factors including foliar anthracnose should exploit some of the advanced genotypes in Kenya. This research also recommends the need to establish whether there is genetic similarity or differences between *Striga* resistant and P-use-efficient genotypes of sorghum.

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