Morphological and Cultural and Variation of *Exserohilum turcicum* isolates in Sorghum (Sorghum bicolour L.)

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Abstract

Sorghum is mainly cultivated for human consumption. Although it is considered as the second most important cereal crop after maize in Africa, this crop is affected by various diseases due to availability of hybrids that are susceptible. Therefore, this research is aimed at characterizing Exserohilum turcicum that is a causative agent of Turcicum leaf blight in Western region of Kenya using cultural and morphological characteristics. Diseased leaf samples of sorghum were collected from two regions (Kibos and Sega) in Western region of Kenya. A number of 6 fungal isolates were studied for both cultural (colony color, pigmentation, sporulation and growth pattern) and morphological (conidia) properties after twelve days of growth on PDA at 25 ^{0}C. Cultural characteristics did show that there was variation among isolates of E. turcicum in colony growth and colour. The colony color was categorized into three categories i.e. gray, dark gray and golden brown. Based on colony pigmentation, the isolates were grouped into black and brown. Based on sporulation, the isolates were classified as; profuse, moderate and fair. On the other hand, the conidial shapes were observed to be curved and fusiform-elliptical. Results also showed significant variations in conidial size, length and width and number of septa in the six isolates of E. turcicum. The largest conidia were reported for isolate Western 1 (103 µm in length and 23.86 µm in width). The average number of septa was 4.5 (range 3-6 septa). Isolates from the two study regions reported similar cultural and morphological characteristics. The characteristics studied which were morphological and cultural showed that, the isolates were E. turcicum. The results of this study will serve as a model for other studies in the future and will also assist in coming up with mitigating losses in sorghum yield.

Keywords: *Exserohilum turcicum*, sorghum, morphological and cultural variations, Turcicum leaf blight

INTRODUCTION

Global climate change has been observed over the past decades and is expected to continue in the future (IPCC, 2007) with extreme events such as droughts becoming common and even worse in arid and semi-arid areas (ASALs). Thus, crops that are drought resistant like sorghum become of significance. Sorghum as a crop is generally cultivated in drier and marginal regions of Africa and it is a source of food for more than 50% of Africa's rural household (FAO, 2008). It is rich in protein, energy, vitamins and minerals for many of the poor people in the African continent (FAO, 1990). FAO (2003) reported that sorghum rated globally as the fifth largest and most significant cereal, after maize, wheat, rice and barley. However, it is ranked the second most important cereal after maize in Africa (FAO, 2008). In Kenya, sorghum is grown in the often drought-prone marginal agricultural areas of Eastern, Nyanza and Coast Provinces (Takuji and Baltazar, 2009). These regions are some of the poorest regions in Kenya therefore sorghum becomes a staple food crop for many low-income households in the regions. In spite of its essential role in the regions, Muui et al. (2013) reported that the current production level of sorghum in Kenya is low. The study further revealed that sorghum production is at less than one ton per hectare and vary significantly from year to year with majority of sorghum growers not producing enough to meet family requirements in most years.

Both abiotic and biotic factors have been reported to affect sorghum production (Ogeto *et al*., 2013). Generally, plant diseases are considered as a major constraint in sorghum cultivation throughout the world and particularly Kenya, (Ramathani, 2010). Precisely, Aden (1991) revealed that leaf blight caused by *Exserohilum turcicum* (Pass.) Leo & Suggs is one of the major diseases that reduce sorghum yield significantly. The fungus pathogen primarily attacks the leaf blade but under extended disease-conducive environment it may also attack leaf sheaths and total leaf wilting occurs under heavy infected conditions significantly reducing the photosynthetic area (Ngugi *et al*., 2000). Turcicum leaf blight (TLB) of sorghum is epidemic in various parts of Kenya and causes severe crop loss depending on cultivar, stage of infection and prevailing environmental conditions. Chandrashekara *et al*. (2014) elaborated that most of the composites and hybrids which are being grown in most sorghum growing regions are found to be susceptible to TLB. The most contributing factors to lack of substantial resilient resistance in the sorghum plant could be ascribed to presence of variability in the population of the turcicum leaf blight pathogen. In order to develop sorghum cultivars that are TLB resistant and high yielding, there is need to determine the variability in the pathogen. Thus, the present study aimed to characterize *Exserohilum turcicum* which is a causative agent of Turcicum leaf blight in Western region of Kenya using cultural and morphological characteristics

MATERIALS AND METHODS

Study area

The study was carried out in two main regions in Western Kenya namely Kibos and Sega. Kibos area is situated near Kisumu at an altitude of 1131m above the sea level and lies between latitude 0° 05' S and longitude 34° 48' E. while, the Sega site is located on the Kisumu-Busia road with an elevation of between 1140m to 1400m above

the sea level and lies between latitude 0° 15'N and longitude 34° 20' E. The regions were chosen because they are known to be Turcicum leaf blight, anthracnose and midge hot-spots (Were, 2012).

Sample collection and isolation of the pathogen

Diseased sorghum leaves were obtained from the two main regions in Western Kenya namely Kibos and Sega. The leaves were wrapped in blotter papers for isolation of the pathogen. These leaves were first washed with tap water followed by sterile distilled water. Manamgoda *et al.* (2012) method on the isolation of fungus from diseased samples was used during the study. Briefly, the diseased portions with some healthy portion were cut into small bits of 3-5 mm size, surface sterilized by dipping them in sodium hypochlorite (5 %) solution for one minute and then 3 bits were transferred aseptically to petri plates containing potato dextrose agar (PDA) medium and were incubated at 25°C in an incubator. The fungus isolates thus isolated from the infected tissue were further purified by single spore isolation.

Cultural characterization of the pathogen

Twelve day old cultures of *E. turcicum* were used to note down the colony color and pigmentation. Observations on colony color, pigmentation, sporulation, growth pattern of each isolate were recorded 12 days after incubation on PDA at 25°C. Colony color and pigmentation of all the isolates grown on PDA was determined with the help of Munsell color charts developed by Soil Survey Staff in 1951 (Reddy *et al.*, 2014). The pigmentation of the colony was recorded from the under surface of the petriplate.
To determine sporulation in each isolate, discs of 5 mm size were cut from 10 day old cultures. Three such discs were placed in a test tube containing 15 ml of distilled water and were vortexed to dislodge the conidia from mycelial mat. Spore load was measured by using hemocytometer (Reddy *et al.*, 2014). Based on the number of spores produced per unit area on culture medium, the isolates were categorized into 4 types namely scanty ($5x10^4$ spores/rnl), moderate ($5\text{-}10xl0^4$spores/ml), good ($l0\text{-}15x\ 10^4$spores/ml) and abundant ($>15x\ 10^4$ spores/ml) sporulating isolates.

Morphological characterization of the pathogen

The conidia of all the isolates of *Exserohilum turcicum*, slides were prepared from twelve days old culture. Temporary slides were prepared in water mount using cotton blue. Data on length, width and septation of conidia were recorded by ocular-micrometer by using a pre-calibrated compound microscope. Twenty spores were observed for each isolate to avoid the error while taking observations.

RESULTS AND DISCUSSION

Cultural characteristics

Pure cultures of six isolates were investigated on cultural characteristics by categorizing the nature of colony appearance on Potato Dextrose Agar medium. The data pertaining to cultural characteristics of *Exserohilum turcicum* was observed 14 days after

incubation on PDA. Details of cultural characteristics such as colony color, pigmentation, and sporulation are described in Table 1.

Based on colony color, the *E. turcicum* isolates were grouped into three categories i.e. gray, dark gray and golden brown. The sorghum isolate Western 1 showed distinctively different colony color than other isolates i.e. golden brown(7.5YR 8/3). On pigmentation, *E. turcicum* isolates were grouped into two categories i.e. black and brown. Isolate Western 1 had a distinct pigmentation which was brown (10YR 7/2). This concurs with the finding of Daniel and Narong (2006) who studied cultural and morphological characteristics of *E. turcicum*. From their results, cultural characterization proved that there is existence of variation in colony growth and colony color.

Based on sporulation, the isolates have three groups i.e. profuse, moderate and fair. The isolates A11 and Western 1 produced excellent sporulation while fair sporulation was observed in Nyanza and Nyadundo Western. The investigation done by Harlapur *et al.* (2007) on sixteen isolates of *E. turcicum* reported that growth in five isolates as fast and profuse while poor and moderate growth was observed in three isolates. Minimum and maximum colony growth was seen following twelve days of incubation

Table 1: Colony characteristics of isolates *Exserohilum turcicum* collected from Sorghum in Sega and Kibos

Isolate	Colony Colour	Pigmentation	Colony Characteristics	Diameter	Sporulation
A11	Gray 2.5Y 5/1	Black 10YR 3/1	Poor restricted growth with irregular margin and dark center	67.88	++++
NYADUNDO WEST.	Black 2.5Y 2.5/1	Black 10YR 3/1	Moderate growth, appressed at the center with circular margin	78.58	++
NYANZA 2	Dark gray 2.5Y4/1	Black 10YR 3/1	Moderate growth, dark at the center with a fluffy margin	78.94	+++
WEST. 1	Golden brown 7.5YR 8/3	Brown 10YR 7/2	Profuse growth, dark brown at the center with circular margin	79.94	++++
G2	Gray 2.5Y 5/1	Black 10YR 3/1	Moderate growth, appressed at the center with circular margin	78.82	+++
NYADUNDO NYANZA	Gray 2.5Y 5/1	Black 10YR 3/1	Moderate growth, appressed at the center with circular margin	76.02	++

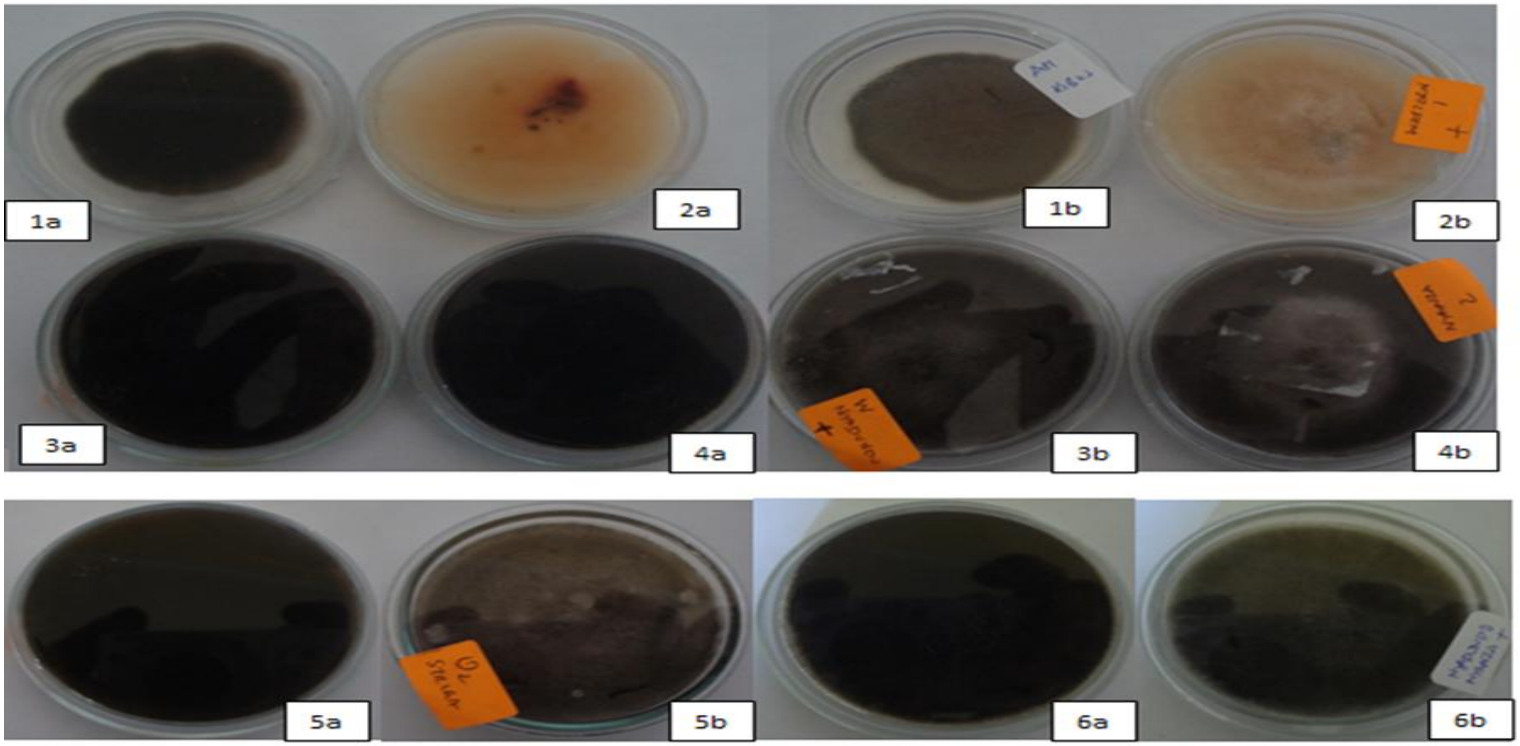

Figure 1: The colony growth characteristics of *Exserohilum turcicum* isolates on PDA. 1a and 1b is the under and upper surface of A11 respectively, 2a and 2b is under and upper surface of Western 1, 3a and 3b is under and upper surface of Nyadundo Western, 4a and 4b is under and upper surface of Nyanza 2, 5a and 5b is under and upper surface of 2a and 6a and 6b is under and upper surface of Nyadundo Nyanza.

Morphological characterization on the pathogen

A pure culture of each isolate was cultured on PDA and then the morphology of the conidia was observed under ocular micrometer by using a pre-calibrated compound microscope and recorded in Figure 2 below. The results indicated that the shape of conidia was distinctively curved for one isolate (Western1) while others were fusiform-elliptical as shown in Figure 2. Variation on conidial size in length and width and the number of septa on 100 conidia samples from six isolates of *E. turcicum* were studied. The results indicated that the largest conidia which were for isolate Western 1 measured 103µm in length and 23.86 µm in width. The average number of septa was 4.5septa (range of 3-6 septa). These findings agree with those of Sivanesan (1987), Harlapur *et al.* (2007) and Rajeshwar *et al*. (2014) who reported variation on conidia and septa size of *E. turcicum*. In addition, a study by Narong (2006) reported that the shapes of the conidia were bended, elongated and spindle. The study found the average conidial length to be 93.97µm and width 13.11 µm, while the number of septa reported ranged between 2 and 7.

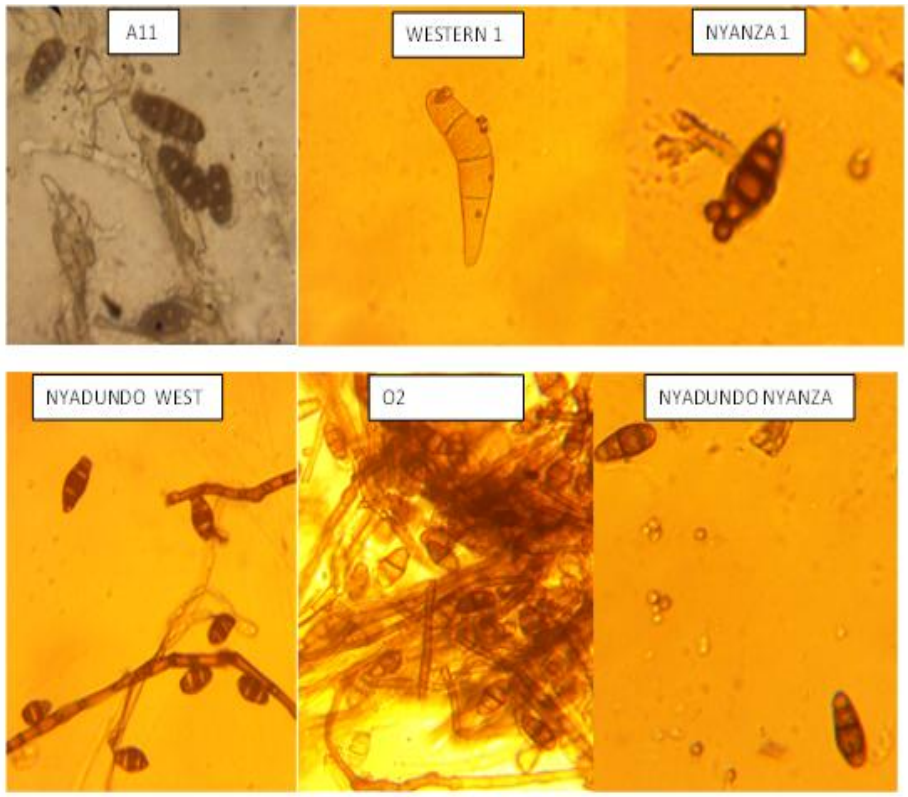

Figure 2: The conidia of the isolates of *Exserohilum turcicum* (400 X)

Morphological diversities and similarities of *E. turcicum* based on the two locations of study (Sega and Kibos)

Exserohilum pathotypes obtained from both Sega and Kibos shared quite a number of features except for Western 1 isolate which was obtained from a farmer's field in Sega as shown in Table 2. This pathotype was very unique in all aspects. Isolates such as Nyadundo Nyanza and Nyadundo Western, and O_2 were isolated from both Sega and Kibos and had very similar cultural and morphological characteristics in both the locations. These findings differ with those of several studies that have been done previously. From the study by Muiru *et al.,* (2008) it is reported that *E. turcicum* from diverse agro-ecological zones showed variation in morphology, pigmentation, growth rate and sporulation rate. Furthermore, Harlapur and Kulakarni (2009) and Gowda *et al.* (2010) reported cultural and morphological variation of the 13 isolates of Turcicum leaf blight (TLB) caused by *E. turcicum*. The key difference between the present results and the previously reported ones could be attributed to the fact that the previous studies were carried out under varying conditions (pH temperature and growth medium), while these conditions were kept constant in the present study.

Table 2: Morphological characteristics of the *Exserohilum turcicum* isolates from Kibos and Sega

Isolate	Conidial Shape	Septa	Width (µm)	Length (µm)	Conidial area (µm)
All	Fusiform-elliptical	5.0	22.48	101.02	2270.93
Nyadundo West.	Fusiform-elliptical	4.7	20.94	99.0	2072.57
Nyanza 2	Fusiform-elliptical	5.2	19.74	99.8	1969.55
West. 1	Fusiform-ellipsoidal	4.8	23.83	103.41	2466.85
O2	Fusiform-elliptical	3.9	20.83	89.20	1857.59
Nyadundo Nyanza	Fusiform-elliptical	3.3	20.36	101.83	2073.16

CONCLUSION AND RECOMMENDATION(S)

The characterization of *E. turcicum* pathogen responsible for causing Turcicum Leaf Blight (TLB) of sorghum was studied. The pathogen was identified as *E. turcicum* using both cultural and morphological and molecular methods. Potato dextrose agar was found to be suitable nutrient media, for supporting the growth of the pathogen. *E. turcicum* isolates were grouped into gray, dark gray and golden brown based on colony color and profuse, moderate and fair basing on sporulation. There was variation in conidia shape. This study will aid in understanding more about the variability of the pathogen, and how to come up with management strategies which can mitigate the incidence of the disease so as to minimize loss of sorghum yield.

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REFERENCES

Abebe, D., & Singburaudom, N. (2006). Morphological, cultural and pathogenicity variation of *Exserohilum turcicum* (pass) Leonard and Suggs isolates in maize (zea mays l.). *Kasetsart Journal (Nat. Sci.)*, *40*, 341-352.

Aden, M. H. (1991). *Studies of sorghum leaf blight incited by Excerohilum turcicum (Pass) Leo. & Suggs* (Doctoral dissertation, Andhra Pradesh Agricultural University).

Chandrashekara, C., Jha, S. K., Arunkumar, R., & Agrawal, P. K. (2014). Identification of new sources of resistance to turcicum leaf blight and maydis leaf blight in maize (Zea Mays L.). *SABRAO J. Breed. Genet*, *46*(1), 44-55.

Food Agriculture Organization (2009). *The state of food insecurity in the world*.

Harlapur, S. I., Kulkarni, M. S., Hegde, Y., & Kulkarni, S. (2007). Variability in Exserohilum turcicum (Pass.) Leonard and Suggs., causal agent of turcicum leaf blight of maize. *Karnataka Journal of Agricultural Sciences*, *20*(3), 665-666.

Harlapur, S. I., Kulkarni, M. S., Kulkarni, S., & Patil, B. C. (2009). Assessment of crop loss due to turcicum leaf blight caused by Exserohilum turcicum (Pass.) Leonard and Suggs in maize. *Indian Phytopathology*, *62*(2), 144-154.

IPCC, A. (2007). Intergovernmental panel on climate change. *Climate change 2007: Synthesis report*.

Manamgoda, D. S., Cai, L., McKenzie, E. H., Crous, P. W., Madrid, H., Chukeatirote, E., ... & Hyde, K. D. (2012). A phylogenetic and taxonomic re-evaluation of the Bipolaris-Cochliobolus-Curvularia complex. *Fungal Diversity*, *56*(1), 131-144.

Muiru, W. M., Mutitu, E. W., Kimenju, J. W., Koopmann, B., & Tiedemann, A. V. (2008). Infectious structures and response of maize plants to invasion by Exserohilum turcicum (Pass). in compatible and incompatible host pathogen systems. *Journal of applied biosciences*, *10*(2), 532-537.

Muui, C. W., Muasya, R. M., & Kirubi, D. T. (2013). Baseline survey on factors affecting sorghum production and use in eastern Kenya. *African journal of food, agriculture, nutrition and development*, *13*(1), 7339-7353.

Ngugi, H. K., Julian, A. M., King, S. B., & Peacocke, B. J. (2000). Epidemiology of sorghum anthracnose (Colletotrichum sublineolum) and leaf blight (Exserohilum turcicum) in Kenya. *Plant Pathology*, *49*(1), 129-140.

Ogeto, R. M., Cheruiyot, E., Mshenga, P., & Onyari, C. N. (2013). Sorghum production for food security: A socio-economic analysis of sorghum production in Nakuru County, Kenya. *African Journal of Agricultural Research*, *8*(47), 6055-6067.

Ogeto, R. M., Cheruiyot, E., Mshenga, P., & Onyari, C. N. (2013). Sorghum production for food security: A socio-economic analysis of sorghum production in Nakuru County, Kenya. *African Journal of Agricultural Research*, *8*(47), 6055-6067.

RAMATHANI, I. (2010). *Characterisation of Turcicum leaf blight epidemics and pathogen populations in the Exserohilum turcicum–sorghum pathosystem of Uganda* (Doctoral dissertation, MAKERERE UNIVERSITY).

Reddy, T. R., Reddy, P. N., Reddy, R. R., & Reddy, S. S. (2014). Cultural and morphological variability among Exserohilum turcicum isolates causing turcicum leaf blight of maize. Environment and Ecology, 32(1), 16-21.

Sivanesan, A. (1987). *Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs*. CAB International.

Takuji, S., & Baltazar, A. A. (2009). Sorghum genome: drought tolerance in genes. *Nature*, *457*, 547-548.

Were J. O. (2012) Diversity and Virulence of Celletotrichum species infecting *Sorghum bicolor* in the lake basin regions of Kenya (MSc. Thesis) Univeristy of Eldoret.