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Prevalence and severity of sorghum leaf blight in the sorghum growing areas of Central Sudan

Mayada Mamoun Beshir[1,2*], Nafisa Elmahi Ahmed[3], Abdelbagi Mukhtar Ali[2,4], Ibrahim Hassabelrasoul Babiker[5], Patrick Rubaihayo[1], Patrick Okori[1]

[1]Department of Agricultural Production, Makerere University (MAK), P. O. Box 7062, Kampala, Uganda.
[2]Biotechnology and Biosafety Research Centre, Agricultural Research Corporation (ARC), P. O. Box 30, Shambat, Sudan.
[3]Crop Protection Centre, Agricultural Research Corporation (ARC), P. O. Box 126, Wad Medani, Sudan.
[4]Plant Breeding and Genetic Laboratory, FAO/IAEA Joint Division of NA, International Atomic Energy Agency (IAEA) laboratories, Seibersdorf, Vienna.
[5]Crop Protection Centre, Plant Pathology Laboratory, Agricultural Research Corporation (ARC), P. O. Box 30, Shambat, Sudan.

*Corresponding Author E-mail: mayadabeshir2015@gmail.com, Tel. +256788705026, +249114470859

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***Turcicum* leaf blight, though a common sorghum (*Sorghum bicolor* L.) disease in Sudan, there is little documented information on its occurrence and distribution in the country. Accordingly, a survey was commenced in 2014 with the objectives to quantify the intensity of *Turcicum* leaf blight in central Sudan, a major sorghum growing area in the country. The results indicated presence of leaf blight in all locations (100%) with incidence 65-100% and severity ranging between 45 - 85% in the 45 fields inspected. The highest disease incidence (100%) was observed in Sennar, Gedarif and Central and South Gezira districts while the lowest was observed in Khartoum district. Disease severity did not follow the same trend and the lowest (45%) was recorded in Shambat (Khartoum district), North Gezira and Sennar. The highest severity was observed in Elrahad (Gedarif district) (100%). All 11 sorghum varieties preferred and grown by farmers in central Sudan were affected by *Exserohilum Turcicum* in all sorghum cultivating districts. These results suggest that leaf blight was important in the study area, and probably in other sorghum growing regions of Sudan.**

Keywords: *Exserohilum turcicum*, sorghum, incidence, severity, Central Sudan.

INTRODUCTION

At least one third of the total cropped area in Sudan is annually placed under sorghum (*Sorghum bicolor* L. Moench), producing about 65% of food grains in the country (Elbashir and Ali, 2014) and making sorghum the number one crop in terms of area and yield (FAOSTAT, 2015). Most of the crop is consumed locally as it is the main cereal food grain for the people; a significant portion is used as fodder, and the remaining is utilised in industry for glucose and starch production (Abdalla and Nour, 2001). All surplus sorghum is exported, especially to Saudi Arabia (Ahmed 2011).

The Gezira scheme having an area of 0.9 million ha, making it the largest gravity irrigated farm under a single management in the world, contributes about 12% of the total sorghum production in Sudan (Zayed et al., 2015). Gedarif district (eastern Sudan) is the most important region for rainfed sorghum production with the principal varieties being Korakolu, Arfa Gadamak, Gadam Elhamam and Wad Ahmed which are relatively high in protein content and hence preferred by exporters (Sulieman and Elagib, 2012).

Although successful and continuous production of sorghum is the key of food security in Sudan, gap between attainable and concrete yields is quite large. This is constrained by biotic and abiotic stress which hinders production. Key bottleneck among the biotic stress is *Turcicum* leaf blight (TLB) caused by the *Ascomycete setosphaeria turcica* (Luttrell) (anamorph: *Exserohlium Turcicum* (Pass) (Leonard and Suggs, 1974).

Occurrence of *E. turcicum* foliar pathogen on sorghum has been very common in Kenya (Ngugi et al., 2001) and Uganda (Ramathani et al., 2011) for a long time however, in Sudan it was first reported in 1970s (ARC report 2013).

Alternative hosts and volunteer crops also provide sanctuary for the pathogens. In case of leaf blight which attacks maize, quick inoculum build up is not uncommon as both crops grow in the same ecologies at the same time and same season (Ngugi et al., 2000).

Under concomitant infection, TLB causes blighting of especially leaf tissues which may lead to coalescence of large patches of the leaf blade reducing the functional leaf area for photosynthesis (Rajeshwar et al., 2014) and ultimately resulting in 70% grain yield losses (Mittal and Boora 2005). Hitherto, no report was available on presence of *E. turcicum* in Sudan and its extent damage to sorghum production.

Ngugi et al. (2000) and Ramathani et al. (2011) found highly resistant genotypes among the five sorghum races (*kafir*, *guinea*, *caudatum*, *bicolor* and *durra*) in East Africa. Therefore, high resistant genotypes are expected to exist in Sudan because it is believed to be one of the centers of diversity for sorghum race bicolor (Kimber, 2000). In East Africa, there are two mating type genes of *E. turcicum* namely MAT1-1 and MAT1-2 which were identified as race 0, 1, 2 and 3 assessed on fungal isolates deriving from both sorghum and maize (Ramathani et al., 2011).

Under severe epidemics, TLB causes significant grain yield losses as high as 70%, through reduced kernel weight (Rajeshwar et al., 2014). It is controlled by use of disease free seeds or seeds treated with chemicals and hot water, following a two to three year crop rotation. Possible, application of fungicides and growing resistant varieties also getting rid of this pathogen (Ramathani et al., 2011). The distribution of TLB and pathotypes of *E. turcicum* have been identified in Uganda (Sserumaga et al., 2013) and Kenya (Ngugi et al., 2000) but work on this aspect was limited in Sudan. Therefore, a survey was undertaken in the major sorghum growing areas in central Sudan to examine the *E. turcicum* pathosystem in terms of disease incidence and severity on sorghum.

MATERIALS AND METHODS

Study area

The study was conducted in four districts namely Khartoum, Sennar, Gedarif and Gezira where sorghum is produced under both irrigation and rainfed systems. These four districts are the main areas for sorghum production in central Sudan (Ahmed, 2011). Disease incidence and severity were evaluated in 45 fields distributed in the nine sorghum growing areas within the four districts. These areas included Elrahad, Doka and Gedarif in Gedarif district, Abu Naama in Sennar district, Wad Elhadad, Wad Elturabi and Wad Medani in Gezira district and Elfaki Hashim and Shambat in Khartoum district (Figure 1).

Field observations and material collection

A hierarchical surveillance structure was used to determine *Turcicum* leaf blight intensity (incidence and severity) in 45 fields, about 10 - 20 days after flowering (Ramathani et al., 2011) during the rainy season of 2014. The surveillance structure consisted of two hierarchical levels; districts and location within district zones. From each location, at least 5 field each averaging one hectare in size was assessed at every 20 km along the main road.

The cultivated varieties assessed included improved varieties released for their high yields (Hageen Dura1, Arfa Gadamak, Tabat and Wad Ahmed) drought tolerant varieties (Bashair, Butane and Yarwasha), and Kurulolu and Gadam Elhamam which are local varieties preferred by farmers. Leaf samples were collected to confirm pathogen identity in the laboratory.

Fungal isolation, culture and DNA isolation

Diseased leaves were collected and used for single spore isolation following sporulation of *E. turcicum* from leaf lesions under aseptic conditions as described by Carson (1995). The pure cultures were subsequently grown on potato dextrose agar (Difco), and the mycelia harvested by scrapping off the plate and directly used in DNA extraction (Ramathani et al., 2011).

Exserohilum Turcicum species-specificity

The *Exserohilum Turcicum* isolates were screened by PCR using the sequence information from the internal transcribed spacer ribosomal DNA (ITS rDNA) of the 5.8S ribosomal RNA gene (GenBank accession number AF163067). The following primers were designed, forward: 5′ - GCAACAGTGCTCTGCTGAAA-3′, reverse: 5′- ATAAGACGGCCAACACCAAG-3′, following Ramathani et al. (2011) method generating a 344 bp fragment. PCR was carried out using 10 ng of template DNA, which was added to a 24 µl mix consisting of sterile H_2O, 2.5 mM $MgCl_2$, 2.5 µl Taq buffer, 0.2 mM of each dNTP, 0.25 µM of forward and reverse primers and 1 U of Taq polymerase. The PCR conditions used were 95°C for 4 min, 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final extension was set at 72°C for 10 min (Ramathani et al., 2011). The PCR products were separated on 1% agarose gels to confirm fragment size and consequently the identity of the isolate.

Data collection and analysis

In each field, disease incidence was assessed for cultivar as the proportion of plants showing symptoms in the field

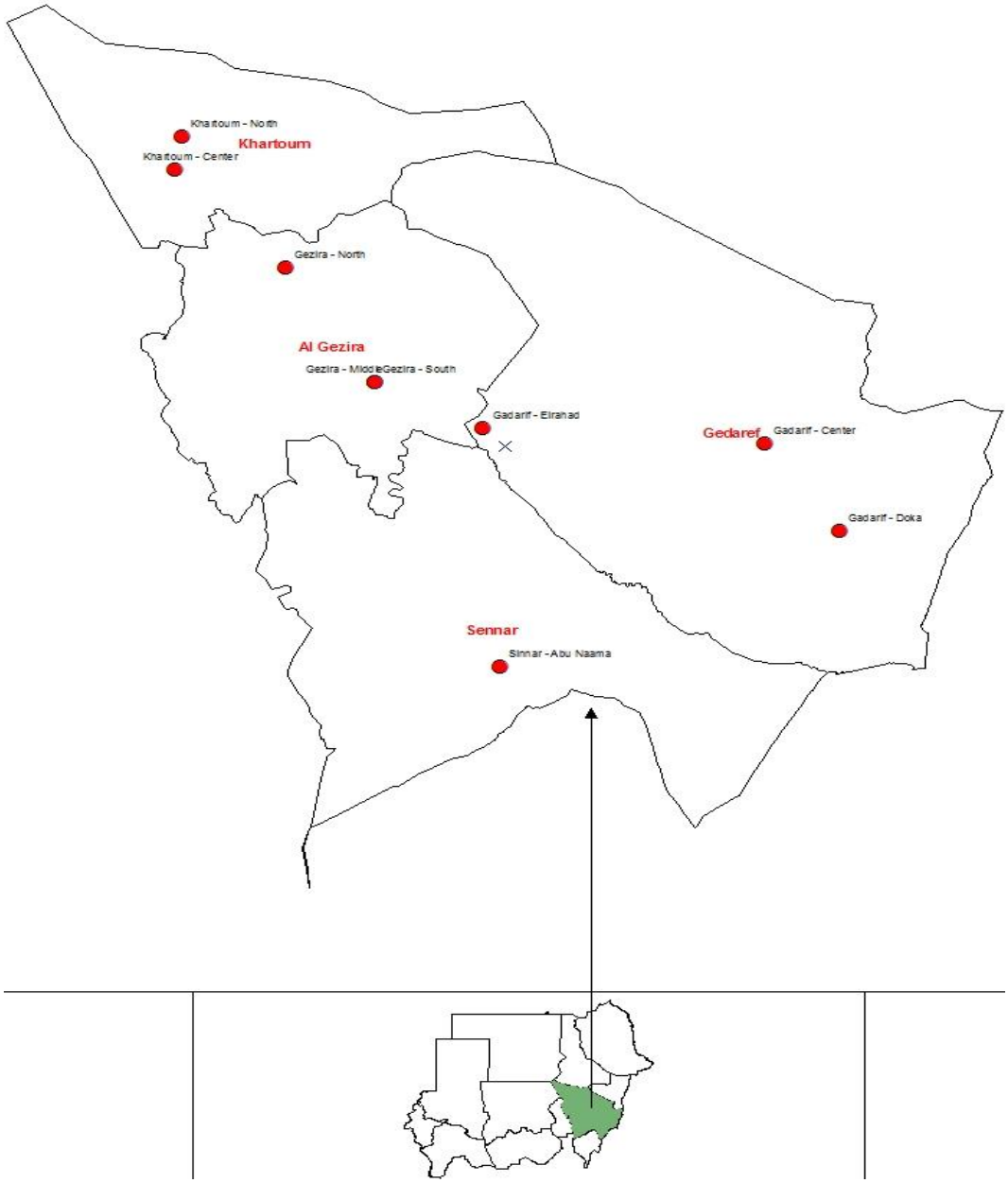

Figure 1. The four main districts showing major sorghum growing agro-ecologies in central Sudan and the nine sampled locations.

(Ramathani et al., 2011) at 10 - 20 days after flowering as TLB symptoms appear before flowering stage (Ngugi et al. 2000). Twenty plants in the middle of each field were randomly selected and the number of plants having *E. turcicum* symptoms were counted on whole plant basis and expressed as a percentage of the plant population (Ramathani et al., 2011). Disease severity was rated using a scale of 0, 3, 5, 10, 25, 50 and >75% leaf area affected (Adipala et al., 1993). Data were recorded on several components, including lesion colour. Means for the different parameters were determined from data collected from the experimental plots. All data were subjected to correlation and analysis of variance (Steel and Torrie, 1997). Data analyses were performed using GenStat 12th Edition (VSN International Ltd., UK).

RESULTS

Confirmation of occurrence of *E. turcuicum*

All diseased leaf samples showed positive reaction for

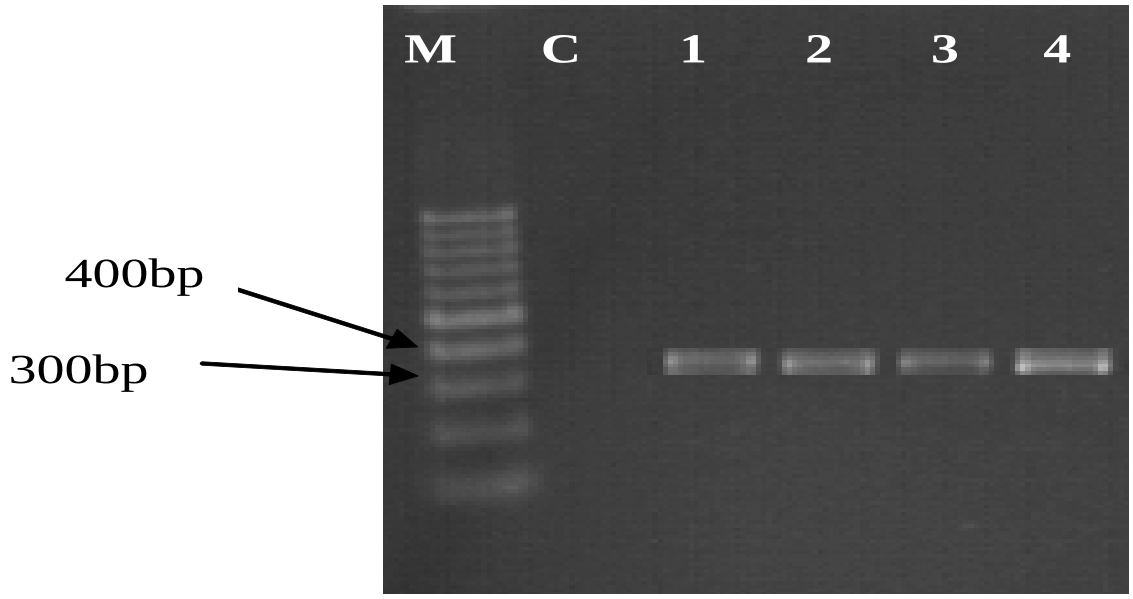

Figure 2. Example of the DNA and PCR product. PCR amplicons of *Exserohilum Turcicum* derived by amplification using rDNA ITS species specific primers: Lane descriptions 1 – Wad Medani, 2 – Shambat, 3 – Gadarif, 4 – Abu Naama, C –Control and M – 100bp DNA ladder.

Table 1. Nested analysis of variance for incidence and severity of *Turcicum* leaf blight on sorghum across districts.

Source	df	Severity			Incidence		
		SS	MS	F [a]	SS	MS	F [a]
District	3	3214.0	1071.3	2.13 [ns]	2187.5	729.2	1.59 [ns]
Location	9	6603.2	733.7	1.46 [ns]	3950.3	438.9	0.96 [ns]
Error	24	12073.5	503.1		11007.4	458.6	
Total	34	18676.7	549.3		15188.6	446.7	

[a] Statistical significant differences = P≤0.05; DF degrees of freedom; SS sum of squares; MS Mean square

species-specific using the internal transcribed spacer ribosomal DNA (ITS rDNA) PCR scoring indicating the presence of *E. turcuicum* across all locations studied (Figure 2). Positive isolates gave a bright band at 344 base pairs. The results of analysis of variances for incidence and severity of TLB in the four districts in central Sudan are presented in Table 1. The analysis of variance revealed non-significant influence of districts on disease incidence and severity. However non-significance was detected, nested ANOVA from districts and locations within districts confirmed the equal occurrence of TLB in central Sudan where sorghum is mainly grown. Mean of disease incidence and severity of TLB in major sorghum growing areas in central Sudan are presented in Table 2. The result of the survey revealed that TLB was prevalent in all districts but with varying intensity on the different varieties.

At the first level of hierarchy, the districts, disease incidence varied from 65% to 100% with the highest mean incidence in Gezira, Gedarif and Sennar (100%). At the second level of hierarchy, the locations, mean disease incidence was highest in Wad Medani, Wad Elhadad, Abu Naama, Doka, Gedarif and Elrahad (100%) and lower in Shambat and Elfaki Hashim (65%) but the lowest (45%) disease severity was recorded in Shambat (Khartoum district). In Gezira, a very high TLB disease incidence and severity were found in the sorghum variety Tabat.

Reaction of sorghum varieties to *E. turcicum*

Analysis of variances for incidence and severity of TLB among farmer's preferred sorghum varieties is presented in Table 3. There was significant variation (P=0.00003) in disease severity while there was no significance in incidence indicating that reaction to TLB varied among what farmers prefer to plant every season. The reaction

Table 2. Mean of disease incidence and severity of *Turcicum* leaf blight in major sorghum growing districts in central Sudan.

Locations	District	Letter	X	Y	Incidence [a]	Severity [a]
Abu Naama	Sennar	P	620185	1404190	100.0	53.8
Doka	Gedarif	P	798645	1494856	100.0	61.3
Elrahad	Gedarif	P	610363	1560701	100.0	100.0
Gedarif	Gedarif	P	758439	1551675	100.0	65.0
Wad Medani	Gezira	P	553265	1590542	100.0	85.0
Wad Elhadad	Gezira	P	553017	1590178	100.0	65.0
Wad Elturabi	Gezira	P	506540	1665986	75.4	51.8
Elfaki Hashim	Khartoum	P	452481	1751375	65.0	65.0
Shambat	Khartoum	P	448233	1729931	65.0	45.0
LSD (P≤0.05)					21.0	22.0
CV%					24.1	41.9

[a] Disease incidence and severity were computed as proportion of plants showing symptoms and percentage leaf area damaged, respectively

Table 3. Analysis of variance for incidence and severity of *Turcicum* leaf blight on sorghum across districts.

Source	df	Incidence			Severity		
		SS	MS	F	SS	MS	F
Variety	14	11075.2	791.1	3.9^{ns}	11420.0	815.7	2.4***
Residual	22	4449.2	202.2		7418.8	337.2	
Total	36	18838.8			15524.3		

[a] Statistical significant differences = P≤0.05 DF degrees of freedom; SS sum of squares; MS Mean square

of farmer's preferred varieties to TLB are presented in Table 4. Hageen Durra 1 (HD1) showed the lowest severity (20%) and incidence (40%) while Yarwasha showed the highest incidence (100%) and severity (85%). Tabat, Wad Ahmed and Abu 70 were cultivated on a relatively large scale in Gezira and Sennar districts while Gadam Elhamam and Wad Ahmed were cultivated mostly in Gedarif district, and the fodder sorghum Abu 70 in Khartoum district. Overall, location effect was non-significant for disease incidence and severity.

DISCUSSION

The results of the laboratory analysis indicated that *E. turcuicum* was the causative agent of leaf blight observed in the study area of central Sudan. Furthermore, the TLB occurred in all the study locations with incidence and severity ranging from 45 to 100, and 65 to 100, respectively. Although location differences were non-significant, varietals differences were highly significant (P=0.00003). In spite of the fact that TLB was widely distributed in the studied area, farmer's knowledge about the disease was limited. In Khartoum district where sorghum is produced mainly as fodder using irrigation, TLB incidence and severity were lowest among the districts. The low incidence and severity levels of the disease in Khartoum district were attributed to high temperature and low humidity levels (Mahgoub, 2014) which are characteristic of Khartoum, unlike other districts.

In Gezira, Gedarif and Sennar, sorghum is normally largely produced for food grain under irrigation and rainfed conditions. These areas are characterized by lower temperature and higher humidity levels during the growing season of sorghum (Mahgoub, 2014), both conditions are conducive to the disease. This in turn explains the high observed levels in incidence and severity of the disease in these districts. Similar results were reported by Ngugi et al. (2000), Mohan et al. (2010) and Ramathani et al. (2011).

Currently in Sudan, about 90% of the total sorghum area is located in the rainfed belt which is characterized with low temperature and high humidity, both favourable to spread of the disease. This perhaps is a factor contributing to low rainfed belt yield levels to only 66% of the total sorghum production since about 90% of total sorghum area in Sudan is located in rainfed belt. Additionally, this study showed that the TLB disease, a yield depressant, was found to be more severe in Gezira district, which is the main irrigated sorghum producing area.

Additionally, the farmer's preferred sorghum varieties exhibited high TLB incidences and severities which rather explain why productivity is low in the country. Although the farmer's preferred varieties showed significantly high

Table 4. Reaction farmer's preferred varieties of sorghum to *Exserohilum Turcicum* across districts.

Variety	Incidence [a]	Severity [a]
Hageen Durra 1 (HD1)	40.0	20.0
Abu 70	76.2	61.0
Wad Ahmed	90.0	35.8
Arfa Gadamak	100.0	60.8
Bashair	100.0	45.0
Butana	100.0	35.0
Gadam Elhamam	100.0	45.0
Korakolu	100.0	45.0
Tabat	100.0	62.5
Wafir	100.0	55.0
Yarwasha	100.0	85.0
LSD (P≤0.05)	22.0	17.0
CV	20.1	25.9

[a]Disease incidence and severity were computed as proportion of plants showing symptoms and percentage leaf area damaged, respectively.

TLB incidences and severities, farmers continued to grow the varieties regardless of risks of high incidence and severity, which lead to reduced yield and income fluctuations from one year to another.

Further work is still needed to gather important information on the detection of changes in the *E. turcicum*population and mating type distribution in order to eliminate this serious threat to sorghum production in Sudan as leading producer in the world.

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