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Screening of Selected Sorghum Genotypes for Resistance to Covered Kernel Smut Disease in Western Kenya

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Abstract

Sorghum is an important food security crop for arid and semi-arid tropics but its production is hampered by many biotic and abiotic factors including covered kernel smut disease (CKSD) caused by fungus *Sporosorium sorghi* in the Ustilaginaceae family. The disease attacks susceptible sorghum genotypes causing yield losses estimated at 43% in Western Kenya. This study determined the response of selected sorghum genotypes to CKSD under field and greenhouse conditions. A total of 15 elite sorghum genotypes were screened under field conditions in Migori and Homa Bay sites and under greenhouse at the University of Eldoret. Data on disease incidence and severity were collected per genotype and analyzed using R-Studio software and means were separated at 1% using Tukey's test. Results showed significant differences among genotypes for disease incidence and severity under fields and greenhouse conditions. Disease incidence varied significantly ($p < 0.001$) among the genotypes ranging from zero (for T53, T30, IS3092, N4 and N68) to 64% (for Nyadundo2) under field conditions but ranged from 0-69% under greenhouse conditions. Similarly, severity followed the same trend with C26 having the worst attack with a score of 5 while T53 recorded the least (score of 1). This study has identified potential sources of resistance for covered kernel smut disease that can be utilized to manage the disease and significantly improve sorghum yields in the target regions.

Keywords: sorghum, covered kernel smut, incidence, severity

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is ranked fifth in importance among cereals in the world and is a major food crop for developing countries (FAO, 2012). It is particularly important in areas with high temperatures and low rainfall due to its resilience (Hayden, 2002). The sorghum grains can be used for syrup production, making of leavened and unleavened bread, bio-energy, bio-ethanol production and preparation of alcoholic beverages (Tonapi et al., 2020). In Sub-Saharan Africa, Middle East, North Africa and India sorghum is mainly used as human food while in Europe, Australia, China, and Western Hemisphere countries it is used as animal feed, forage, and for industrial purposes including ethanol production. Sorghum production is mainly concentrated in Asia, Sub-Saharan Africa and the Americas and Caribbean (FAO, 2012).

Its global consumption is estimated to be 61.0 million metric tons per year (USDA, 2019). However, grain yield in most parts of the world is relatively low, estimated at 0.925 t/ha compared to 5 t/ha reported from experimental stations (ICRISAT, 2004). The low yield is attributed to a number of factors including biotic, abiotic and socio-economic factors (Esele, 2013). The most important diseases and pests of sorghum include shoot fly, stem borer, shoot bug, aphids, sorghum midge, head bug and covered kernel smut disease. Collectively, these constraints limit sorghum production and hamper its productivity across regions of the world (Tonapi et al., 2020).

Covered kernel smut disease (CKSD) caused by *Sporisorium sorghi* in the Ustilaginaceae family *is* a major constraint in sorghum production (Mtisi & McLaren, 2008). The fungus is seed-borne and develops systemically as the sorghum crop grows. In Kenya, its incidence is exacerbated by the informal sorghum seeds system whereby small-scale farmers continue to share and exchange retained own untreated seeds for planting the next season's crop among the communities (Gwary et al., 2007). According to Howard et al. (2005) maturing fruiting bodies of the fungus called sori rupture and release teliospores that infects seeds on the same or other sorghum plants. The teliospores of the fungus replace the grain in the panicle causing direct crop losses in grains.

According to Sisay et al. (2012) the fungus *Sporisorium sorghi* in the Ustilaginaceae family can grow and develop at 10-32 ºC, but the optimum soil temperature conducive for the disease development is 18-25 ºC. The infection is established in warmer and wet soils with humidity of between 15-20%. More importantly, periods of delayed seed germination and emergence are optimal for the infection (Ashok et al., 2011) which enhances its incidence. In the year 2012, Gautam et al., recorded more than 50% disease incidence in Ethiopia. In general disease incidence varies from place to place. Annual yield losses due to CKSD in Africa reaches 10% with localized losses of 60% or more (Sisay et al., 2012). In Kenya, CKSD also causes significant yield losses ranging from 42-48% (Okongo et al., 2019). However, in Migori and Homa Bay Counties, little is known about its incidence, severity and distribution. Some new improved sorghum varieties that were introduced in the area by the Rongo University Sorghum Improvement Team in 2017 were infected by the disease (http://www.ccrp.org) raising the issue of its management.

To minimize yield losses due to CKSD, several methods can be used such as chemicals, cultural, biological and through breeding for tolerant crop varieties. Chemical method includes the use of fungicides such as Captan and Carboxin+Thiram (Vitavax) which assist in reducing the incidence and severity of the disease on sorghum but does not completely control the disease (Jere, 2004). Moreover, most of these fungicides are extremely expensive and unaffordable to the smallholder farmers.

Several cultural methods are available for controlling the disease including soaking of seeds in water for four hours followed by drying of seeds under shade, collection of smutted ear heads and incinerating them (IPM, 2008). According to Adane and Gatam (2000), CKSD can also be controlled by use of fermented cattle urine and botanicals from Abeyi (orm) *Maesa lanceolata*. However, the two methods are not widely used and their efficiency in different regions need to be established. Moreover, Abeyi plant is not readily available for farmers in Western Kenya (Okongo et al., 2019).Therefore the CKSD remains a major threat to food security in western region despite the chemical, biological and cultural methods currently in use owing to their labour intensive nature and or cost.

The use of resistant genotypes is one of the most viable strategies for the control of covered kernel smut disease (Kutama et al., 2013). This is because orphan crops like sorghum has a low return to investment and therefore, the introduction of resistant varieties remains the most cost-effective and sustainable option to control covered kernel smut disease (Wilson, 2011). In Kenya, there is lack of smut resistant genotypes, creating a need to identify stable sources of resistance through screening which could be utilized directly or used in breeding programs to develop other resistant varieties. Therefore, this study seeks to improve production of sorghum through screening and selecting resistant genotypes to be used for management of covered kernel smut disease.

2. Materials and Methods

2.1 Plant Materials

A total of 15 plant materials were used which included the newly released sorghum varieties by Rongo University Sorghum Breeding Program, commercial variety and farmers' cultivars (Table 1) collected through field survey by Okongo et al. (2019).

Table 1. Sorghum genotypes used in the study

Plant Material	Source	Colour
NYADUNDO 1	Rongo University	Red
NYADUNDO 2	Rongo University	Red
C26	Rongo University	Cream
MUK27	Makerere University	Brown
MUK60	Rongo University	Red
T53B	Rongo University	Brown
N68	Rongo University	Brown
T30B	Rongo University	Brown
E117B	Rongo University	Brown
MUK154	Makerere University	Red
IS3092	Kalro Katumani	Brown
N4	Rongo University	Red
JOWI	Farmer	Red
OCHUTI	Farmer	Red
SEREDO	Kenya Seed Company	Brown

2.2 Description of Experimental Sites

The study was conducted in two counties, Homa Bay and Migori. The first site is located at 0°42′S and 34°50′E, 1221 m above sea level, has an average annual temperature of 21.2 °C with a humidity of between 20-28% and annual precipitation of 1369 mm per year with Vertisol soil type.

Migori site is located at 1°07′S and 34°42′E. It has an elevation of 1281 m above sea level with daily temperature ranging between 26-34 °C with humidity of between 18-20% and average annual rainfall estimated at 1100 mm with granite type of soil.

The greenhouse screening was done at the University of Eldoret located at 0.52°N and 35.27°E which has an elevation of 2090 m above sea level, average temperature of 15.8 °C and average rainfall of 1263 mm.

2.3 Experimental Design and Procedures

2.3.1 Preparation of Sporidial Inoculum Suspension

The CKSD inoculum was prepared by collecting 5 grams of dry teliospores from mature panicle smut infected sorghum genotypes from on-farm trials by shaking them out of the heads and sieving to remove the debris, The teliospores were then washed in 70% ethanol to sterilize then suspended on 250 ml sterile water and plated on Potato Dextrose Agar (PDA) and incubated in the dark at 28 °C for 3 days. The sporidial colonies were then transferred in flask containing 100 ml potato Dextrose Broth (PDB) and incubated on a rotary shaker for 4 days. The suspension was then filtered using a cheese cloth, which was then used to inoculate the seedlings with a hypodermic syringe according to procedures described by Frederiksen (2000).

2.3.2 Germination of Sorghum Seeds in Pots

Ten seeds of each of the fifteen sorghum genotypes (Table 1) were planted and grown in pots arranged in a Completely Randomized Design (CRD) replicated three times in the greenhouse, each pot was filled with 1.5 kg forest soil + 0.15 g teliospores and mixed with a handful of organic matter. The seedlings were then thinned when they were I month old to three seedlings per pot.

2.3.4 Inoculation of Seedlings

The inoculum suspension was then used to inoculate the seedlings with the help of a hypodermic syringe when they were 10 cm in height (4 weeks old). An inoculum suspension was injected into each seedling continuously until drops of the inoculum were seen at the top of the leaf.

For field screening, fifteen genotypes (described in Table 1) were planted in CKSD hotspots in Migori and Homa Bay sites. The experiments were set up in a Randomized Complete Block Design (RCBD) with three replications. Each genotype was planted in a (2.25 × 4) m plot with 4 rows at a spacing of (75 × 20) cm. Standard agronomic practices were followed to raise a healthy crop.

2.4 Data Collection and Statistical Analysis

2.4.1 Covered Kernel Smut Disease Incidence

This was assessed on infected panicles by determining the proportion of sorghum plants showing the symptoms of the covered kernel smut disease compared to the total number of plants in the plot, and the incidence expressed as a percentage as described by Chaube and Punder (2005) using the formula:

$$\text{Disease incidence per variety} = \frac{\text{Total number of diseased plants in the plot}}{\text{Total number of plants in the plot}} \times 100 \quad (1)$$

2.4.2 Covered Kernel Smut Disease Severity

Covered kernel smut disease severity was scored on the infected plants using disease resistance classification scale described by Gwary et al. (2001) and Marley et al. (2002) on a scale of 1-5 where 1 is immune showing no disease symptoms on the panicle, 2 is resistant showing 1% panicle area infected, 3 is moderately susceptible showing 2-10% head area attacked, 4 is susceptible with 11-25% head area covered with smut and 5 more than 26% with severe head damage as follows:

Table 2. Disease resistance classification scale

Severity resistance rating	% Panicle area infected	Description
1	0	Immune
2	1	Resistant
3	2-10	Moderately susceptible
4	11-25	Susceptible
5	> 26	Very susceptible

Data collected on disease severity and incidence was transformed using square root transformation method and analyzed using R-Studio. Analysis of variance was done for the two sites and the greenhouse according to K. Gomez and A. Gomez (1984). Differences were accepted as significant at $p < 0.001$ and the means separated at 1% using Tukey`s range test.

3. Results

3.1 Disease Incidence Under Field Conditions

At Migori site, there were significant differences($p < 0.001$) on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes(Figure 1, Table 3) but replication and residuals had no effect on disease incidence, Four varieties namely N4, MUK24, N68 and IS3092 showed significant variation in disease incidence compared to the rest of the varieties. C26 had the highest mean disease incidence (60%) compared to N68 (3%) while the local checks (Ochuti and Jowi), Nyadundo1 and Nyadundo2 showed statistically similar disease incidence.

At Homa Bay site, there were significant differences ($p < 0.001$) on incidence of covered kernel smut disease amongst the fifteen sorghum genotypes (Figure 2, Table 3). Replication and residuals had no effect on disease incidence levels. Ochuti and Jowi, the local checks had the highest mean incidence of 56.7% while IS3092 had the lowest mean incidence of 3% (Figure 1b). Nyadundo2 and Nyadundo1 had a mean incidence of < 50% which compared well with the commercial checks, Seredo which showed a mean incidence of 43.3%.

Table 3. Mean square for covered kernel smut disease incidence for sorghum genotypes tested in Migori and Homabay sites

SOV	DF	Mean Squares	
		Migori	Homabay
REP	2	73.16	13.89
Genotype	14	1936.2***	1690.02***
Residual	28	50.56	47.65
CV		9.2	4.3
SED		5.8	5.64
LDS		11.89	11.55

Note. SOV: source of variation; DF: degree of freedom; SED: standard error deviation; LSD: least significance difference; CV: coefficient of variation.

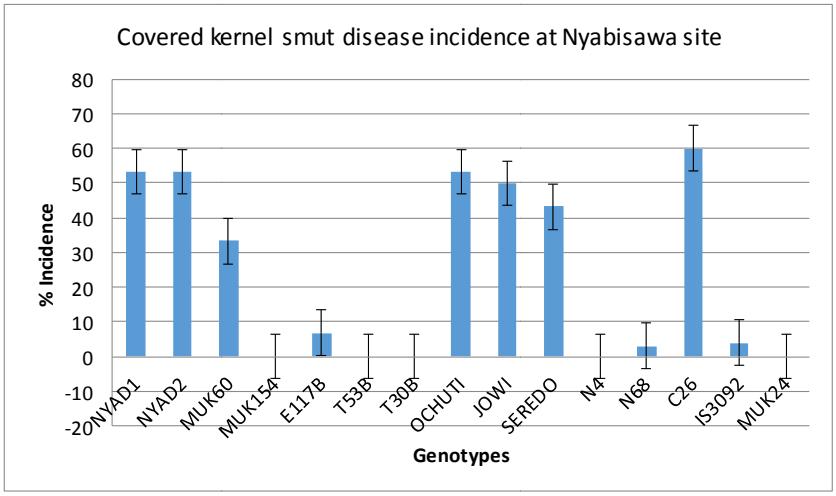

Figure 1. Covered kernel smut disease incidence at Migori site

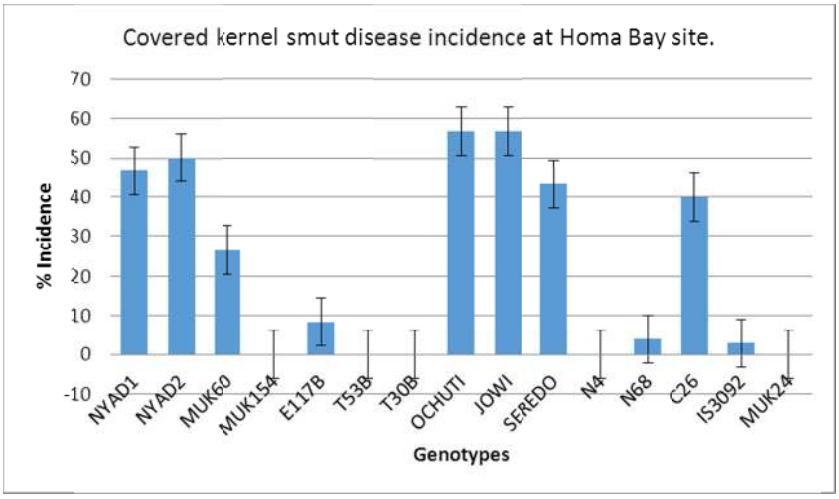

Figure 2. Covered kernel smut disease incidence at Homa Bay site

3.2 Disease Incidence Under Greenhouse Conditions

There were significant differences ($p > 0.001$) on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes screened (Figure 3, Table 4) C26 and Nyadundo 2 had the highest mean disease incidence (63.3%) which was statistically different from that of IS3092 and MUK154 (3%). Ochuti and Jowi the local checks showed statistically similar means of disease incidence level.

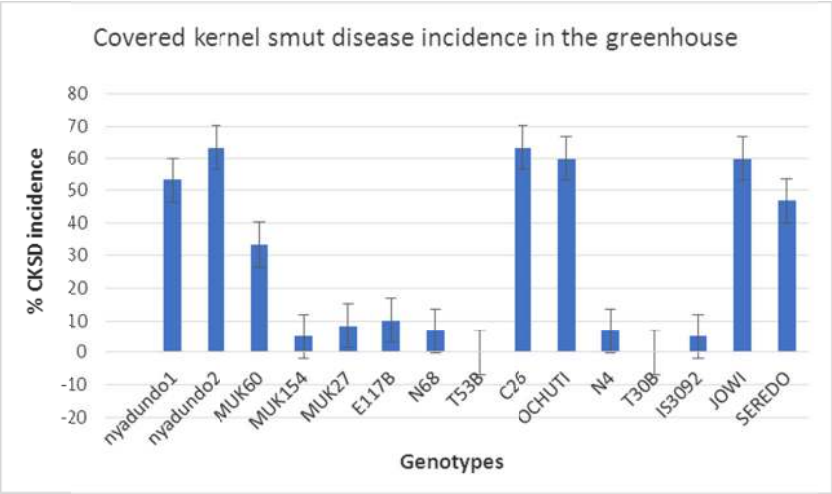

Figure 3. Covered kernel smut disease incidence in the greenhouse

Table 4. ANOVA table for covered kernel smut disease incidence in the greenhouse

SOV	DF	Sum of Squares	Mean Square	F Value
Genotype	14	29431.1	2102.2	64.14***
Residual	30	983.38	50.56	
Total	44	33414.4		
SED	**4.68**			
LSD	**9.55**			
CV	**7.3**			

Note. SOV: source of variation; DF: degree of freedom; SED: standard error deviation; LSD: least significance difference; CV: coefficient of variation.

3.3 Severity Score of Covered Kernel Smut Disease on Sorghum Genotypes Under Field Conditions

The differences on the severity of covered kernel smut disease were significant ($p > 0.001$) among the fifteen sorghum genotypes tested (Table 5). Five varieties MUK154, MUK27, T53B, N4 and T30B recorded the lowest disease damage rating of 1 and were considered immune to covered kernel smut disease (Figure 5).

Genotypes E117, N68 and IS3092 had a score of 2 and were regarded as very resistant. C26 on the other hand had the highest mean severity score of 5, and therefore was recorded as very susceptible. Nyadundo1, Jowi, Ochuti, Nyadundo2 and Seredo had a score of 4 and therefore were considered susceptible.

At HomaBay site,the differences on the severity of covered kernel smut disease were significant ($p < 0.001$) among the fifteen sorghum genotypes tested. MUK154, MUK27, T53B, N4 and T30B did not show significant variation in terms of disease severity and recorded the lowest score of 1. Therefore, these genotypes were considered immune to covered kernel smut disease. Another set of genotypes consisting of E117B, N68 and IS3092 had a disease severity score of 2 and hence were considered as very resistant. Amongst all the genotypes, the local checks, ochuti and jowi had the highest severity score of 4.7 and were classified as susceptible to the disease.

Table 5. Mean square table for sorghum genotypes tested for covered kernel smut disease severity in Migori and Homabay sites

SOV	DF	Meansquares	
		Migori	Homabay
REP	2	0.0889	0.0222
Genotype	14	7.327***	6.422***
Residual	28	0.1603	0.1651
CV		2.8	1.5
SED		0.32	0.332
LDS		0.66	0.679

Note. SOV: source of variation; DF: degree of freedom; SED: standard error deviation; LSD: least significance difference; CV: coefficient of variation.

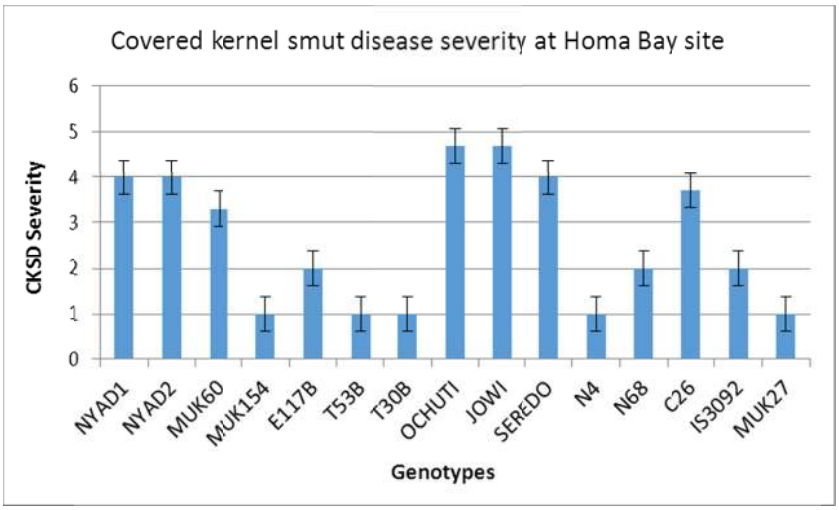

Figure 4. Covered kernel smut disease severity at Homa Bay site

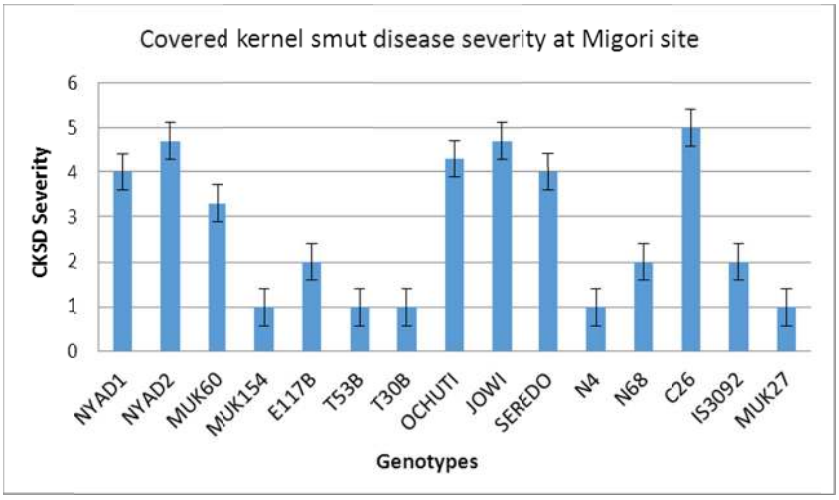

Figure 5. Covered kernel smut disease severity at Migori site

3.4 Severity Score of Covered Kernel Smut Disease on Sorghum Genotypes Under Green House Conditions

Under controlled conditions,the differences on the severity of covered kernel smut disease were significant ($p < 0.001$) among the fifteen sorghum genotypes tested (Table 5). MUK154, T53B, N4 and T30B were not

statistically different ($p < 0.001$) with regard to CKSD to the disease damage and obtained the lowest score of 1 (Figure 6) and were classified as immune to CKSD. Genotypes E117, N68 and IS3092 had a score of 2 and were classified as very resistant. Nyadundo1 and Seredo had a score of 4 and were considered as susceptible while C26, Ochuti, Jowi and Nyadundo2 on the other hand had the highest mean severity score of 5 and were considered as very susceptible.

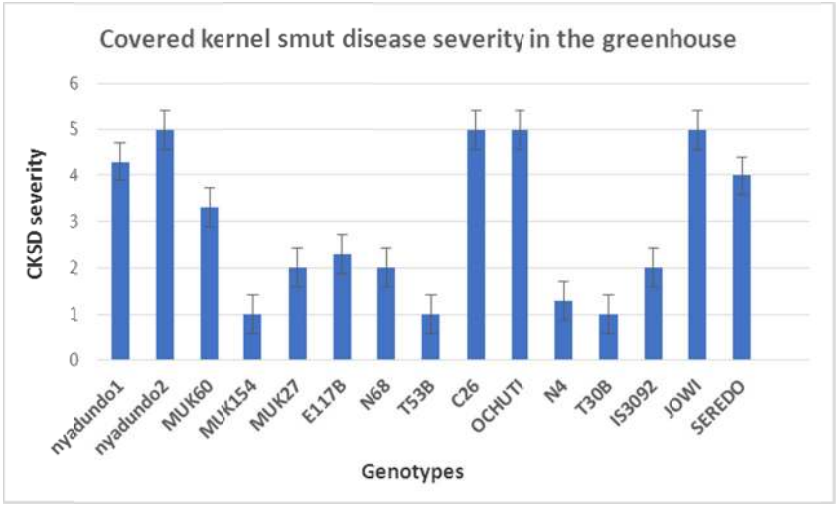

Figure 6. Covered kernel smut disease severity in the greenhouse

Table 6. ANOVA table for covered kernel smut disease severity in the greenhouse

SOV	DF	Sum of Squares	Mean Square	F Value
Genotype	14	111.244	7.9460	57.08***
Residual	30	4.667	0.1556	
Total	44	115.91		
SED	**0.3220**			
LSD	**0.6577**			
CV	**13.3**			

Note. SOV: source of variation; DF: degree of freedom; SED: standard error deviation; LSD: least significance difference; CV: coefficient of variation.

Table 7. ANOVA table for genotypes, sites and interaction

SOV	DF	Sum of squares	Mean square	F Value
Site	1	4.2008	4.2008	13.4391**
Genotype	14	11.4126	0.8152	2.607*
G × E	14	4.3761	0.3126	
Residual	14	4.3761	0.3126	
SED	**0.163**			
LSD	**0.830**			
CV	**0.559**			

Note. SOV: source of variation; DF: degree of freedom; SED: standard error deviation; LSD: least significance difference; CV: coefficient of variation.

There were significant differences ($p < 0.01$) on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes, while sites were significantly different at $p < 0.001$ but G × E interaction and residuals had no effect on disease incidence (Table 7).

Figure 7. Different sorghum genotypes responding to covered kernel smut disease attack under greenhouse conditions

4. Discussions

4.1 Variation in Disease Incidence Under Field and Glasshouse Conditions

In this study, there were significant variation in disease incidence among genotypes and enviroments ranging from 0-64% at Homa Bay, Migori and in the greenhouse. The presence of highly significant differences between the two test sites for all the fifteen genotypes indicated that the genotypes performed differently across the test environments. There was no significant difference on G × E interaction therefore no emphasis on evaluating the sorghum genotypes across the environments.

The higher disease prevalence in some genotypes in Migori compared to HomaBay or greenhouse cannot be readily explained at the moment but could be due to different enviromental factors at the three sites such as soil type, temperature, moisture or variation in pathogen isolates. Previous research have attributed site to site incidence differences to the pathogens biology and enviromental factors that affect germination of teliospores (Gwary et al., 2007). Similar trends in disease incidence was also reported by Sisay e al. (2005) who emphasized that CKSD thrives better in enviroments with tempratures between 18-25 ^{o}C and a relative humidity of 15-20% which is similar to the conditions in which we did this experiment.

The inherent genetic differences among genotypes could be the second reason for observed variation in disease incidence observed that the host species specificity to the pathogen vary according to different genotypes of sorghum. Similarly Gwary et al. (2007) attributed the variations in disease incidence to the inherent genetic differences among individuals.

4.2 Severity Score of Covered Kernel Smut Disease on Sorghum Genotypes Under Field and Greenhouse Conditions

Significant variations in disease severity were observed in the fifteen genotypes in the current study. Phenotypically, the varieties were categorised as immune (MUK154, T53B, T30B, N4 and MUK24), very resistant varieties (E117B, N68 and IS3092), susceptibles (Seredo, Nyadundo1 and Nyadundo 2) and Ochuti and Jowi, very susceptible as described by Marley et al. (2002) depending on the degree of damage. Resistance to CKSD is also dependent on genetic difference among genotypes and enviromental conditions.This result also agreed with the early report by Nzioki et al. (2000) that most studies for resistance to sorghum covered kernel smut disease is controlled by single gene and therefore,weather resistant or susceptible a variety is will depend on the parent used.

In general the response of the genotypes to the disease followed a similar trend both under the two fields and green house conditions indicating that all conditions were conducive for detecting the occurrence of the diseases. However the disease severity in the greenhouse was higher compared to the one recorded under field conditions probably due to variations in the enviromental factors and uneven distribution of innoculum in the soil. This was in agreement with the findings of Thakur et al. (2007) who suggested that field screening using trials at hotspot

and relying on natural infection has not been effective due to variations in environmental factors and uneven distribution of inoculum in the soil. Although for our case, the field screening was effective in the season when the experiment was done.

5. Conclusion

The study has identified sorghum genotypes that are tolerant, moderately tolerant and susceptible to covered kernel smut disease in Western Kenya through field and greenhouse screening. All the commercial and farmer varieties were found susceptible to the CKSD. The tolerant varieties included MUK27, T53B, N68, T30B, E117B, MUK157, IS3092 and N4 while the susceptible ones were, Nyadundo 1 and 2, Ochuti, Jowi and C26.The observed large variation in incidence and severity indicates possibility of managing the disease through selection and breeding for resistant varieties. We recommend further breeding for genetic improvement of sorghum using the identified resistant lines.

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