SCREENING OF SORGHUM (*Sorghum bicolor*) GENOTYPES FOR RESISTANCE TO COVERED KERNEL SMUT DISEASE (*Sporosorium sorghi)* FOR WESTERN KENYA

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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DECLARATION

I declare that this thesis is my original work and it has not been presented for award of degree in any other university or college to the best of my knowledge. No part of this thesis may be reproduced without the prior written permission of the author and or Rongo University

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DEDICATION.

This thesis is dedicated to my family; my loving and caring husband Mr. Omogi, my two loving daughters Victorine and Cate, and my brother Kennedy all who have become the source of my motivation and inspiration.

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ABSTRACT.

Sorghum is an important food security crop for arid and semi-arid tropics but its production is hampered by covered kernel smut disease (CKSD) which is a seed borne panicle disease caused by fungus *Sporosorium sorghi*. The fungus attacks susceptible sorghum genotypes causing yield losses estimated at 43% in Western Kenya posing a major threat to sorghum production. The current control measures involve the use of chemical, cultural and biological methods but they are costly, and environmentally unfriendly, laborious and ineffective and hence not sustainable. Most researchers have proposed the use of resistant genotypes which is affordable and sustainable to small scale farmers, but such varieties are not available. Thus, a study was conducted in 2019 growing seasons in order to determine the response of selected sorghum genotypes to CKSD under field and greenhouse conditions, and determine heterosis for agronomic traits in sorghum single crosses developed from tolerant and susceptible varieties to control CKSD as a first step to initiate introgression breeding for tolerance to the disease. A total of 15 genotypes were evaluated in two disease hotspot areas of Migori and Homa Bay counties in a Randomized Complete Block Design (RCBD) replicated thrice. Each genotype was planted in a 2.25 X 4m plot at spacing of 75 X 20cm. For controlled experiment in the greenhouse, the 15 genotypes were planted in pots in a Completely Randomized Design (CRD) also replicated thrice. In both cases, data on disease incidence, severity and grain yield was collected per genotype and analyzed using R for windows (version 3.6.2) and means separated using Tukey's test. Resistant genotypes were identified then crossed with the susceptible lines to incorporate covered kernel smut disease resistance through hand emasculation. Results showed significant differences among genotypes for disease incidence, severity and yield parameters. The disease incidence was evenly distributed and it varied significantly ($p<0.001$) between the sorghum genotypes per location. A range of 0-60% and 0-69% disease incidence was recorded under field and greenhouse conditions with Nyadundo2 and C26 having 60% and 69% respectively, while T53, T30, IS3092, N4 and N68 had 0% incidence. Similarly, severity also followed the same trend with Nyadundo 1 having a score of 5 while T53 scored 1. T53 produced the highest mean grain yield of 3.63t/ha while Seredo had the lowest mean grain yield of 0.20t/ha. Significant heterosis for seed weight, panicle traits, plant height and 50% days to flowering were observed on the eight F1 crosses. Various crosses showed significant heterosis in different traits. For instance, MUK60 X N13 had negative heterosis and heterobeltiosis for 50% days to flowering and plant height while NYADUNDO1 X IESV92038/SH had a positive heterosis and heterobeltiosis for panicle traits. This study has identified and developed six crosses which are potential sources of resistance for covered kernel smut disease that can be utilized to significantly improve yields in hotspot areas of western Kenya or for further breeding.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BP	Better-Parent Heterosis
CKSD	Covered Kernel Smut Disease
CRD	Completely Randomized Design
CV	Coefficient of Variation
DF	Degree of Freedom
F1	First Filial Generation
FAO	Food and Agriculture Organization
IPM	Integrated Pest Management
LSD	Least Significance Difference
MP	Mid-Parent Heterosis
RCBD	Randomized Complete Block Design
REP	Replications
SOV	Source of Variation
USDA FAS	United States Department of Agriculture Foreign Agriculture Service

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CHAPTER ONE: INTRODUCTION

1.1 Background

Sorghum *(Sorghum bicolor* (L) Moench) is a self-pollinating, diploid (2n=2x=20) species belonging to the *Poaceae* family with a genome size of 730Mb Ashok *et al*., (2011). The plant is an annual crop but some cultivars are perennial which grow in clumps that may reach over 4m high. Its grain is small, ranging from 2 to 4mm in diameter and a C_4 plant with higher photosynthetic efficiency with a higher abiotic stress tolerance therefore can grow in a wide range of environments around the world Reddy *et al*., (2009).

Sorghum (*Sorghum bicolor* (L) Moench) is ranked fifth in importance among cereals after rice, wheat, maize and barley Ashok *et al*., (2011). It is very important in areas of high temperatures and low rainfall as the crop is drought tolerant. Due to its physiological and morphological characteristics it can survive in drier environments. The following are some of the features attributed to the crop: Produces many roots, has reduced leaf area thus reducing water loss through transpiration, can remain dormant during drought and resume growth when conditions are favorable, the leaves have a waxy coating and the ability to roll in during drought thus effectively reducing transpiration and compete favorably with weeds ICRISAT, (2018).

According to FAO (2012), sorghum crop has two main uses including human consumption and animal feed. The sorghum grains are used as human nutrition all over the world, the grain is rich in carbohydrates, zinc and iron in which other cereals like maize do not supply all the three minerals in human food. The grain is used for flour production, preparation of side dishes and porridges, malted and distilled beverages production, preparation of special dishes such as popped grain and syrup production

from sweet sorghums (Gwary *et al* 2007). Sorghum plant is also considered to be a very significant crop for animal feeds as it can be used as fodder. Grain sorghum is also used for silage, for example, the sweet sorghums have a higher silage yield. Some other uses of sorghum fibers include making of wallboards, fences, biodegradable packaging materials, solvents, broom making and thatching house roofs (Taylor, 2003).

Sorghum (*Sorghum bicolor* (L) Moench) is ranked the fifth most important cereal crop in the world with a recorded annual production of over 60 million metric tonnes (FAO, 2012). As a result, Africa produces 20 million metric tonnes over an exceeding area of 40 million hectares, accounting for 14% of the total area of cereal production (Tonapi *et al*., 2020). In the semi-arid tropics of the developing countries, sorghum accounts for 70% of the total world area cultivated although in most parts of the area the crop is grown on a relatively small scale by small-holder farmers where it serves as a risk-reducing crop (Jere, 2004). In Kenya, sorghum is a crop of both the small holder mostly residing in the Arid and Semi-Arid parts of the country and commercial farmers in higher rainfall areas (Mtisi and McLaren, 2008). According to the United Nations Relief and Recovery Unit (2004), it is reported that the area under sorghum production during the 2003/4 season was 207 000 hectares, which is an increase of close to 300% from 2002/3 season in Kenya which was 621000 hectares. Sorghum production in most parts of the world is relatively low, estimated at 0.925 tonnes per hectare compared to 5 tonnes per hectare reported from experimental stations (ICRISAT, 2004).

The low yields are attributed to a number of factors like; Biotic, Abiotic and Socio-Economic factors (Esele, 2013). The most common pests in sorghum include; Sorghum midge *(Contarinia sorghi cola)* is a serious pest of sorghum during flowering, it destroys developing seeds thus preventing seed development (Sharma, 2012). Birds are

one of the most important pests of sorghum worldwide. They are capable of causing heavy losses, the most common species is the *Quelea quelea*, other minor pests also include; army worms, corn aphids, sorghum head caterpillars and moths (IPM, 2018). The most important diseases of sorghum include rust, grey leaf spot, leaf blight, head smut, loose kernel smut, covered kernel smut and anthracnose. Collectively, these diseases have caused yield losses which are varied from one region to the other (Esele, 2013).

Covered kernel smut disease caused by *Sporisorium sorghi* is a major constraint in sorghum production (Mtisi and McLaren, 2008). The fungus *Sporosorium sorghi* attacks sorghum during planting, it is seed-borne and develops systemically as the sorghum crop grows. According to Howard *et al*, (2005) the mature fruiting bodies of the fungi called sori ripen and rupture releasing teliospores that infect seeds on other plants. The teliospores of the fungus are seed borne and therefore germinate within the seedling plants that are infected, but symptoms generally do not appear until flowering or heading. The pathogen grows within the plant to the shoot apex and invades floral tissues where individual ovules are replaced by smut fruiting bodies that resemble the glumes. Most sori are conical or oval and resemble an elongated sorghum seed causing losses in proportion to the area of the panicle infected (Howard *et al*., 2005).

According to Sisay *et al*, (2012) annual yield losses due to covered kernel smut in Africa is estimated at 10% with local losses within the countries estimated at 60% or more. The occurrence of covered kernel smut disease varies from place to place, In Eastern African countries for example Ethiopia; the incidence was estimated to be about 50% (Sisay *et al*., 2012) while in Kenya, covered kernel smut disease is also significant with yield losses of 42-43 % (Okong'o *et al*., 2019).

To minimize yield losses due to covered kernel smut disease, several methods can be used such as chemicals, cultural and biological control measures to minimize yield losses due to covered kernel smut disease. Chemical method includes the use of fungicides which assist in reducing the incidence and severity of the disease on sorghum but does not completely control the disease. Healthy looking seeds should be treated with Carboxin x Thiram (Vitavax) at about 2 g active ingredients per kg of seed or elemental Sulphur at about 5g per kg of seed (Sisay *et al.*, 2012). The seeds can also be treated with fungicides such as Captan at 0.3% per kg of seed (Jere, 2004). However, most of these fungicides are extremely expensive to purchase hence this method is unaffordable to the smallholder farmers. Culturally, covered kernel smut disease can also be controlled by soaking the seeds in water for four hours, followed by drying the seeds, first in the shade then under the sun. This procedure kills germinating smut spores and does not impair seed viability (IPM, 2008). One can also collect the smutted ear heads of sorghum in cloth bags and destroy the fungus by dipping in boiling water for 30 minutes, incineration of infested samples should be done by removing and burning the heads infested before the spores are scattered (IPM, 2008). This method is affordable but tends to be labour intensive for most farmers therefore not applicable.

According to studies conducted by Adane and Guatam, (2000) in Ethiopia, covered kernel smut disease can also be controlled botanically this is by use of fermented cattle urine and botanical Abeyi (orm*) Maesa lanceolata*. Where the Smut inoculated sorghum seeds are treated with aqueous extracts of the leaves of the botanical Abeyi (orm) extract which is diluted with water, seeds are treated and then air dried before planting. Abeyi and fermented cattle urine seed treatments reduced the prevalence of the disease. They proposed that this could be used as a substitute for fungicides and are

potentially useful for resource poor farmers; however, the plant Abeyi is not locally available for farmers in western Kenya.

Despite the available control measures, the disease has continued to persist in small holder farmers who grow sorghum continually in their farms, there is therefore a need to develop and explore alternative strategies to minimize loses to sorghum yields due to the covered kernel smut disease. The use of resistant genotypes has been proposed as the most cost-effective strategy for the control of covered kernel smut disease given that sorghum in general has a low return to investment. Kutama *et al*, (2013) and Wilson, (2011). Currently, there are no reports on availability of covered kernel smut disease resistant genotypes in Kenya therefore, farmers continue to use and share seeds that have been infected with the smut fungus within the communities. Gwary *et al.,* (2007). Therefore, this study seeks to improve sorghum production by identifying potential sources of resistant varieties of sorghum to covered kernel smut disease.

1.2 Statement of the Problem

Covered kernel smut of sorghum caused by *Sporisorium sorghi* is a serious problem to sorghum production worldwide and a major cause of yield reduction and hunger among communities which depend on sorghum as a staple food. According to Gwary *et al* (2007) and Sisay *et al*, (2012), annual yield losses due to covered kernel smut disease in Africa ranges between 10 - 60% and about 60-70 % in Western Kenya (Okongo *et al*., 2019). According to these authors, there is lack of resistant varieties to the disease as most of the cultivated sorghum varieties in Western Kenya including commercial and local varieties are susceptible to covered kernel smut disease. Besides, most of these farmers plant untreated sorghum seeds hence aggravating the occurrence and spread of

the disease in the region. Because of these reasons, farmers have continued to incur huge yield losses from this disease leading to chronic food insecurity in the region. The pathogen infects sorghum flowers preventing seed development which directly reduces yields. It also causes molding of sorghum grains lowering the grain quality and also affects marketability making sorghum fetch very low prices or unable to be sold at all. It is therefore paramount for stable sources of resistance to be identified and used for breeding programs for improvement of sorghum genotypes in Western Kenya.

1.3 Justification of the Study

Sorghum crop is the second most important cereal crop after maize in Western Kenya where it accounts for 60% of the total area sown owing to its tolerance to high temperatures, and drought. Most of Western Kenya regions is semi-arid and sorghum is the most adapted crop that effectively combats food insecurity in the area. However, Western Kenya has reported yield losses of between 30-70% as a result of covered kernel smut disease (Gwary *et al*., 2007) owing to lack of tolerant varieties to the disease. The existing control methods including chemical, cultural, biological and breeding for tolerant crop varieties have failed to adequately minimize yield losses. The use of fungicides has low adoption rate among farmers owing to high cost and damage to the environment, cultural methods are labour intensive and hence inappropriate to small scale farmers, while biological control measure are less effective. Therefore, screening of sorghum genotypes would facilitate the identification of genetic sources of resistance to covered kernel smut disease which can be introgressed into the adapted but susceptible sorghum varieties. This is a more sustainable way of managing the disease, especially among smallholder farmers. Currently, there are no reports on availability of covered kernel smut disease resistant genotypes in Kenya therefore farmers continue to

share seeds that have been infected with the smut fungus within the region. Therefore, this study seeks to improve sorghum production by identifying potential genetic sources of resistance to covered kernel smut disease which could be used to control the disease and hence improve grain yield under small hold farms, especially in western Kenya where the disease is common.

1.4 Research Objectives.

1.4.1 General objective.

To identify sources of resistance and develop sorghum genotypes that are resistant to covered kernel smut disease for improved yields in Western Kenya.

1.4.2 Specific objectives

1. To determine response of selected sorghum varieties to covered kernel smut disease under field and greenhouse conditions.
2. To determine heterosis for agronomic traits in sorghum single crosses developed from tolerant and susceptible varieties to covered kernel smut disease as a first step to initiate introgression breeding for tolerance to the disease.

1.5 Research hypotheses

1. Genetic differences exist in sorghum genotypes, in that way they could respond differently to covered kernel smut disease under field and greenhouse conditions.
2. Genetic differences exist in sorghum genotypes and are transferable into farmer preferred varieties and better F1s in agronomic traits than the parents can be developed in the sorghum single crosses.

1.6 Significance of the study

The results of the current study on sorghum covered kernel smut disease resistance will contribute additional information to breeders to the continuing effort to develop sorghum lines which are resistant to the disease.

With increased sorghum production this current study, is set to benefit both human and livestock nutrition since fodder and silage will be made from sorghum which will be used for livestock forage and human food will also increase reducing losses due to the disease.

It will reduce the dependence of fungicides which is very expensive to small scale farmers hence increase in income.

In general, the study will provide information to farmers on how to improve yields attributed to covered kernel smut disease resistance hence increased food production.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sorghum Production

Sorghum (*Sorghum bicolor* (L) Moench) is indigenous to Africa belonging to the grass family *Poaceae* with many morphological differences (Hariprasanna and Patil 2015). It is referred to as the "poor man's crop with a recorded annual production of over 60 million tonnes FAO, (2012). As a result, Africa produces 20 million tonnes over an exceeding area of 40 million hectares, accounting for 14% of the total area of cereal production Taylor, (2003). This makes the sorghum crop the second most important cereal crop after maize in Africa (Gwary *et al*., 2007). Sorghum is not ranked high in importance compared to other cereals such as maize, wheat and rice. Whereas these key staple cereals perform well in high productive areas, sorghum is grown in low to mid potential areas. Majority of the sorghum produced in Kenya is grown the Coastal, Eastern and Western parts of the country (Mwema and Mulinge, 2013). According to USDA, 2019 data, World sorghum production was 57.6 million tons in the 2012/13 season, increased to 60.9 million tons in 2014/15 season. Production decreased to 61.4 million tons in the 2015/16 season but increased again in the 2016/17 season reaching 63million tons. The latest USDA report that includes 2017/18 season forecasts suggests that the sorghum production will be 60.6 million tons. Kenya is ranked last in sorghum production compared to other East African countries. Over the last four years Ethiopia has been ranked the first in sorghum production FAO, (2018). According to Food and Agricultural Statistics, (2018), it is reported that the area under sorghum production during the 2010/11 season was 250 000 hectares, which is an increase of close to 300% from 2009/10 season in Kenya, sorghum is a high yield potential crop and this was

evident when Kenya produced 1.3 tonnes per hectare in 2015.

2.2 Sorghum Utilization

According to FAO, (2012), like many grains; sorghum has two main uses which include human consumption and animal feed. The sorghum grains are used as human nutrition all over the world; it is rich in carbohydrates, zinc and iron nutrients, Grain sorghum is also grind into flour to make porridge and ugali, malted and distilled beverages production for example beer, preparation of special dishes such as popped grain and syrup production from sweet sorghums. According to Howard *et al.,* (2013), Health benefits of sorghum include: (1) High nutrient value, Sorghum provides vitamins, magnesium, iron, copper, calcium, phosphorus and potassium when included in the diet. (2) Improves digestion, Sorghum is one of the best foods for dietary fiber, single serving of sorghum contains 48% of daily recommended intake of fiber this keeps the food moving along the digestive tract rapidly preventing cramping, bloating, constipation stomach aches, excess gas and diarrhea. Moreover, excess amount of fiber in the body helps to scrape off dangerous cholesterol which helps improve heart health and protects the body from heart attack and stroke. (3). Prevention of cancer, The bran layer of sorghum grains contain antioxidants that help reduce various types of cancer e.g. esophageal cancer, antioxidants neutralize and eliminate free radicals in the body which cause healthy cells in the body to mutate into cancerous cells. (4) Controls diabetes, Tannin rich bran of sorghum has enzymes that inhibit the absorption of starch by the body which helps regulate insulin and glucose levels in the body. By keeping these levels balanced thereby preventing diabetic shock and other health complications. (5) Healthy bones, Magnesium is in high quantities in sorghum, hence calcium levels will be properly maintained as magnesium increases calcium absorption in the body

these two minerals are integral in the development of bones tissues and speed up the healing of damaged or aging bones. This helps prevent arthritis thereby keeping the body active and healthy in old age (Howard *et al*, 2013).

Sorghum is also a very important crop for domestic feeds as it used as fodder. The grains are processed by cracking, rolling or grinding because of the high tannin content before they are fed to livestock, when processed the nutritional value of sorghum increases considerably. Grain sorghum is also used for silage, for example, the sweet sorghums have a higher silage yield. Some other uses include; sorghum fibers are used in making of wallboards, fences, biodegradable packaging materials, solvents, broom making and thatching house roofs (Wilson, 2011).

2.3 Sorghum agro-ecological requirements

Sorghum grows on many different soils, however best yields are realized on deep fertile, well drained loamy soils. However, it's also tolerant to shallow soils and droughty conditions (Gwary *et al.,* 2017). Sorghum grown on deep well drained permeable (Wilson, 2011). Sorghum can moderately tolerate salt, it does well in pH range of 6.0-8.5. Sorghum is sensitive to aluminum toxicity and soils with acid saturation higher than 20% can pose problems (Esele, 2013).

Sorghum grows under a wide range of climatic conditions and can still yield well even under unfavorable conditions of drought stress and high temperatures. It is widely grown in temperate regions and at altitudes of up to 2300m in the tropics. It can tolerate high temperatures for good growth and the minimum temperature for the germination of the sorghum seed is 7-10 degrees Celsius. Sorghum is best adapted to areas having an average rainfall between 450-600mm. Although, it can respond to good moisture supply therefore, it is one of the toughest, drought tolerant crops available and this tends to

maintain its popularity in the regions where the weather is very unpredictable (Kudadjie *et al*., 2014).

The ability of sorghum to grow in drier environments is due to the following physiological and morphological characteristic which include: (1) Production of many roots than other cereals, (2) Has reduced leaf surfaces thus reducing the surface area exposed for water loss through transpiration, (3) Can remain dormant during drought and regain growth when conditions are favorable for growth, (4) The leaves have a waxy cuticle hence reducing the rate of transpiration and the ability to roll in during drought thus effectively reducing transpiration, (5) Competes favorably with many weeds (Borell *et al*., 2014).

2.4 Sorghum production constraints

Sorghum production in Kenya is under-utilized. It's grown in drought-prone marginal areas of Coast, Eastern and Western counties of the country, even though it's suitable in the semiarid areas its production is still low (ICRISAT, 2004). The low yields are attributed to factors which include; Biotic, Abiotic and Socio-Economic factors Esele (2013).

2.4.1 Abiotic factors

Unreliable and insufficient rainfall is a big challenge for farmers since agriculture is dependent on rain. Very low rains and high temperatures in the post flowering stage in the growth period of sorghum reduces yields. Delays in rain during the planting period also affects the yields as late maturing varieties are always disadvantaged Kudadjie *et al.,* (2004). Very low moisture content inhibits imbibition and reduce the ability of sorghum seeds to germinate.

Poor soil quality affects sorghum yields. Sorghum varieties tolerant to aluminum are

used on acidic infertile soils of Western, Eastern and Coastal counties. Poor soils are also caused by wind erosion and poor farming practices by farmers, e.g. continuous cropping which has led to exhaustion of nutrients from the soil, even though, Sorghum production usually takes place in marginal areas that are prone to infertility and water stress conditions as it is drought tolerant Thakur *et al.*, (2004).

2.4.2 Socio-Economic factors

The majority of sorghum farmers, especially in the coastal, eastern and western parts of Kenya do not produce enough sorghum to meet family requirements. Furthermore, sorghum farming is a semi-subsistence enterprise that offers smaller returns than other investments such as livestock. As a result, less attention is paid to invest in the use of seeds from improved varieties to boost production (FAO, 2012).

Most farmers rely on family labor to work on their farm, this has affected labor size needed to produce more yields. Children also who have gotten education do not consider sorghum farming as a source of stable and reliable income most of the educated men and women have migrated to cities to look for white collar jobs leaving old people to work in the farms. Land ownership is still bestowed with the males putting them the sole owners hence top decision makers in the family, women who may want to own a piece of land to plant sorghum are viewed with suspicion this variation brings a power difference in the household hence low yields experienced in sorghum production. Men prefer maize to sorghum while women prefer sorghum because of the food security and other health benefits. These factors lower the production yields of sorghum. A survey carried out in western Kenya showed that farmers bought agricultural inputs e.g. seeds and fertilizers but they have a perception that because they already have crops that were grown from certified seeds, there is no need to procure the

same seasonally hence use seeds from previous harvests Okongo *et al*., (2019).

2.4.3 Biotic factors

Sorghum plants grown in local areas are always attacked by bacterial, viral, parasites and pests, weeds which include: Couch Grass (*Cynodon dactylon*), Black Jack (*Bidens pilosa*) Wondering Jew (*Tradescantia zebrina*) and many others but the common and most devastating one is *striga* and fungal diseases

2.4.3.1 Striga

Striga is a major threat to sorghum production in Kenya, it's prevalent in Western Kenya and farmers lose up to 90% of the crop due to this weed. The weed limits the productivity of the crop by siphoning off water and nutrients from the crop for its own growth and causes rampant damage to sorghum. It damages sorghum crop upon attachment to its roots thus resulting into wilting, yellowing of the leaves, curling of leaves, and stunted growth resulting into less sorghum yields Mtisi and McLaren, 2008). *Striga* species which are very common in the region are *Striga haemonthica benth* and *Striga asiatica* which greatly reduce yields. Some *Striga*-resistant sorghum varieties have been developed for example Serena, further crossing of Serena produced Seredo which had some resistance during screening trials in western Kenya (Kiriro, 1991). High *striga* susceptibility was observed for Ochuti, Jowi and Andiwo II which are local varieties grown by farmers (Ayiecho and Nyabundi, 2000), but these generally offer lower yields than improved (but *Striga*-susceptible) varieties (Ashok *et al*., 2012). The effect of *Striga* has been found to decrease when sorghum is grown in conjunction with legumes (Carsky *et al.*, 2009).

2.4.3.2 Other pests and diseases

The most important diseases of sorghum include rust, grey leaf spot, leaf blight,smuts

and anthracnose. They feed at different stages of sorghum developmental cycle these diseases have caused yield losses and also affect the quality of sorghum grain which are varied from one region to the other (Carsky *et al*., 2009).

Sorghum midge *(Contarinia sorghi cola),* is a serious pest of sorghum during flowering, it destroys developing seeds thus preventing seed development, resistant varieties are available and widely used (Sharma, 2012).

Birds are also one of the most important pests of sorghum in Kenya. They are capable of causing heavy losses hence causing economic damage. The most common species is *Quelea quelea,* it causes extensive damage to sorghum in western, Eastern and Coastal regions of Kenya, various strategies have been employed to control the bird including use of repellants, cultivating long glumes and goose necked varieties, chemical control on cultivars with bird resistance characteristics to help maintain high crop yield and productivity. Breeding for resistance to bird damage has been an effective control measure Merwine, (1963) reported a hybrid RS617 that has a bitter taste at milk or dough stage and confers resistance to birds. The bird damage on developing grains can result in near total crop loss.

2.5 Sorghum smut diseases.

Smuts are one of the diseases common in sorghum production areas in Kenya which limit grain crop production in these areas (Vinceli and Hershman, 2011). Damage is confined on the head or panicle area reducing grain yield and quality of the grain. Three types of smuts are common in Kenya in areas where sorghum is grown and are caused by different species of fungus *Sporosporuim,* which are commonly named, the covered kernel smut, loose kernel smut and head smut (Frederiksen *et al*., 2000). Smuts are one of the most significant diseases in sorghum production areas especially where untreated

seed is planted. ICRISAT carried out surveys in Southern Africa and concluded that the sorghum crop in most countries was affected from the same smut diseases with some variation due to climatic factors and the level of improvement in the sorghum genotypes (Wilson, 2011).

2.5.1 Head smut

It is not common in sorghum growing region compared to the kernel smut. The pathogen has increased due to cultivation of susceptible cultivar. It is caused *by sporosorium relinium.* Smutted plants have weakened root system and severe stalk and root rots compared to healthy crops. Infection appears when the young head, enclosed in the boot is completely replaced by a large smut gall covered by a thick brown to white membrane. The membrane then raptures exposing a mass of black powdery teliospores intermingled with a network of long thin dark broom-like filaments of vascular tissue. Wind or rain scatter the smut spores to the soil where they remain viable for a very long period of time. When the infected sorghum seeds are planted the following planting season the viable smut pores already in the soil germinate along with the seed. The fungus develops only in actively growing meristematic tissues, the spore may also cling to the surface of sorghum seed introducing the smut fungus into the soil not previously affected Fredericksen *et al*., 2000. In western Kenya, according to a survey done by Okongo *et al*, 2019, Up to 5% of the plants were affected but overall infection didn't exceed 1-2% and it is considered of minor importance at this time. Control of head smut is through the use of resistant varieties, crop rotation, treating seeds with fungicides and destroying infected heads.

2.5.2 Loose kernel smut

This is caused by the fungus *Sporisorium sphacelotheca cruenta*, it is not also very

common in sorghum producing areas in Kenya. Mostly all kernels in an infected panicle are normally smutted, partial destruction of the kernels is very rare. The infected kernels may be transformed into a leafy structure or escape infection, individual kernels are replaced by small smut galls that are 2.5cm longer, pointed and surrounded by a thin gray membrane, the membrane bursts after the panicle emerges from the boot and the powdery black spores are blown away by wind or rain leaving a long black pointed conical structure in the center of the gall, some smut pores may cling to the surface of healthy kernels (Fredericksen *et al.,* 2000). When such infested kernels are planted the teliospores germinate along with the seed, grows with the plant until booting when a long black pointed smut galls develop in place of a normal kernel. The plants affected are stunted, have thin stalks and heads emerge earlier than healthy plants. The losses due to loose kernel smut is relatively lower in western Kenya not exceeding 5% even in hotspot areas. The best control for this fungus is planting certified seeds. Seeds from fields with even a small amount of loose smut should not be planted without treating with Carboxin or Carboxin+Thiram which provide good control of the loose smut (Sharma *et al*., 2000).

2.6 Covered kernel smut disease

Of all the three smuts covered kernel smut is the most serious and common in most of the sorghum growing areas in Kenya where prophylactic control measures are not used. It is caused by the fungus *Sporosorium sorghi*. Assessment of the occurrence of CKSD in Western Kenya from a survey conducted by Okongo *et al*, 2019 was estimated at about 10%, Up to 60% was observed in hot spot areas. Covered kernel smut disease may be controlled by the application of fungicide (seed treatment), but the chemicals may be unavailable or unaffordable to most of the smallholder farmers. This study seeks

to provide an effective non chemical and affordable practice through breeding sorghum lines that are resistant to covered kernel smut disease.

Covered kernel smut is a seed borne panicle disease caused by the fungus *Sporisorium sorghi* which is classified within the Ustilaginales, class Basidiomycetes (Perez, 2002). The disease occurs in Western region of Kenya where sorghum is produced and causes greater grain loss in yield (Frederisken and Odvody, 2000). All the kernels on the head of the infected crops are destroyed and replaced by a dark brown teliospores covered in a tough grey membrane which bursts when mature and adhere to the surface of healthy seeds. The disease is only apparent after heading where individual ovules are replaced by smut fruiting bodies that vary in size. The smut sori are smooth, oval, conical or cylindrical in shape and vary in size from those small to be covered by the glumes to those that are one cm long, but vary in color from white to grey or brown (Howard *et al.,* 2005).

2.6.1 Lifecycle of *Sporisorium sorghi* pathogen

The fungus *Sporisorium sorghi* produces diploid teliospores which are spherical with a diameter of 3-8µm, when the diploid teliospores germinate they produce a four celled basidium, which bears monosporodium that fuses together to produce the pathogenic dikaryon (Wilson, 2011).

Literature indicates that *Sporisorium sorghi* originated from other crop smuts, *Ustilago maydis, Ustilago scitaminea* and *Sporisorium reilanum*, and this occurred before domestication and modern agriculture Munkacsi *et al*., (2007).

Normally all the kernels in affected head are replaced by dark brown powdery masses of teliospores covered with a tough greyish brown membrane which bursts open during harvesting time. The sori when mature burst and the microscopic spores adhere to the

surface of healthy seeds. When a smut infested seed is planted the teliospores germinate along with it forming a basidium which infect the germinating seedling and at that time the teliospores replace kernels and are surrounded by a tough grey membrane. At maturity the membrane raptures releasing spores that contaminate seeds and soil Fredericksen *et al*., 2000.

2.6.2 Environmental factors favoring the development of covered kernel smut disease

The fungus *Sporisorium sorghi* germinates and develops at 15-37ºC. The soil optimum temperature favorable for covered kernel smut disease development is 15-37ºC and infection is optimum in warmer, dry soils with a humidity of 10-15%, during this period, delayed seed germination is experienced that is optimal for the contamination of crop. These conditions are prevalent in Western Kenya especially during the growing seasons when the area experiences insufficient rains and this may have caused the prevalence of the disease in the area. The infection decreases at temperatures between 35-40ºC Selveraj, (2012). Spore germination varies with the genotype, under the optimum temperature for germination is from 20-30ºC, and the spores retain viability for a long period when kept in dry conditions, covered kernel smut pathogen retains its viability in the soil and the crop residues.

Ashok *et al*., (2011), stated that infection takes place before the seedlings emerge out ant therefore the conditions suitable for prolonged germination of seeds is suitable for the pathogen infection and establishment this was also the case in Western Kenya because of heavy rains which were experienced immediately after planting Okongo *et al*., (2019). Genotype variety, temperature of the soil, moisture content in the soil and depth of planting are also factors known to affect the incidence and severity of the

pathogen. High temperatures after sowing will reduce CKSD infection on the crops.

Sisay *et al*., (2011), stated that high temperatures and low soil moisture content encourage faster seed germination hence the seedling escapes from the pathogen invasion of the germinated radicle of the crop. Low temperatures, high soil moisture content and deeper planting of sorghum seeds increases infection rates.

2.6.3 Effects of covered kernel smut disease on the sorghum crop production.

2.6.3.1. Effects of the CKSD on sorghum growth

When smut infected sorghum seed is grown, the teliospores grow along with the seedling. The pathogen invades the developing seedling where it continues to grow systemically inside the plant before booting (Howard *et al.,* 2005). The teliospores or the smut galls which have formed and replaced the kernels are only noticed after heading. The infected plants appear to be normal growing plants until the emergence of the panicle or the head, the diseased kernels are all replaced by the dark brown powdery masses of teliospores (sorus) covered by a greyish brown tough membrane (Ashok *et al*., 2011). Therefore, to some extend growth of the sorghum plant infected by covered kernel smut disease is never affected, infected plants grow normally in terms of height and size as compared to healthy plants (Wilson, 2011).

2.6.3.2 Effects of CKSD on sorghum yield and quality

Covered kernel smut disease destroys all of the kernels in the head and replaces them with cone-shaped gall or may affect only portions of the panicle hence yield is reduced. When the galls are broken; the spores disseminate and contaminate the outer surface of the kernels (Howard *et al.,* 2005). Damage is confined only to the head or panicles, thus the reduction in yield is 42 -43% in western Kenya which is of economic value (Jere, 2004). The CKSD of sorghum reduces seed production seriously and it also affects

forage yields substantially due to stunted growth of the infected crops. The quality of the yield is also reduced by the presence of the black teliospores which adhere on the surface of the healthy kernels Thakur *et al*., (2007). Patil and Padule (2000), also reported that smutted seeds of sorghum showed reduced seed germination by 54% and seedling vigor index (664) as compared to healthy seeds and the increased chaffiness of the seeds fetched low prices in the market.

2.6.4 Control of Covered kernel smut disease of sorghum

To reduce sorghum yield losses due covered kernel smut disease it is of importance to identify the suitable methods to employ to help curb the disease. Covered kernel smut disease can be controlled by practicing some methods namely the chemical, cultural, biological control methods (Wilson, 2011).

2.6.4.1 Chemical control method

Chemical method is the use of fungicides which help in reducing the occurrence of the disease on the sorghum however it does not completely control the disease. Sorghum seeds can be completely protected from covered kernel smut disease and this can be achieved through proper seed treatment (Howard *et al.*, 2005). Covered kernel smut disease is effectively controlled by treating the seed with a protecting fungicide, this prevents introducing the kernel smut fungus into clean fields free from the fungus (Selveraj, 2013). It also provides protection against seedling smut fungi which may still be in the soil and also viable in the soil (Thakur *et al.,* 2007). The seeds that appear healthy should be treated with carboxin (Vitavax) at 2g per kg of seed or elemental Sulphur at 5g per kg of seed. The seeds can also be dusted with fungicides such as Captan or Thiram at 0.3% per kg of seed (Jere, 2004). Systemic fungicide Apron also reduces the risk of CKSD. According to Wright and Fulleton (2006), they stipulated

that carboxin + thiram when applied to sorghum failed to reduce severity of the disease compared with untreated seeds it also resulted in the lowest plant vigor in the first month of growth this suggested that there was a degree of phytotoxity from the chemical. All other fungicides used in the experiment provided greater control than carboxin + thiram the best control was achieved by propiconazole and flutriafol + imazalil sulphate both of which reduced the proportion of smutted plants by 70% compared with untreated seeds. Based on my research flutriafol+ imazalil sulphate and propiconazole both offer viable alternatives to carboxin + thiram for control of CKSD but neither can be found in the local shops in western Kenya, therefore due to the higher costs and unavailability, the usage of fungicides under small scale farmers is very rare.

2.6.4.2 Biological control method.

Farmers also the use the locally available botanical plants as bio pesticides and other materials like cattle urine against covered kernel smut disease. According Adane and Guatam (2000), they stipulated that, fermented cattle urine and botanical Abeyi (orm) (*Maesa lanceolate*) reduced the occurrence of covered kernel smut disease. Smut inoculated sorghum seeds were treated with aqueous extracts of the leaves of the botanical Abeyi (orm) at the rate of 20ml extract diluted with the same amount of water and 200g of healthy seeds were also treated with fermented cattle urine and then air dried before planting the result showed that both Abeyi and fermented cattle urine seed treatments reduced the prevalence of the disease. This could be used as a substitute for fungicides as it is potentially useful for resource poor farmers. Although, the method is economically feasible, socially acceptable and environmentally safe, the plant Abeyi is not available in western Kenya therefore making the method inapplicable

2.6.4.3 Cultural control method

Cultural methods used in controlling CKSD generally involves field hygiene which includes; soaking the seeds in water for then drying the seeds, first under the shade and then in the sun. This procedure kills germinating teliospores without impairing seed viability (IPM, 2008). The practices of crop rotations and cultivation have little effect on controlling the disease, since the smut teliospores can persist and remain viable in the soil for long periods of time and years (Perez, 2002).

Sorghum ratooning is also not advisable to practice as most of the ratooned crops exhibit higher incidences of covered kernel smut as the spores remain viable for long (Wilson, 2011). Smutted panicles of sorghum can also be collected in cloth bags and the pathogen destroyed and killed by dipping in boiling water or burning of infested heads in the field should be done before the spores are scattered (Wilson, 2008). Since the covered kernel smut pathogen may live in the soil for many years then the farmer can only grow sorghum in the same field only once after many years (Howard *et al*., 2005). Due to these limitations which are laborious and ineffective control of CKSD in western Kenya is not feasible using the cultural methods.

2.6.4.4 Breeding method

The use of breeding method through incorporating resistant genes is one of the control measures that can effectively be employed for the control of CKSD, however progress in this has been very slow especially in Western Kenya. Breeding method is cost effective and a biologically safe means of protection which can be applied in controlling covered kernel smut disease since it gives a possibility for the recombination of genes. Differences on disease incidence in different sorghum genotypes screened for covered kernel smut disease could be due to the differences in the individual inherent reaction

the pathogen Gwary *et al*, (2007). Lindsay Phiri (2016), recommended that breeders to further screen other sorghum genotypes for resistance to covered kernel smut disease in order to improve susceptible sorghum genotypes to covered kernel smut disease by incorporating a resistant ability to the pathogen infection. Sorghum varieties which were newly released from Rongo University Sorghum Project were distributed to farmers in the western Kenya. They were high yielding, multi stress tolerant and had high productivity but most of the varieties were attacked by the pathogen. This study therefore proposes to use breeding as a measure to reduce the covered kernel smut disease occurrence in sorghum. The genotypes that are to be used in this study are improved sorghum varieties that are being promoted by the institution's sorghum project and most of them were infected by the pathogen.

2.7 Screening techniques for resistance to covered kernel smut disease

According to Thakur *et al*., (2007), the following Screening techniques have been developed for sorghum covered kernel smut disease, these methods help to differentiate lines as either resistant or susceptible.

2.7.1 Field screening method

Field screening method uses experiments at hotspots and rely on natural infection to ascertain resistant and susceptible genotypes, this has not been effective due to differences in environmental factors and uneven distribution of teliospores in the soil. Therefore, in addition to natural infection an inoculation technique in which seedlings are injected with sporidial suspension of the pathogen using a modified and simplified hypodermic syringe technique based on the method developed by Hayden, (2013) can be followed. Genotypes to be screened are sown in a plot comprising infector rows of highly susceptible lines. However, the greenhouse screening method is more reliable

and effective than field screening technique.

2.7.2 Laboratory screening (inoculum preparation)

Smut Sori are collected from mature panicles of CKSD infected sorghum genotypes by threshing affected panicles which are collected from farm trials. The teliospores are collected at the maturity stage. Threshing is done by lightly pounding the affected sorghum heads in paper bags. A sieve is used to collect the smut galls by removing plant materials and other debris. The affected panicles remain in the paper bags to prevent the dissemination of the teliospores and are stored at a temperature of less than 21°C and relative humidity of less than 12% to prevent the teliospores from desiccation and germination respectively. The seeds are inoculated using the teliospores of covered kernel smut pathogen at a ratio of 100 seeds to 0.l5g of teliospores in small envelopes. The inoculation is done by shaking the envelopes to facilitate proper seed coating prior to planting Lindsay Phiri, (2017).

2.7.3 Glass house screening.

This is useful as a controlled environment. The seeds are coated with teliospores before planting. They are then grown in pots and at the booting stage they are injected with the inoculum and the panicles are bagged to promote infection. Sprinkler irrigation is provided to enhance infection. The bags are opened after fifteen days after inoculation to allow the panicles to dry for 3 days scoring each panicle for the percentage of florets bearing smut sori. Panicles with no infection are selected to obtain mature seeds from these and re-evaluated to confirm the resistance. However, this technique has limitations in identifying moderate levels of resistance (Nzioki *et al* 2008).

2.8 Heterosis

Heterosis refers to the process by which a cross exhibits better characteristics than both parents (Chen, 2010). Potential of sorghum crosses is estimated from the percentage increase or decrease of their performance over the mid parent and better parent (Holchholdienger and Hoecker, 2007). Heterosis over better parent (heterobeltiosis) is the increase in character of the cross compared to that of the better parent for that character. It is more realistic and practical because it shows the performance of the cross in comparison with the best parent unlike mid parent heterosis which is the increase in the character of the cross compared to the mean of the parents in relation to that character. It compares the cross with the mean of the two parents (Lakshmi et al 2011). In this study both mid parent and better parent heterosis were worked out. Heterosis is expressed as a percentage increase or decrease of F1 cross over the mid parental value. The superiority of F1 cross over the better of two parents is known as heterobeltiosis while mid parent heterosis is the superiority of the F1 over the average of both parents. Heterosis can be positive or negative and both are always useful for crop improvement. Positive heterosis in general, is desirable for yield while negative heterosis for early maturity. Observations by various breeders on some quantitative characters in sorghum are briefly reviewed hereunder.

Ringo *et al*, (2015) reported that desired heterobeltiosis for days to 50% flowering varied from -5.23 to -14% indication of early maturing material. Lowest (desired) heterobeltiosis for plant height was -53.61% with crosses ICSA15 × Tegemeo and ATX623 × KARI-MTAMA1 most promising for this trait. Grain yield showed average heterosis and heterobeltiosis of up to 81.90% and 77.18% respectively both expressed in ICSA11 × S35. Average heterosis for plant height varied from -17.2% to -55.67%

whereas heterobeltiosis for the same traits ranged between -11.44 to -53.61%. ICSA15×Tegemeo and ATX623×KARI-MTAMA1 were the most promising as they were short in stature indicative of dwarfness. Short sorghums require relatively shorter period to maturity compared to taller ones and withstands lodging as well as easiness during harvesting as also reported by Madhusudhara and Patil, (2013). Tall plants can easily lodge but are beneficial in areas where more priority is for fodder, biomass fuel and thatching. Ringo *et al*, (2015) observed that heterobeltiosis for the range for panicle length was 10.6 to 17.1% while that of panicle width was 21.0 to 41.4%. However, Hemlata and Vithal (2006) reported relatively higher heterobeltiosis ranging from 39.6 to 48.4% for panicle length and low, 13.1 to 17.9% for panicle width respectively. Positive and significant average heterosis for panicle width ranged between 18.9 expressed in ICSA 12 × IESV 23019) to 54.7 in ICSA 88001 × KARI MTAMA 1. Heterobeltiosis for the same trait varied from 20.9 (CK60A × KARI MTAMA 1) to 40.8 (ICSA 293 × ICSR 24009) Ringo *et al*, (2015). Panicle exertion (length of panicle from ligule flag leaf to base of inflorescence) is an important characteristic that often determines the quality of the grains. Poor panicle exertion is disadvantageous because the leaf sheath provides favorable conditions for fungi and insects to develop at the base of the panicle hence extend to the whole panicle as also reported by Dogget, (1988).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sorghum materials

Plant materials were selected under field conditions through a survey which was conducted in the farmer fields. The varieties had been known to perform better as they were high yielding and preferred by farmers, however there reaction to covered kernel smut disease was unknown. The prevalence of the disease was high, the disease symptoms were observed in all local varieties, existing commercial varieties and the improved varieties from Rongo University (Okongo *et al*, (2019). The survey covered six counties located in Western Kenya along the shore of Lake Victoria where sorghum is grown in substantial quantity by smallholder farmers. The counties were Busia, Siaya, Kisumu, Vihiga, Homa Bay and Migori. These counties were selected because a good number of farmers in these counties had been given improved sorghum seed which were being promoted by Rongo University Sorghum Project (MC Knight Sorghum technical report, 2018).

Table 3-1: 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
N13	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	CREAM

3.2 Site description

The experiments for this study were conducted at Adiedo in Homa Bay County, Nyabisawa in Migori County, Kibos in Kisumu County and Eldoret in Uasin Gishu County.

The study to determine the response of selected sorghum varieties to covered kernel smut disease was conducted in two field sites, Adiedo and Nyabisawa during the 2018/2019 farming season. The sites were selected because they were among the six counties where the covered kernel smut disease prevalence and occurrence were high. They are located in Western Kenya along the shores of Lake Victoria.

Adiedo is located at 0 42S and 34 50E in Migori County. It has an elevation of 1221m above sea level, has a tropical climate, average annual temperature is 21.2 degrees celcius and a precipitation of 1369mm per year (Homa Bay Meteorological Station).

Nyabisawa is located at 1 07S and 34 42 E in Migori County. It has an elevation of 1281m above sea level, has a semi- arid climatic condition with daily temperature ranging between 26 degrees Celsius during coldest month (April and November) and 34

degrees celsius during hottest months (January-March). It receives between 250mm and 1200mm of rainfall annually, with average annual rainfall estimated at 1100mm, has two rainy seasons March-April-May (long rains) and September-November (short rains) (Migori Meteorological Station, 2020.).

The greenhouse screening was done in Eldoret University Research Farm, Eldoret. This experiment was to act as a control for the fields screening for the occurrence of the smut pathogen. It is also located in Western Kenya on the Uasin Gishu plateau west of the Great Rift Valley at 0.52N and 35.27E. It has an elevation of 2090 m above sea level. It has a warm and temperate climate, there is a great deal of rainfall even in the driest months. The temperature has an average of 15.8 degrees Celsius and the average amount of rainfall is 1263mm (University of Eldoret Meteorological Station, 2020.).

F1 Crosses were done at Kibos Research Station, Kisumu. This was to initiate the introgression of covered kernel smut disease into adapted farmer preferred varieties and heterosis observed in different agronomic traits in the developed crosses. The site lies within coordinates 0.59 S and 37.04 E at an altitude of 1548 meters above sea level. Its climate is modified by the presence of Lake Victoria. It has an annual relief rainfall that ranges between 1200mm and 1300mm, the rain mainly falls in two seasons. It's warm throughout the year with mean annual temperature of 23 degrees Celsius. Temperature ranges between 20 and 32 degrees Celsius, the humidity is relatively high throughout the year (Kibos Sugar Factory Meteorological Station, 2020.).

3.2.1 Field evaluation for covered kernel smut disease

The field evaluations were conducted in Homa Bay (Adiedo) and in Migori (Nyabisawa) hotspots of covered kernel smut disease during the May-July 2019 cropping season. A total of fifteen sorghum genotypes (described in section 3.1) were

used for covered kernel smut disease resistance screening. The land was cleared and farm yard manure incorporated into the soil at the rate of 2t/ha. The experiment was set up in a randomized complete block design (RCBD) with three replications at both sites. Each genotype was planted in a (2.25 X 4) M plot with 4 rows and 5 seeds were sown manually at a spacing of 25x75 cm per hole.

After 4 weeks of sowing the seedlings were thinned to 2 vigorous plants per hill while the missing stands were filled with good and healthy seedlings. Weeding was done twice, after the third and the sixth weeks. During the first weeding, that is after three weeks, top dressing was done using urea at the rate 0t two table spoonful per heel. When the crop was fully mature, harvesting was done manually by severing the panicle while the stalk is standing.

3.2.2 Inoculum collection and preparation.

Inoculum was prepared by collecting teliospores from mature sorghum panicles which showed symptoms of covered kernel smut disease from the field experiments in Adiedo and Nyabisawa sites by bagging the individual panicles. They were then allowed to dry for a week, this was done at the physiological maturity stage of sorghum. The panicles were then cut from the crop. Threshing was then done by pounding the affected sorghum heads in paper bags. A sieve was used to collect the teliospores by removing plant material and other debris. The affected panicles remained in the paper bags to prevent the teliospores from being disseminated and then stored in a refrigerator to prevent the teliospores from desiccation and germination.

Inoculum to be used in the greenhouse experiment was prepared by washing the teliospores in 80% ethanol plated on Potato Dextrose Agar (PDA) and the plates incubated in the at 28 degrees centigrade for 5 days. The colonies were then transferred

in flask containing 150 ml potato Dextrose Broth (PDB) and incubated on a shaker for 7days. The suspension was then filtered using a cotton cloth, this was then used to inoculate the seedlings with a hypodermic syringe when they were 20 days old (Frederiksen, 2000).

3.2.3 Greenhouse screening

Green house evaluation was conducted in Eldoret University Research Farm. This was used as a control experiment for the occurrence of the covered kernel smut disease in the fifteen genotypes used. Humidity was 20% and temperature 25 degrees Celsius, these conditions were kept constant throughout the growing period. The seeds were also sterilized by dusting with fungicides to prevent them from being attacked by diseases. Fifteen sorghum genotypes were grown in pots arranged in a completely randomized design (CRD) with three replications. Five seeds of each of the fifteen sorghum genotypes, each pot was filled with 1.5kgs forest soil + 0.15g 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. The inoculum suspension was then used to inoculate the seedlings with the help of a hypodermic syringe when they were 10 cm height (20 days old seedlings) according to the procedures of Fredericksen, 2000. The innoculum was injected into each seedling continuously until drops of the inoculum were seen at the top of each of the leaf. The genotypes were allowed to grow and mature then each panicle was scored for the percentage of florets bearing the smut at booting stage.

3.3 Data collection

3.3.1 Covered kernel smut disease incidence on the sorghum genotypes

Covered kernel smut disease incidence was assessed on infected panicles by determining the proportion of sorghum plants showing the symptoms of the covered kernel smut disease to the total number of sorghum plants in the plot, and the results expressed as percentage. While in the green house, each panicle was scored for the percentage of florets bearing smut teliospores as demonstrated by Chaube and Punder, (2005) using the formula:

Equation 3-1: Disease Incidence per Variety

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

3.3.2 Covered kernel smut Disease severity on the sorghum genotypes

Covered kernel smut disease severity was scored on the infected plants using a disease resistance classification scale described by Madhusudhan, *et al.*, 2011 and House, (1985) on a scale of 1-5 where 1 is immune showing less than 5% disease symptoms on the panicle, 2 is resistant showing 5 - 20% panicle area infected, 3 is moderately susceptible showing 20-40% head area attacked, 4 is susceptible with 40-60% head area covered with smut and 5 more than 60% with severe head damage as follows:

Table 3-2: Disease resistance classification scale

Severity resistance rating	**% panicle area infected**	**Description**
1	0%	Immune
2	1 -10%	Resistant

3	11 – 25%	Moderately susceptible
4	36 – 40%	Susceptible
5	>40%	Very susceptible

Note: Disease classification adopted from House (1985)

3.3.3 Effect of covered kernel smut disease on grain yield of different sorghum genotypes.

Grain yield was measured in each plot, the grain was then combined per plot, dried and weighed and mean yield data recorded.

3.4 To determine heterosis for agronomic traits in sorghum single crosses developed from tolerant and susceptible varieties to covered kernel smut disease as a first step to initiate introgression breeding for tolerance to the disease

A total of fifteen genotypes were used in this experiment. The resistant parents to covered kernel smut disease were; N4, MUK154, T30B, N13, E117B, IS3092, T53B and MUK27 while the farmer preferred varieties which were susceptible to the pathogen included: NYADUNDO1, NYADUNDO2, C26, MUK60, OCHUTI JOWI and SEREDO.

Table 3-3: List of crosses developed and used in the study

s/n	**Resistant parent(P1)**	**Susceptible parent(P2)**	**F1 Crosses**
1	MUK154	JOWI	JOWI X MUK154
2	MUK154	MUK60	MUK60 X MUK154
3	N13	SEREDO	SEREDO X N13
4	N13	JOWI	JOWI X N13

5	N13	C26	C26 X N13
6	N13	OCHUTI	OCHUTI X N13
7	N13	NYADUNDO2	NYADUNDO2 X N13
8	N13	MUK60	MUK60 X N13
9	IS3092	C26	C26 X IS3092
10	IS3092	NYADUNDO1	NYADUNDO1 X IS3092
11	IS3092	SEREDO	SEREDO X IS3092
12	IS3092	JOWI	JOWI X IS3092
13	IS3092	MUK60	MUK60 X IS3092
14	N4	MUK60	MUK60 X N4
15	N4	NYADUNDO1	NYADUNDO1 X N4
16	N4	SEREDO	SEREDO X N4
17	N4	C26	C26 X N4
18	N4	NYADUNDO2	NYADUNDO2 X N4
19	N4	OCHUTI	OCHUTI X N4
20	N4	JOWI	JOWI X N4
21	T30B	OCHUTI	OCHUTI X T30B
22	T30B	NYADUNDO2	NYADUNDO2 X T30B
23	T30B	C26	C26 X T30B
24	T53B	MUK60	MUK60 X T53B
25	T53B	NYADUNDO1	NYADUNDO1 X T53B
26	T53B	JOWI	JOWI X T53B
27	E117B	SEREDO	SEREDO X E117B

28	E117B	MUK60	MUK60 X E117B
29	MUK27	JOWI	JOWI X MUK27
30	MUK27	OCHUTI	OCHUTI X MUK27

Selfing was done during the flowering stage by bagging the resistant varieties with butter papers, this ensured that there was transfer of pollen grains of a floret to the stigma of same floret or another floret within the same panicle but no transfer of pollen to another panicle of a different plant. Bagging was done to the susceptible genotypes when few florets at the tip were open and at that time the tip was clipped off and the panicle bagged. The florets which had complete anthesis were clipped off and branches on the lower portion of the panicle also clipped leaving few florets in the central portion, after emasculation they were covered with paper bags and stapled. On the 4th day after emasculation, the pollen from the male parent which were the resistant varieties were taken into butter paper bag and slowly inserted into the emasculated panicle for the pollen to stick to the stigmas of the susceptible genotype. The crossed seeds were harvested after seed filling and maturation.

Determination of heterosis in agronomic traits in the F1crosses was then done by planting the parental varieties together with the F1 crosses along each other in a Randomized Completely Block Design replicated thrice. Recommended cultural practices were followed to raise a good crop. The experiment was conducted in Kibos research station, Kisumu. The F1 crosses had very few seeds per panicle which were true crosses, therefore, from each entry, only four plants which acted as the sample size of each plot were tagged randomly and data collected systematically from plants as follows; (i) *Number of days to 50% flowering*, the number of days taken from the date

of sowing to the date when 50% of the plants in the plot showed anthesis. (ii) *Plant height (4 plants sampled),* this was recorded in centimeters from base of the plant to the tip of the panicle at the time of physiological maturity. (iii) *Panicle length (4 plants sampled);* Length from the base of the panicle to its tip was measured and recorded in centimeters. (iv) *Number of leaves (4 plants sampled);* total number of leaves in the plant were counted and recorded. (v) *Hundred seed mass;* hundred grains were counted at random from each genotype and weighed in grams. (vi) *One panicle grain weight;* unthreshed panicle from each genotype was dried and weighed in grams and recorded. (vii) *Panicle width (4 plants sampled);* width of panicle was measured at the broadest point in centimeters. (viii) *Internodes' length (4 plants sampled);* length between the nodes was measured and recorded in centimeters.

3.5 Data Analysis

Data collected on disease severity and incidence was transformed using square root transformation method according to Berry (1987). While grain yield converted to t/ha and analyzed using R-Studio. Analysis of variance was done for each site according to Gomez and Gomez (1984). Differences were accepted as significant at $p<0.005$ and the means separated using Tukey's test.

All data on heterosis were collected as per standard sorghum descriptors (IPIGRI, 1993). The mid parent (relative heterosis) and better parent (heterobeltiosis) were computed according to Alam *et al*, (2004). The following formulae were used for the estimation of relative heterosis and heterobeltiosis for all the characters.

Equation 3-2: % mid parent heterosis (relative heterosis).

$$H\% = \left(\frac{F1 - MP}{MP}\right) \times 100$$

Equation 3-3: % better Parent heterosis (heterobeltiosis).

$$HB\% = \left(\frac{F1 - BP}{BP}\right) \times 100$$

Where H is mid parent heterosis (%), HB is heterosis over better parent (%), F1 is mean performance of F1 cross, BP is mean performance of better parent, MP =mean mid-parent value= (P1+P2)/2, P1=mean performance of parent one, P2=mean performance of parent two.

Test of significance for heterosis needs computation of standard error (SEm) for MP and BP, SEm was calculated based on error mean square (EMS) from the ANOVA table.

The significance of heterosis was then tested by comparing the calculated t value with the tabulated student t value for appropriate error degrees of freedoms at 5% and 1% levels of significance respectively.

Equation 3-4: t_{calc} for BP and MP$= \frac{\overline{F1} - \overline{BP}/\overline{MP}}{SEm}$

Where $SEm = \sqrt{\frac{2EMS}{r}}$

Where Ems = Error mean of squares

r = replications

CHAPTER FOUR: RESULTS

4.1 Covered kernel smut disease incidence on sorghum genotypes.

To determine the percentage mean incidence of covered kernel smut disease on the fifteen sorghum genotypes screened at Adiedo, Nyabisawa and in the greenhouse the following results were obtained.

4.1.1 Adiedo Site.

To determine the percentage mean incidence of covered kernel smut disease on the fifteen sorghum genotypes screened at Adiedo site the results obtained indicated that, Ochuti and Jowi, the local checks had the highest mean incidence of 56.7%, N13 and IS3092 had a mean incidence of 3% while MUK154, T53B, T30B, N4 and MUK27 had the lowest mean of 0% (Figure 4-1). Nyadundo2 and Nyadundo1 had a mean incidence of < 50% which compared well with the commercial check, Seredo which showed a mean incidence of 43.3%.

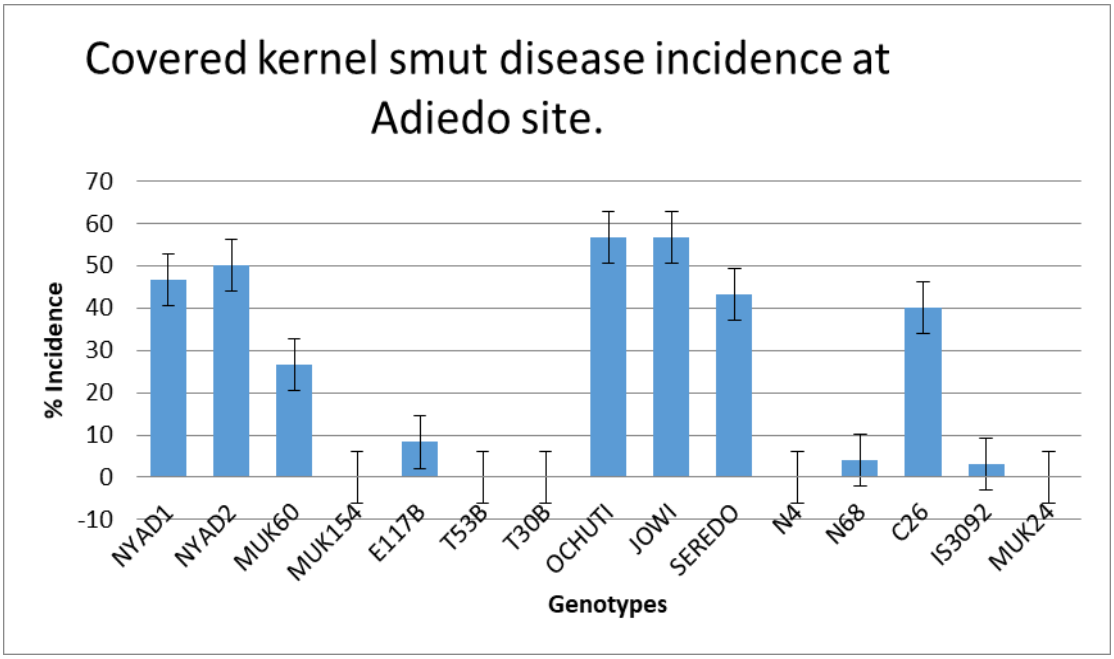

Figure 4-1: Covered kernel smut disease incidence at Adiedo site

There were significant differences ($p<0.001$) on the percentage incidence of covered kernel smut disease amongst the fifteen sorghum genotypes screened. (Table 4.1).

Table 4-1: Analysis of Variance for covered kernel smut disease incidence at Adiedo site.

SOV	**Df**	**Sum of squares**	**Mean square**	**F Value**
REP	2	27.78	13.89	0.29
GENOTYPE	14	23660.31	1690.02	35.47***
RESIDUAL	28	1334.22	47.65	
TOTAL	44	25022.31		
Grand mean	**SED**	**LSD**	**CV**	
22.4	**5.64**	**11.55**	**4.3**	

SOV-source of variation, df-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation.

4.1.2 Nyabisawa Site.

To determine the percentage, mean incidence of covered kernel smut disease on the fifteen sorghum genotypes screened at Nyabisawa site the results obtained indicated that, C26 had the highest mean incidence of 60 % which was statistically different from N13 and IS3092 which had a mean incidence of 3% while T53, MUK154, T30B N4 and MUK27 had the lowest mean of 0% (Figure 4-2). Ochuti and Jowi the local checks, Nyadundo1 and Nyadundo2 showed statistically similar mean of < 53.3%.

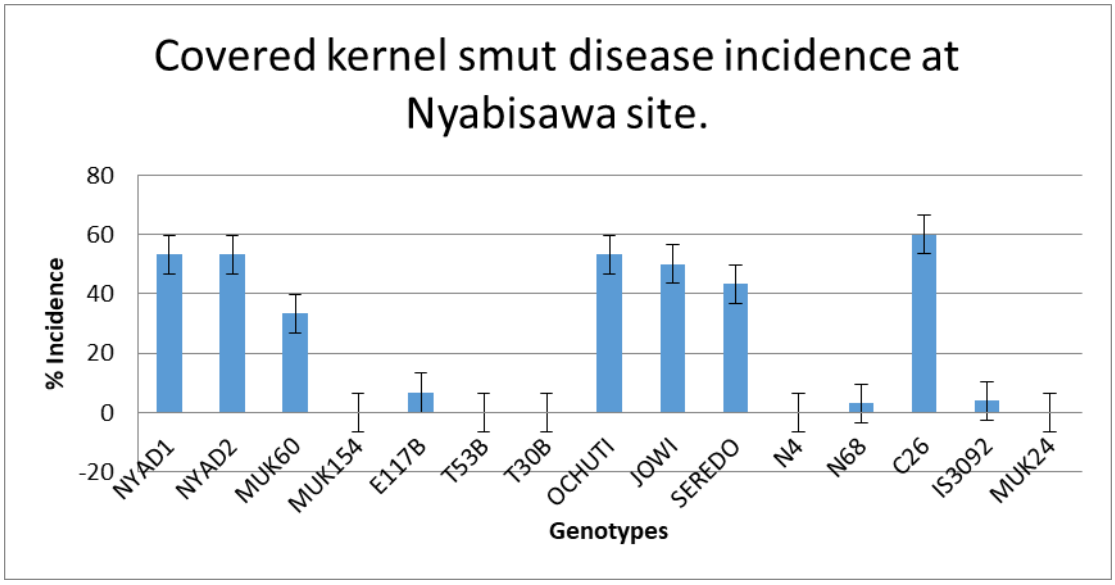

Figure 4-2: Covered kernel smut disease incidence at Nyabisawa site

There were significant differences ($p<0.001$) on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes screened.

Table 4-2: Analysis of Variance for covered kernel smut disease incidence in Nyabisawa site

SOV	DF	Sum of	Mean square	F Value

		squares		
REP	2	146.31	73.16	1.45
GENOTYPE	14	27106.98	1936.21	38.30***
RESIDUAL	28	1415.69	50.56	
TOTAL	44	28668.98		
Grand mean	**SED**	**LSD**	**CV**	
24.0	**5.81**	**11.89**	**9.2**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.1.3 Green house

To determine the percentage mean incidence of covered kernel smut disease on the fifteen sorghum genotypes screened in the greenhouse the results obtained indicated that, C26 and Nyadundo 2 had the highest mean incidence of 63.3% which was statistically different from IS3092 and MUK154 which had a mean incidence of 3% while T53B and T30B had the lowest mean of 0%. (Figure 4.3). Ochuti and Jowi the local checks showed statistically similar means of 60%.

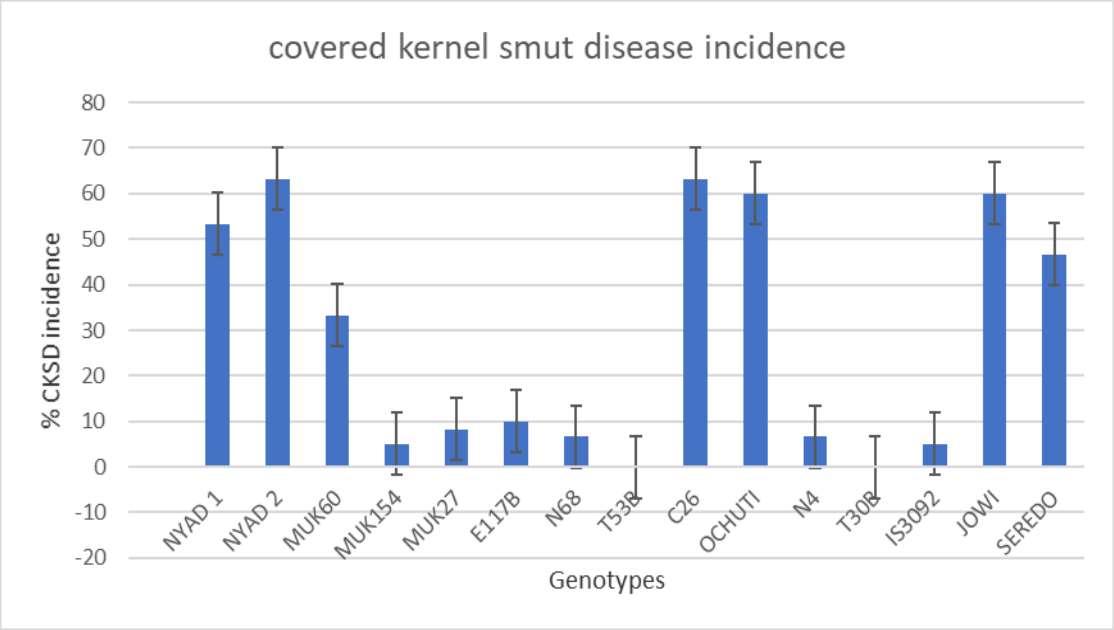

Figure 4-3: Covered kernel smut disease incidence in the greenhouse

There were significant differences ($p<0.001$) on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes screened.

Table 4-3: Analysis of Variance 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		
Grand mean	**SED**	**LSD**	**CV**	
28.11	**4.68**	**9.55**	**7.3**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.2 Severity score of covered kernel smut disease on sorghum genotypes.

The severity of covered kernel smut disease on the fifteen sorghum genotypes was determined by measuring the area of the panicle affected and scored using disease resistance classification scale and the following results were obtained from the two sites, Adiedo and Nyabisawa and in the greenhouse.

4.2.1 Adiedo Site.

MUK154, MUK27, T53B, N4 and T30B were statistically similar in terms of disease severity with the lowest score of 1. Therefore, these genotypes were considered immune to covered kernel smut disease. Another set of genotypes consisting of E117B, N13 and IS3092 had a disease severity score of 2 and hence were considered as 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.

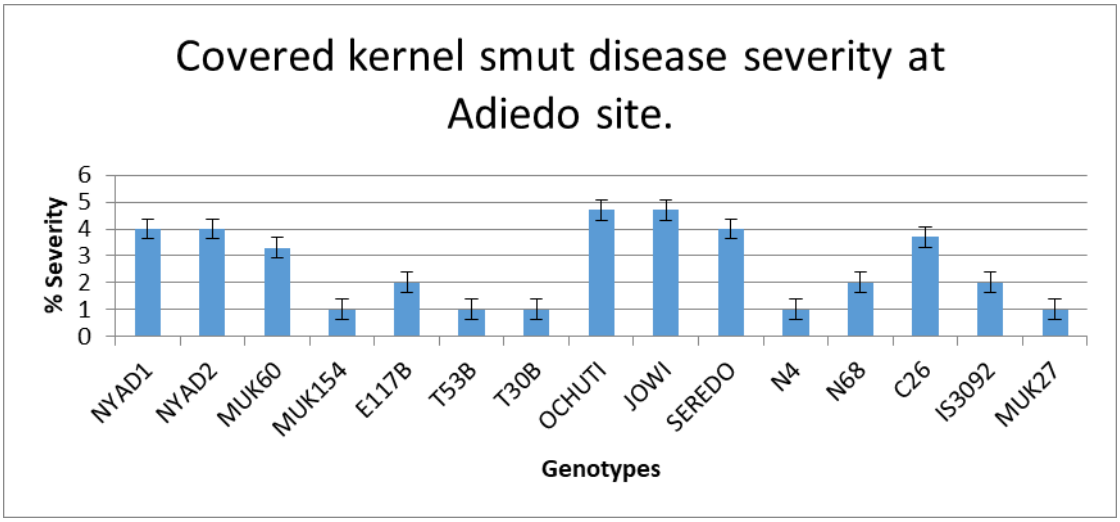

Figure 4-4: Covered kernel smut disease severity at Adiedo

The differences on the severity of covered kernel smut disease were significant ($p<0.001$) among the fifteen sorghum genotypes tested.

Table 4-4: Analysis of Variance for covered kernel smut disease severity at Adiedo site

SOV	**DF**	**Sum of squares**	**Mean square**	**F Value**
REP	2	0.0444	0.0222	0.13
GENOTYPE	14	89.9111	6.4222	38.90***
RESIDUAL	28	4.6222	0.1651	
TOTAL	44	94.5778		
Grand mean	**SED**	**LSD**	**CV**	
2.622	**0.3317**	**0.6795**	**1.5**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.2.2 Nyabisawa site.

MUK154, MUK27, T53B, N4 and T30B were considered statistically the same as they had the lowest score of 1 which make them to be classified as immune to covered kernel smut disease (Figure 4.5). Genotypes E117, N68 and IS3092 had a score of 2 and were

regarded as 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.

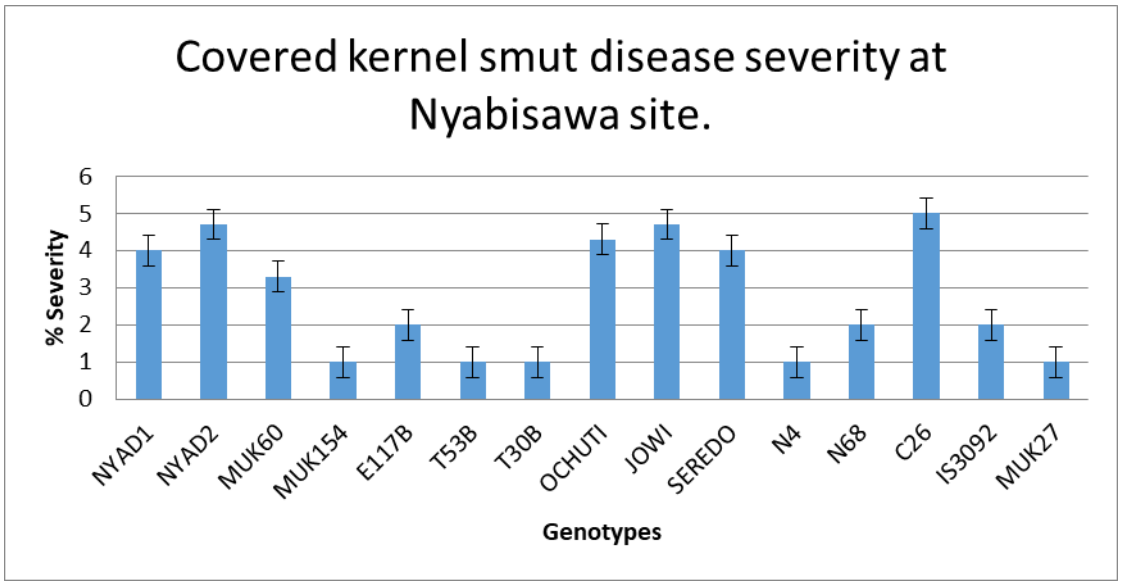

Figure 4-5: Covered kernel smut disease severity at Nyabisawa site

The differences on the severity of covered kernel smut disease were significant ($p<0.001$) among the fifteen sorghum genotypes tested.

Table 4-5: Analysis of Variance for covered kernel smut disease severity in Nyabisawa site.

SOV	DF	Sum of squares	Mean square	F Value
REP	2	0.1778	0.0889	0.55
GENOTYPE	14	102.5778	7.3270	45.70***
RESIDUAL	28	4.4889	0.1603	
TOTAL	44	107.2444		
Grand mean	**SED**	**LSD**	**CV**	

2.711	**0.3269**	**0.6697**	**2.8**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.2.3 Green house

MUK154, T53B, N4 and T30B were statistically the same as they had the lowest score of 1 which make them to be classified as immune to covered kernel smut disease (Figure 4.6). Genotypes E117, N68 and IS3092 had a score of 2 and were classified as resistant. C26, Ochuti, Jowi and Nyadundo2 on the other hand had the highest severity score of 5, and therefore were recorded as very susceptible. Nyadundo1, and Seredo had a score of 4 and therefore were considered susceptible.

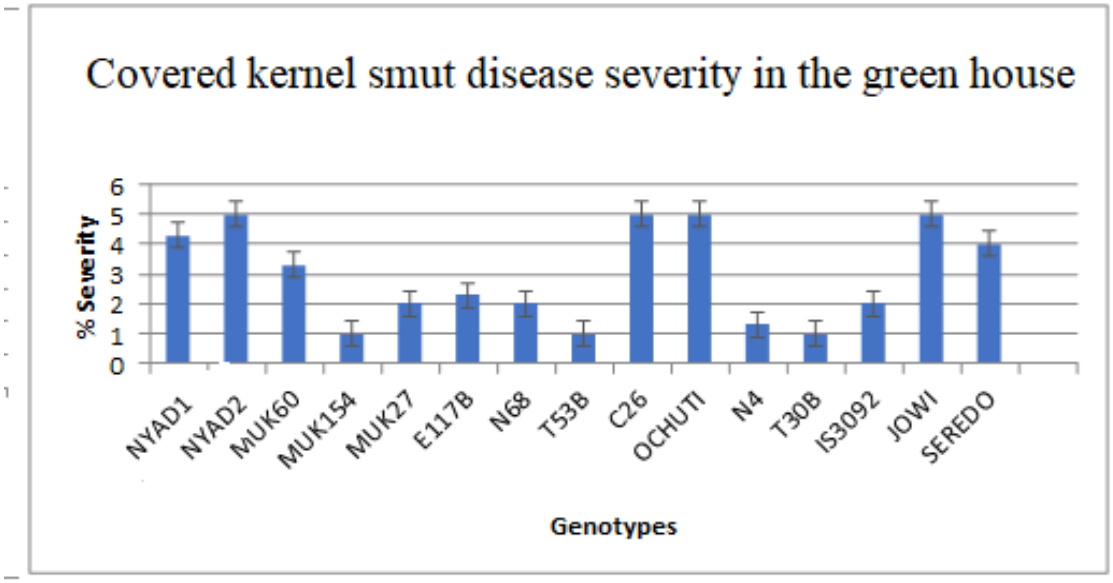

Figure 4-6: Covered kernel smut disease severity of the inoculated sorghum genotypes in the greenhouse**.**

The differences on the severity of covered kernel smut disease were significant ($p<0.001$) among the fifteen sorghum genotypes tested.

Table 4-6: Analysis of Variance 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		
Grand mean	**SED**	**LSD**	**CV**	
2.956	**0.3220**	**0.6577**	**13.3**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

Table 4-7: Analysis of Variance for covered kernel smut disease incidence in Migori and Homabay site

SOV	DF	Sum of squares	Mean square	F Value
REP	2	81.49	40.74	0.83
GENOTYPE	14	49989.29	3570.66	72.86***
LOCATION	1	62.50	62.50	1.28***
G X L	14	778.00	55.57	1.13***
RESIDUAL	58	2842.51	49.01	
TOTAL	89	53753.79		

SOV-source of variation, DF-degree of freedom, REP- Replication, G X L –Genotype by location

Analysis of variance (ANOVA) across locations is presented in Table 4-7. The differences on the incidence of covered kernel smut disease amongst the fifteen sorghum genotypes showed that Genotypic, Location and G X L interactions effects were highly significant ($p<0.001$) among the sorghum genotypes tested.

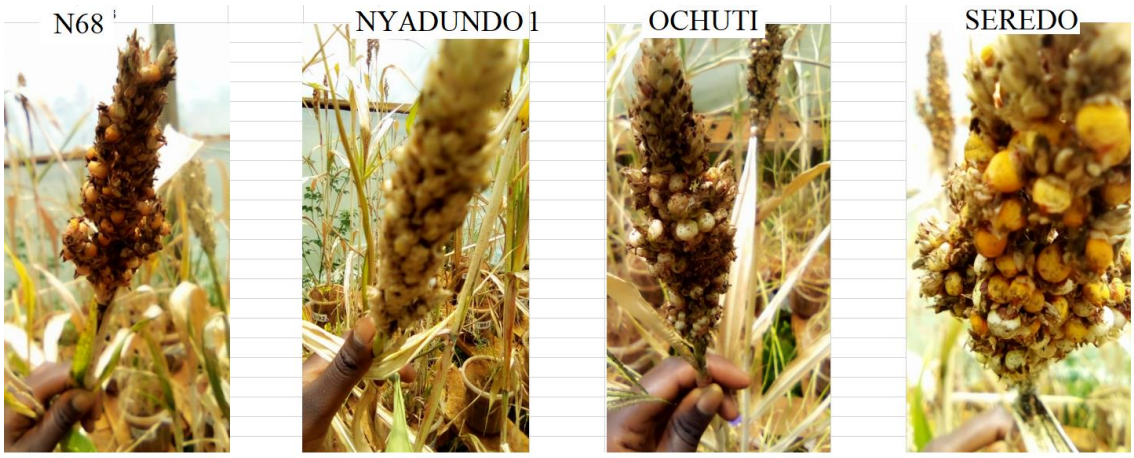

Plate 4-1: Different sorghum genotypes responding to covered kernel smut disease attack under greenhouse conditions.

4.3 Effect of covered kernel smut disease on the grain yield of different sorghum genotypes

To determine the effect of covered kernel smut disease on the grain yield of the fifteen sorghum genotypes, the following results were obtained from Adiedo and Nyabisawa sites.

4.3.1 Adiedo Site sorghum yields

T53 yielded the highest with a mean grain yield of 3.63t/ha while the least yielding was Seredo with a total mean yeild of 1.09t/ha (Fig.4-7).

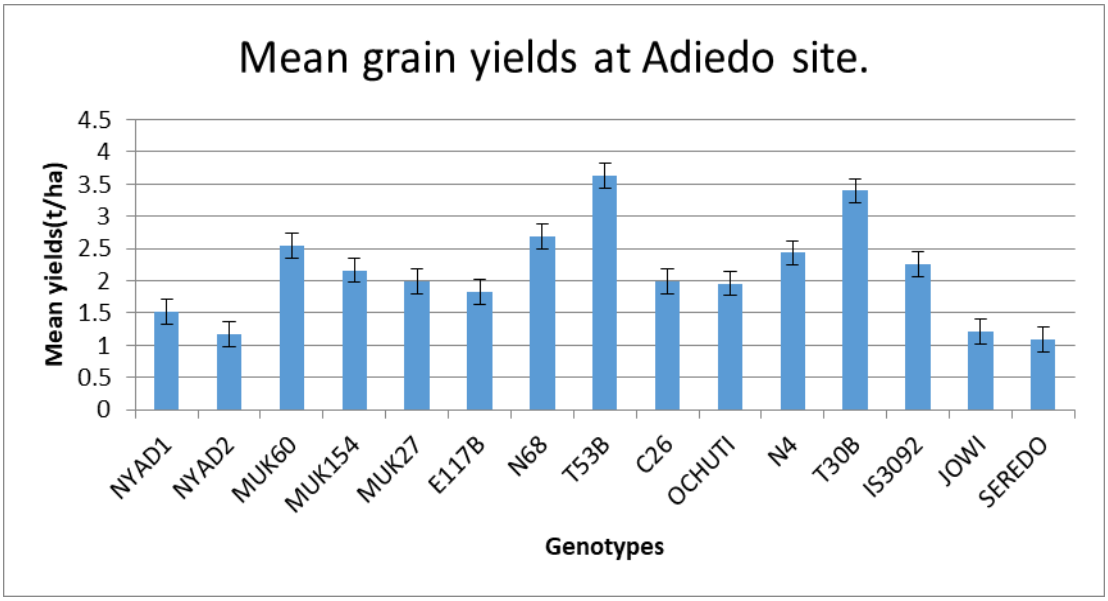

Figure 4-7: Mean grain yields of sorghum at Adiedo site

There were significant differences in the grain yield ($p<0.001$) among the different sorghum genotypes tested after harvesting, threshing, drying and weighing (Table 4-7).

Table 4-8: Analysis of Variance for covered kernel smut disease on the grain yield in Adiedo site.

SOV	DF	Sum of squares	Mean square	F Value
REP	2	0.02509	0.01254	0.50
GENOTYPE	14	3.27646	0.23403	9.34***
RESIDUAL	28	0.70158	0.02506	
TOTAL	44	4.00312		
Grand mean	**SED**	**LSD**	**CV**	
0.559	**0.1292**	**0.2647**	**5.2**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.3.2 Nyabisawa Site sorghum yields

N68 yielded the highest with a mean grain yield of 2.108t/ha, while the least significant grain yield was obtained from Seredo with a total mean yeild of 0.197t/ha (Figure 4-8).

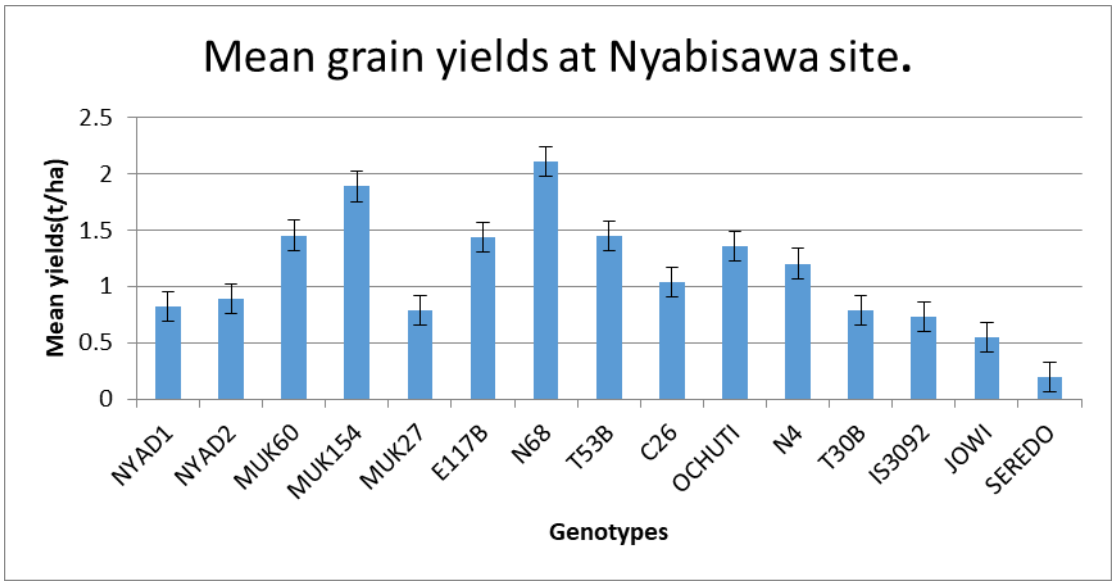

Figure 4-8: Mean grain yields of sorghum at Nyabisawa site.

In Nyabisawa there was significant differences in the grain yield among the different sorghum genotypes(Table 4-8).

Table 4-9: Analysis of Variance for covered kernel smut disease on the grain yield at Nyabisawa site

SOV	**DF**	**Sum of squares**	**Mean square**	**F Value**
REP	2	0.001522	0.000761	0.37
GENOTYPE	14	0.109609	0.007829	3.84***
RESIDUAL	28	0.057134	0.002040	
TOTAL	44	0.0168265		
Grand mean	**SED**	**LSD**	**CV**	
0.113	**0.037**	**0.076**	**6.4**	

SOV-source of variation, DF-degree of freedom, SED-standard error deviation, LSD-least significance difference, CV-coefficient of variation

4.4 To determine heterosis for agronomic traits in sorghum single crosses developed from tolerant and susceptible varieties to covered kernel smut disease as a first step to initiate introgression breeding for tolerance to the disease

The resistant varieties were crossed with the susceptible genotypes then the F1 crosses were harvested after seed filling and maturation. The F1s were the planted together with the parents to determine heterosis in some selected agronomic traits and the following results obtained. A total of 30 crosses were developed out of which 8 were true crosses (Table 4.10).

Table 4-10: Parental lines and the F1 crosses

Sn	Parent 1	Parent 2	F1 Cross
1	MUK60	N13	MUK60 X N13
2	MUK60	IS3092	MUK60 X IS3092
3	SEREDO	E117B	SEREDO X E117B
4	JOWI	MUK154	JOWI X MUK154
5	NYADUNDO	N4	NYADUNDO1 X N4
6	JOWI	N13	JOWI X N13
7	SEREDO	N13	SEREDO X N13
8	JOWI	MUK27	JOWI X MUK27

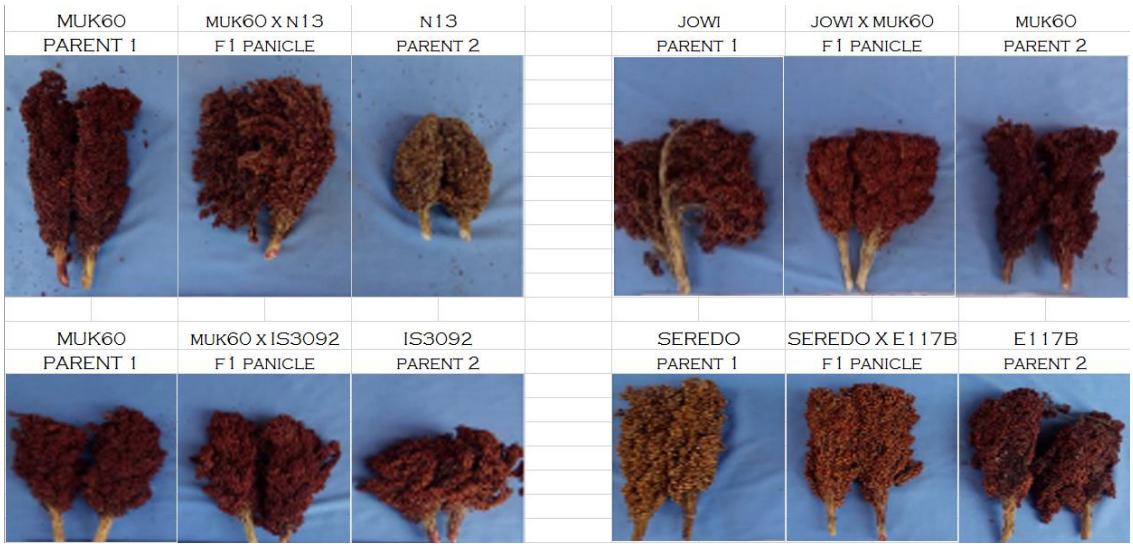

Plate 4-2: Some selected F1 crosses developed for resistance to covered kernel smut disease.

4.5 Description of F1 crosses and heterosis in various agronomic traits.

4.5.1 Analysis of variance of parents and F1s in different agronomic traits.

To determine heterosis in the number of days to 50% flowering, plant height, panicle length, number of leaves, seed mass, panicle weight, and panicle width and internodes length. The analysis of variance of parents and F1s for the agronomic traits are presented in Table 4-10. The mean sum of squares due to genotypes (parents and crosses) were highly significant for all the traits.

Table 4-11: ANOVA for various agronomic traits in sorghum.

	Mean sum of squares								
SOV	**Df**	**DF**	**PH (cm)**	**NL**	**INL (cm)**	**PL (cm)**	**PWI (cm)**	**PWE (g)**	**100SW (g)**
Replication	2	2.49	162.2	0.08	0.71	0.61	2.12	0.14	0.02
Genotypes	16	278.31*	6307.5*	44.05*	134.54*	40.16*	8.44*	2641.9*	2.80*
Error	32	0.59	204.8	0.31	0.54	0.76	0.39	0.39	0.23

Total	50	4476.98	107796.4	714.75	2171.29	668.63	151.65	4228.98	3.05
Mean		69.31	195.6	11.16	18.12	20.78	9.35	76.02	2.56
SED		0.63	11.68	0.45	0.6	0.72	0.51	0.51	0.07
LSD		1.28	23.80	0.92	1.22	1.46	1.04	1.04	0.14
CV		0.60	1.60	0.60	1.10	0.90	3.80	0.10	0.80

SOV-source of variation, **df**-degree of freedom, **PH**-plant height, **DF**-days to 50% flowering, **NL**-number of leaves, **INL**-internode length, **PL**-panicle length, **PWI**-panicle width, **PWE**-panicle weight, **100SW**-100 seed weight, **SED**-standard error deviation, **LSD**-least significance difference, **CV**-coefficient of variation, * significant at 1% level.

4.5.2 Mean performance of parents and crosses

For each of the parents and the F1 crosses, four plants which were tagged and data on the eight agronomic characters collected on them, there mean was calculated. The mean performance for the parents (Table4-11) and F1 crosses (Table4-12) for eight characters are presented below and discussed hereunder character wise.

Table 4-12: Mean performance of parental lines in agronomic traits.

SORGHUM LINES	**Days to 50% flowering**	**Plant height (cm)**	**Number of leaves**	**Inter-node length (cm)**	**Panicle length (cm)**	**Panicle width (cm)**	**Panicle weight (g)**	**100 Seeds weight (g)**
MUK60	77.00	173.00	12.00	12.00	22.33	6.70	72.00	3.00
N13	58.00	208.00	6.00	26.00	11.70	8.50	23.00	3.00
IS3092	82.00	211.30	11.00	15.00	16.33	12.60	61.00	3.00
SEREDO	75.00	178.00	10.00	6.00	23.00	8.70	74.00	4.00
E117B	77.00	175.00	10.00	11.00	18.70	11.70	79.00	3.00
JOWI	71.00	265.00	11.00	27.00	20.70	11.00	65.00	3.00
MUK154	67.00	156.00	11.00	12.00	22.70	7.70	76.00	3.00

NYAD1	53.00	128.00	8.00	16.00	20.30	7.70	48.00	3.00
IESV92038	73.00	125.00	9.00	15.00	24.00	8.30	71.00	4.00
Mean	**70.33**	**179.92**	**9.78**	**15.56**	**19.97**	**9.21**	**63.22**	**3.22**

Table 4-13: Mean performance of F1 crosses in respect to different agronomic traits

HYBRIDS	**Days to 50% flowering**	**Plant height (cm)**	**Number of leaves**	**Internode length (cm)**	**Panicle Length (cm)**	**Panicle width (cm)**	**Panicle weight (g)**	**100 Seeds weight (g)**
MUK60 X N13	53.00	253.00	9.00	24.00	21.50	10.50	73.00	3.00
SEREDO X E117B	79.00	240.00	12.00	9.00	21.70	6.30	68.00	4.00
JOWI X MUK154	77.00	230.00	10.00	24.00	25.30	10.70	97.00	3.00
NYAD1 X IESV92038	63.00	220.00	20.00	23.00	28.50	8.00	153.00	4.00
JOWI X N13	63.00	178.30	9.00	25.00	18.70	9.30	44.00	3.00
JOWI X MUK60	72.00	156.30	11.00	22.00	20.00	12.00	126.00	3.00
SEREDO X N13	63.00	135.00	10.00	25.00	18.70	10.00	77.00	4.00
MUK60 X IS3092	82.00	120.00	21.00	20.00	21.00	9.33	87.00	3.00
Mean	**69.00**	**191.58**	**12.75**	**21.50**	**21.93**	**9.52**	**90.63**	**3.63**

4.5.2.1 Days to 50% flowering

Among the 9 parents evaluated, NYADUNDO1 was the first to flower at 53 days followed by N13 (58 days), whereas Seredo (75 days) took comparatively more numbers for 50% flowering followed by E117B (76 days) and IS3092 (82 days). Among the 8 F1 crosses, the cross MUK60 X N13 (53 days) was the earliest to flower followed by SEREDO X N13 (63 days) and JOWI X N13 (63 days). However, MUK60 X IS3092 (82 days) took longest duration to 50% flowering followed by JOWI X MUK154 (77 days).

4.5.2.2 Plant height (cm)

JOWI was the tallest with a mean plant height of 265cm followed by IS3092 (211.3cm) while NYADUNDO1 was the shortest with a mean plant height of 125cm followed by MUK154 (155cm), MUK60 (173cm) and E117B (175cm). Maximum plant height was recorded by F1 cross MUK60 X N13 (253cm) which differed significantly from all other crosses. On the other hand, lowest plant height was recorded by the cross MUK60 X IS3092 (120cm) followed by SEREDO X N13 (135cm) and F1 cross of NYADUNDO1 X IESV92038/SH (156.3cm).

4.5.2.3 Panicle length (cm)

Among the 9 parental lines the highest mean panicle length was observed in IESV92038/SH (24cm) followed by SEREDO (23cm) and MUK154 (22.7cm). The lowest panicle length was observed in N13 (11.7cm). The cross NYADUNDO1 X IESV92038/SH (28.5cm) exhibited highest panicle length followed by JOWI X MUK154 (25.3cm) then JOWI X MUK60 (20cm) and MUK60 X N13 (21.5cm) whereas the lowest panicle length was observed in cross JOWI X N13 (18.7cm).

4.5.2.4 Panicle weight (g).

Among 9 parental lines, the line E117B recorded highest mean performance of 79g followed by MUK154 (76g) whereas other lines significantly differed from these two lines by having a mean performance range of MUK60 (72g) to N13 (23g). Out of the 8 F1 crosses, two exhibited above 100g weight per panicle. NYADUNDO1 X IESV92038/SH recorded the highest weight of 153g followed by JOWI X MUK60 (126g), The performance of F1 crosses in terms of panicle weight varied from to 97g-44g for F1 crosses of JOWI X MUK154 and JOWI X N13.

4.5.2.5 Panicle width (cm).

The highest panicle width was recorded in IS3092 (12.6cm) followed by E117B (11.7cm) which are statistically different, on the other hand the lowest panicle width was recorded in MUK60 (6.7cm). Among the F1 crosses, JOWI X MUK60 (12cm) recorded highest panicle width followed by MUK60 X N13 (10.3cm) and SEREDO X E117B (10.7cm) which were statistically different. However, lowest panicle width was recorded in NYADUNDO1 X IESV92038/SH (8cm).

4.5.2.6 100 grain weight (g).

Among the 9 lines, SEREDO and IESV92038/SH recorded maximum 100 grain weight of 4g and the lowest was 3g recorded in MUK60, N13, IS3092, JOWI and NYADUNDO1 which were statistically significant. Maximum 100 grain weight was recorded by SEREDO X E117B (4g), JOWI X N13 (4g) and SEREDO X N13 (4g) which were also statistically significant. Minimum 100g weight was recorded by the crosses MUK60 X N13, MUK60 X IS3092, JOWI X MUK154 and JOWI X MUK60 which were 3g.

4.5.3 Estimates of Heterosis for different agronomic traits of parents and F1 crosses

To draw the valid conclusions regarding the extent of heterosis for the eight agronomic traits in sorghum, the overall means of parents, and F1 crosses were computed to obtain relative heterosis and heterobeltiosis for all the characters.

Table 4-14: Estimates of Heterosis

F1 cross	**Days to 50% flowering**		**Plant height (cm)**		**Number of leaves**		**Internode length (cm)**	
	H%	**HB%**	**H%**	**HB%**	**H%**	**HB%**	**H%**	**HB%**
MUK60 X N13	-21**	-31**	-30**	-44**	0	-25**	26.3**	-8.3**
MUK60 X IS3092	-9.4**	13.9**	2.99**	-9.6**	82.6**	75**	48.1**	33.3**
SEREDO X E117B	3.27**	2.6**	4.57**	1.89**	20**	20**	5.88**	-18**
JOWI X MUK 154	-6.3**	6.94**	14.3**	-10**	10*	-9.1*	54.3**	-13**
NYAD1 X IESV92038/SH	29.1**	6.67**	-23**	-36**	13.5**	122**	48.4**	50**
JOWI X N13	2.23**	11.3*	-38**	-49**	5.88	-22**	-5.7*	-7.4**
SEREDO X N13	-6**	-3.9**	28.7*	25.7**	25**	0	56.3**	-3.8
JOWI X MUK60	16.2**	-19	-16**	-18**	9.09	-8.3*	12.8**	-19**

CONTINUATION

Cross	Panicle length (cm)		Panicle width (cm)		Panicle weight (g)		100 seeds weight (g)	
	H%	HB%	H%	HB%	H%	HB%	H%	HB%
MUK60 X N13	26.4	-3.7	38.2**	23.5**	53.7**	1.39*	38.89*	25
MUK60 X IS3092	8.64**	-6	-3.3	-26**	30.8**	6.94**	-9.68	-33.33**
SEREDO X E117B	4.08*	-5.7	-38**	-46**	-11	-14**	-10.34	-38.10**
JOWI X MUK 154	16.6**	11.5**	14.4*	-2.7	37.6**	27.6**	-14.29	-28.57**
NYAD1 X IESV9203 8/SH	28.7**	18.8**	0	-36	157**	11.5**	-16.67	-6.67
JOWI X N13	16.9**	-7.9**	6.29**	-15	0**	-32	33.3**	33.3**
SEREDO X N13	7.78	-19**	16.3**	14.9*	58.8**	4.05**	-6.90	-10.00
JOWI X MUK60	-5.7*	-10**	35.6**	9.09	83.9**	75**	-11.11	-23.81**

Hb-Heterosis over better parent, ** significant at 1%, Ht-Heterosis over mid parent

4.5.3.1 Plant height (cm).

The per cent heterosis for plant height ranged from -38 % (JOWI X N13) to 28.7% (SEREDO X N13) over mid parent, -49 % (JOWI X N13) to 25.7 % (SEREDO X N13)

over better parent. The cross SEREDO X N13 exhibited highest positive mid parent (28.7%) and better parent (25. 7%). F1s that exhibited negative significant relative heterosis and heterobeltiosis were 4 and 6 respectively (Table 4-13). MUK60 X N13 was the shortest.

4.5.3.2 Panicle length (g).

The magnitude of heterosis varied from -5.7% (JOWI X MUK60) to 28.7% (NYADUNDO1 X IESV92038/SH) over mid parent -19% (SEREDO X N13) to 50% (NYADUNDO1 X IESV92038/SH) over better parent. The cross NYADUNDO1 X IESV92038/SH had the highest positive mid parent (28.7%) and better parent (18.8%). F1s that exhibited both significant positive heterosis and heterobeltiosis were 7 and 2 respectively (Table4-13).

4.5.3.3 Panicle weight (g).

The magnitude of heterosis ranged from -11% (SEREDO X E117B) to 157% (NYADUNDO1 X IESV92038/SH) over mid parent, -32 % (JOWI X N13) to 115 % (NYADUNDO1 X IESV92038/SH) over better parent. The cross NYADUNDO1 X IESV92038/SH had the highest positive midparent (157%) and better parent (115%). F1 crosses that exhibit both positive relative heterosis and heterobeltiosis were 7 and 6 respectively. (Table 4-13).

4.5.3.4 Panicle width (cm).

The range of per cent heterosis for panicle width ranged from -38 % (SEREDO X E117B) to 38.2% (MUK60 X N13) over mid parent, -46 % (SEREDO X E117B) to 23.5% (MUK60 X N13) over better parent. The cross MUK60 X N13 had the highest positive heterosis in midparent (38.2%) and better parent (23. 5%).

4.5.3.5 100 grain weight (g).

The magnitude of heterosis ranged from -6.90% (SEREDO X N13) to 38.89% (MUK60 X N13) over mid parent and -38.10 % (SEREDO X E117B) to 33.3% (MUK60 X IS3092) over better parent. The cross MUK60 X N13 had the highest positive midparent (38.89%) and better parent (25%).

4.5.3.6 Days to 50% flowering.

Relative heterosis for days to 50% flowering varied from -21% (MUK60 X N13) to 29.1% (NYADUNDO1 X IESV92038/SH) whereas, heterobeltiosis ranged between -31% (MUK60 X N13) to 13.9% (MUK60 X IS3092). Negative relative heterosis and heterobeltiosis was expressed in MUK60 X N13 indication of early maturity.

4.5.3.7 Number of leaves.

The percent heterosis for the number of leaves ranged between 0% (MUK60 X N13) to 82.6 (MUK60 X IS3092) for mid parent, while, -25% (MUK60 X N13) to 75% (MUK60 X IS3092) for better parent. Positive significant relative heterosis and heterobeltiosis was exhibited by 1 and 3 F1 crosses respectively.

4.5.3.8 Internode length (cm).

For internode length, the F1s recorded a mid-parental heterosis ranging from -5.7% (NYADUNDO1 X IESV92038/SH) to 56.3% (SEREDO X N13) and heterobeltiosis ranged from -19% (JOWI X MUK60) to 50% (NYADUNDO1 X IESV92038/SH) (Table 4.13).7 F1 crosses expressed significant positive average heterosis, while 2 crosses manifested significant positive heterobeltiosis.

CHAPTER FIVE: DISCUSSIONS

5.1 Effects of Covered Kernel Smut Disease on sorghum genotypes.

The fifteen sorghum genotypes responded differently to covered kernel smut disease in Nyabisawa site in Migori, Adiedo site in HomaBay and in the greenhouse, with the highest incidence of 64 % in Nyadundo 2 and C26 while the least incidence was 0% in T53B and T30B, this is an indication of genetic differences among the sorghum genotypes tested.

The disease infection occurred at both field sites and followed a similar trend which indicated that the two sites exhibited conducive enviroment such as temprature of 18-25 degrees celcius and warm soils with a humidity of 15-20% for the covered kernel smut disease development (Selveraj, 2013). The presence of highly significant differences between the two test sites and the greenhouse for all the fifteen genotypes indicated that the genotypes performed differently across the three test environments, the highest disease incidence was 64%, 60% and 57% in the green house, Migori and Homa Bay sites respectively.

Analysis of variance (ANOVA) across locations showed that Genotypic, Location and G X L interactions effects were highly significant ($p<0.001$) among the sorghum genotypes tested (Table 4- 7). These differences suggested that there was high variation among the genotypes, locations and their interaction. Genotypic effects exhibited the highest (92%) contribution to the total of the total sum of squares (TSS), Sites contributed 0.12% of the TSS for variation and GXL contributed 1.45% of TSS while the rest were unexplained variance which went to Residual sum of squares. These findings compare well with those of Shunmugavali, (2005) who also recorded higher

genotypic contribution to the TSS and this was attributed to genotypic differences among the germplasm used. In our case, this was expected since the sorghum germplasm used were of diverse origin and their response to disease was varied. The highly significant differences between the two locations for the occurrence and distribution of covered kernel smut disease, suggested that the pathogens occurrence and distribution was different in the two sites probably due to differences in environmental conditions and edaphic factors such temperature, soil moisture and humidity that could have ether accelerated or decelerated disease progression (Mortazaviain *et al*., 2014). It also suggests that there was significant contribution of environmental variance to the observed differences with regard to disease reactions. The significant GXL interaction implied that different varieties could be selected for different agro ecologies (Derera et al., 2008). This gives the plant breeder the choice of either developing specific genotype that can perform well under variable conditions as suggested by Dehghani *et al.,* (2006).

The disease mean incidence varied from 0 – 60 % among the genotypes tested which was associated with the pathogen specificity to the various sorghum genotypes, MUK154, T53B, N4 and T30B had an incidene of 0% in all the test sites therefore the covered kernel smut disease was only specific to the other eleven genotypes. The findings compare well with that of Wilson, (2011) who suggested that the host species specificity of the pathogen could vary according to different genotypes of sorghum. Further, the variations could have been due to the differences in the individual inherent reaction to covered kernel smut pathogen (Gwary *et al*, 2007).

The higher disease prevalence in some genotypes in the greenhouse than in Nyabisawa site in Migori and Adiedo site in Homa Bay was associated with delayed seed

germination that occurred in greenhouse due to the cool conditions which could have been optimal for further contamination of the plants. It might also be attributed to the pathogens biology, the fungal pathogen *Sporosorium sorghi* which transmit CKSD can remain viable in the soil for a very long time as teliospores germinating with the seeds, which is systemic beccause the soils in the pot were also mixed with the teliospores (Gwary *et al*, 2007).

Significant variations in disease severity were observed in the fifteen genotypes. The varieties were categorised as immune (1), resistant (2), moderately susceptible (3), susceptible (4) and very susceptible (5) as described by Marley *et al* (2002). The varieties that were immune included MUK154, T53B, T30B, N4 and MUK24, resistant varieties were E117B, N68 and IS3092, Susceptibles were Seredo, Nyadundo1 and Nyadundo 2 while Ochuti and Jowi were very susceptible. This was expected because of the variations in the genetic make-up of the different genotypes. This compares well with the findings of Gwary *et al*., (2007), that the differences obtained on disease severity may be due to the differences in the individual genotype inheritance reaction to the pathogen. 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 different sorghum 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.

The reaction of covered kernel smut disease was different in both fields and in the greenhouse, Disease severity in the greenhouse was higher compared to the fields. The

highest severity score in the green house was 5 will in the field it was 4.3 this could be due to variations in the enviromental factors and uneven distribution of innoculum in the soil. This was in line with the findings of Thakur *et al*., (2007) 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 even though in our case it was successful. Although for our case, the field screening was effective in the season when the experiment was done.

Covered kernel smut disease significantly reduced grain yield on different sorghum genotypes in the two fields. The most affected was Seredo which had a mean yield of 1.09t/ha, with a disease incidence of 50% and severity score of 4 therefore described as susceptible to the pathogen while the least affected was T53B which had a mean yield of 3.63t/ha with a disease incidence of 0% and a severity score of 1, therefore immune to the disease. Covered kernel smut destroys all of the kernels in the head and replaces them with fruiting bodies or may affect only portions of the panicle hence yield is reduced. Similar findings were also reported by Hamilton *et al.,* and Merkuz *et al*., (2011) that, sorghum genotypes responded differently to covered kernel smut disease attack hence yielding differently and caused heavy reduction in grain yield of sorghum genotypes.

The greenhouse was used as a control experiment to minimize the effects of variables which were not of interest to the study. The results obtained were very significant in the determination of resistant and the susceptible genotypes.

5.2 To determine heterosis for agronomic traits in sorghum single crosses developed from tolerant and susceptible varieties to covered kernel smut disease as a first step to initiate introgression breeding for tolerance to the disease

A total of 30 crosses were developed, however only 8 were true crosses, these included: JOWI X N13, JOWI X MUK60, MUK60 X N13, SEREDO X E117B, JOWI X MUK154, SEREDO X N13, MUK60 X IS3092 and NYADUNDO1 X N4. The rest were unsuccessful. This low success rate is normally expected in sorghum when using inbred lines which are open pollinated due to sorghum being a self-pollinating plant. Other issues could be related to temperature variations which could have led to pollen death before successful pollination. In our case temperature variations partly affected the process besides excessive rains that also impaired the fertilization process. These results compared well with those of Wilson, (2012) who reported 30% seed set with hand emasculated sorghum plants, premature emasculation of the florets and temperature variations were some of the reasons cited for the low success rates. Muraya *et al* (2011), reported higher seed set of 80%, this was in contrast with the results from this study because they used male sterile lines which could not release functional pollen therefore self-pollination was inhibited on the crosses.

Analysis of variance showed that there were highly significant variations among the parents and the crosses for all the traits studied. Mean squares due to parents and crosses were also significant for plant height, days to 50% flowering, number of leaves, internode length, panicle length, panicle width, panicle weight and 100 seeds weight. These phenotypic differences among the various F1s can be attributed to genetic variations for reactions to covered kernel smut disease that existed among sorghum genotypes. In most cases, means of the F1s exceeded the means of the parents for all the

agronomic traits which is attributed to heterosis, Springer and Stupar, (2007). In all the cases, the F1s showed positive, negative and no heterosis of which the former two shows transgressive inheritance of covered kernel smut disease. The genetic basis of heterosis include dominance, overdominance or epistatic effects Birchler *et al*, (2003).

Negative heterosis for traits such as Days to 50% flowering, for instance in MUK60 X N13 was highly desirable as it is an indication of an early maturing hybrid. Similar findings have been documented by Helmata and Vithal, (2006) who reported low heterosis of (-3.55to -22.45) on days to flowering which they used as a basis of their selection. In order to overcome covered kernel smut disease, breeders tend to select early maturing sorghum as delayed maturity always gives the fungus time to establish itself in the plant Sisay *et al*, (2004).

For other traits such as panicle weight, panicle length and panicle width, positive heterosis and heterobeltiosis was highly desirable as it was a sign of genetic gain in yield which was a sign of some level of resistance to the disease.

Hybrids that exhibited positive heterosis and heterobeltosis for plant height such as MUK154 X JOWI and JOWI X N13 were tall in stature which was in agreement with the findings of Okongo *et al*, (2019) who reported that most of the tall sorghum varieties were resistant to the fungi across many environments.

Different sorghum genotypes responded differently to covered kernel smut disease under field and greenhouse conditions making it possible to select the resistant and susceptible genotypes for use in managing the disease, this was attributed to genetic differences which existed in the different genotypes. The susceptible and resistant genotypes were then crossed resulting into better performing F1s in agronomic traits

than the parents which can be used in further breeding programs to obtain sorghum varieties that are resistant to covered kernel smut disease.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS.

The following conclusions were drawn from the study:

1. In this study, there was great genetic variation observed among the sorghum genotypes in response to Covered kernel smut disease. The fungus did not affect all sorghum genotypes tested. T53, N4, T30, IS3092 and MUK154 showed some level of immunity while Nyadundo 1 and 2, Ochuti, Jowi and C26 were susceptible to the disease.
2. The study developed eight crosses; MUK60 X N13, MUK60 X IS3092, SEREDO X E117B, JOWI X MUK154, JOWI X N13, SEREDO X N13 and JOWI X MUK60 and NYADUNDO1 X N4 which were confirmed to contain relevant traits introgressed by detecting both positive and negative heterosis for several agronomic traits which is an indication of transgressive inheritance of covered kernel smut disease resistance.

This study recommends;

1. Promotion and adoption of the high yielding and resistant sorghum genotypes for direct utilization by farmers in covered kernel smut disease hot spots.
2. Further advancing and testing of the new crosses for development of covered kernel smut disease resistant varieties and determine genetic control and inheritance of the pathogen in sorghum.

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APPENDICES

Appendix I : Letter from the Host Institution

RONGO UNIVERSITY

OFFICE OF THE DEAN
SCHOOL OF GRADUATE STUDIES

Tel. 0771349741

P.O. Box 103 - 40404
RONGO

Our Ref: **MPLB/6001/2015**

Date: Thursday, January 23, 2020

The Chief Executive Officer,
National Commission for Science, Technology & Innovation,
off Waiyaki Way, Upper Kabete,
P.O Box 30623-00100,
Nairobi-KENYA.

Dear Sir,

RE: RESEARCH PERMIT FOR MS. OKONG'O CAROLINE AUMA-MPLB/6001/2015

We wish to inform you that the above person is a bona fide graduate student of Rongo University in the School of Agriculture Natural Resources and Environmental Studies pursuing a Master degree in Plant Breeding. She has been authorized by the University to undertake research titled; **"Breeding for Resistance to Covered Kernel SMUT(Sporisorium sorghi) Disease in Sorghum(Sorghum bicolor)."**

This is, therefore, to request the commission to issue her with a research permit to enable her proceed for field work.

Your assistance to her shall be highly appreciated.

Thank you.

RONGO UNIVERSITY
THE DEAN
23 JAN 2020
SCHOOL OF GRADUATE STUDIES
P. O. BOX 103 - 40404, RONGO

Dr. Edward Anino
DEAN, SCHOOL OF GRADUATE STUDIES

Copy to: Vice Chancellor
Deputy Vice Chancellor (Academic and Student Affairs).
Dean, School of Agriculture Natural Resources and Environmental Studies
HoD, Agronomy and Environmental Sciences

Appendix II: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 799020

Date of Issue: 16/December/2020

RESEARCH LICENSE

This is to Certify that Ms.. Caroline Okong'o of Rongo University, has been licensed to conduct research in Kisumu, Migori, Uasin-Gishu on the topic: Breeding for Resistance to Covered Kernel SMUT (Sporisorium sorghi) Disease in Sorghum (Sorghum bicolor) for the period ending : 16/December/2021.

License No: **NACOSTI/P/20/8145**

799020

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

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