

REACTIONS OF MAIZE GENOTYPES TO *USTILAGO MAYDIS* (DC) CORDA

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<https://doi.org/10.35410/IJAEB.2022.5768>

ABSTRACT

Maize production in Tanzania has still been relatively low due to limiting factors such as diseases. Maize smut caused by *Ustilago maydis* is among those diseases. This study aimed to determine response to *U. maydis* by maize genotypes cultivated in Sengerema district, Tanzania. Five (5) maize genotypes, namely SEEDCO SC 419, ZAMSEED ZMS 402, PIONEER PHB 3253, LUBANGO HYBRID, and GEMBE were selected for use in the study. Control plots were established for yield loss quantification. 2mls and 3mls (1×10^6 teliospores mL⁻¹) were injected into apical node of 60cm tall plant and into the ear silk of plant before pollination respectively to ensure uniform infection and thus eliminate disease escapes. Maize smut disease was assessed based on its symptoms and signs. Data on the smut incidence, severity, number of kernels per cob, total grain weight, yield, and yield loss were collected. Smut disease incidence, severity and yield loss had significant impact among the tested genotypes ($p < 0.001$). The highest and lowest average disease incidence was in GEMBE (5.22%) and ZAMSEED ZMS402 (0.66%) respectively. While the highest and lowest mean severity were in GEMBE (2.48cm) and SEEDCO SC 419(0.07cm) respectively. The average yield loss for SEEDCO SC 419 was 51.89% recorded as the lowest. While for the ZAMSEED ZMS402 was 65.17% recording as the most susceptible. Based on the results SEEDCO SC419 was the most tolerant genotype to *U. maydis*. Differences in susceptibility levels among the maize genotypes is an important factor influencing *U. maydis* infection success.

Keywords: Incidence, Losses, Severity, *Ustilago maydis*, Yield.

Highlights

- Smut incidence and severity levels are variable among the maize genotypes
- Yield reductions due to maize smut are influenced by susceptibility levels of the genotypes

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the major food crops in Tanzania due to being cultivated and consumed in higher amounts (Luzi-Kihupi et al., 2015; Nassary et al., 2020). Regardless of maize cultivation being practiced in every region in Tanzania, relatively higher in Lake Zone (Kiruwa et al., 2020). The maize production rate in Tanzania has been relatively lower compared to other maize-growing countries like South Africa (Kihara et al., 2021) due to various factors such as drought, pest infestations, and diseases. Maize smut (common smut) disease caused by a fungus (*Ustilago maydis*) (Milisavljevic et al., 2018) is among the major diseases affecting maize production (Suleiman et al., 2017). It occurs in

every growing area and season, causing significant yield losses and damages (Skibbe et al., 2010). The yield of infected maize plants can be reduced by 40 to 100% (Matei and Doehlemann, 2016). With maize being an important food source for both animals and humans, a 40% loss of yield may prove devastating for the supply of food (Suleiman et al., 2017). Yield loss cases in maize production due to maize smut disease have been reported mostly in Lake Zone regions (Nyambo, 2009). This is because maize growers in the Sengerema district have relatively low or completely no knowledge of maize smut and how to manage the disease to reduce the impacts of *U. maydis* infections (Suleiman and Rosentrater, 2015). Likewise, already no effective fungicide against maize smut is known (Aydoğdu, 2015). Consequently, it is crucial to benefit from tolerant varieties in the breeding and management of the pathogen (Aydoğdu and Boyraz, 2016). The impact degree of *U. maydis* infection in inducing damages and yield losses vary in the growing season, location, and among the maize varieties or cultivars, depending on the susceptibility level of the particular variety or cultivar (Agrios, 2005). Besides reducing yield, smut disease can cause significant losses to the processing industry by adversely affecting product quality (Mueller et al., 2016). Ears can be shorter, smaller in diameter, and weigh less, while kernel depth and the number of kernels per cob also are usually reduced (Frommer et al., 2018). Smutty galls are not the only quality issue related to maize smut infection. Mueller et al. (2016) state that in highly susceptible maize varieties or cultivars, *U. maydis* has been found growing on the ears of maize with galls absence. But also discoloured areas on the inner ear leaf sometimes associated with *U. maydis* growth on the kernels, are usually verified by isolations. Damage to individual kernels is difficult to detect visually at harvest but becomes recognisable when the kernels were blanched (Mohan et al., 2013). *U. maydis* infection on kernels increases their vulnerability to other pathogens such as *Fusarium verticillioides* which causes ear rotting (Boyraz et al., 2018). Getting information on yield loss due to maize smut disease is an essential prerequisite for both the maize growers and pathologists to take any decisive action towards managing it in maize protection (Mafuru et al., 1999).

Therefore, this study was designed to determine resistant or tolerant maize genotypes that can be recommended to maize growers to be grown alongside or instead of the currently grown varieties in Sengerema district, Mwanza region of Tanzania, since is considered to be among the maize smut hotspot areas, particularly in Lake zone regions.

2. MATERIALS AND METHODS

Ecological nature of research area

The field study was carried out at Nyamazugo village, Sengerema district, in Mwanza region. Nyamazugo village was selected for the experimental field, because was found to be among the hotspot areas of maize smut disease in terms of the number of maize fields affected (Mafuru et al., 1999). The area is located at the latitude 1.0 to 4.45 south and longitude 30.25 to 35.15 east. An altitude of 1200-1300 m above sea level, with a mean annual rainfall of 1000-1200 mm (Masuki and Mbogoni, 2016). The general soil texture comprises sandy and loamy (Pauw, 1982). The area was fertilized with DAP (Di-ammonium phosphate NP 18:46) applied at a rate of 0.5 g per plant during the sowing of maize seeds on flat seedbeds and Urea fertilizer (32% Nitrogen) applied at a rate of 0.5 g

per plant during the vegetative stage. The average temperature and humidity when the inoculations were performed were 24°C and 65% respectively. Laboratory activities were done at the Plant Pathology laboratory, in the Department of Crop Science and Horticulture, Sokoine University of Agriculture, Morogoro.

Maize genotypes

Maize genotypes used in this study were certified seeds of LUBANGO HYBRID, SEEDCO SC 419, ZAMSEED ZMS 402, and PIONEER PHB 3253 (TOSCI, 2020) which were obtained from Agro-shops, and GEMBE, a local variety (landrace) taken from farmers as their self-saved stocks in Sengerema district, Mwanza region.

Source and isolation of *Ustilago maydis*

Maize smut galls were collected from diseased plants from maize growing areas in Sengerema district in 2022. The galls collected in the fields were chopped and chlamydospores (teliospores) were separated from the gall tissues by sieving through a tea strainer. The galls collected in the fields were chopped and chlamydospores (teliospores) were separated from the gall tissues by sieving through a tea strainer. Afterward, the teliospores were surface-sterilized by submerging in a 1% copper sulfate solution in Erlenmeyer flasks for 20 to 60 hrs, and filtered through two layers of sterile cheesecloth not allowing the teliospores to pass through (Tunçdemir, 1985).

Preparation of inoculum

Teliospore suspensions were diluted to appropriate concentrations (5 g/l) (El-Fiki et al., 2003). Teliospore suspensions in the Erlenmeyer flasks were stirred to get a homogeneous solution and the teliospores were counted by using a hemocytometer (Fig. 1). Teliospore suspensions were adjusted to 1×10^6 teliospores mL⁻¹ as described by Tunçdemir (1985).

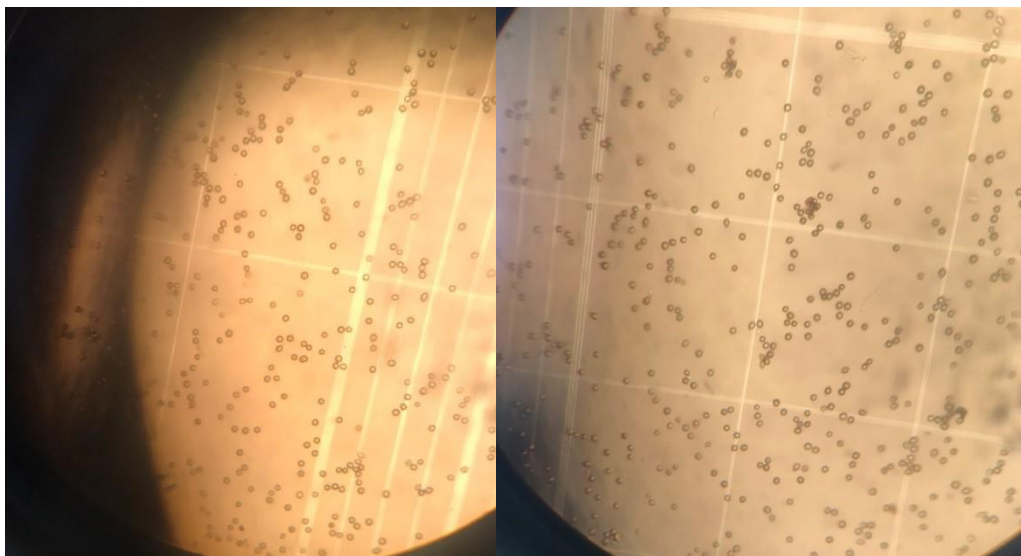


Fig.1: Counting number of *U. maydis* teliospores using hemocytometer observed under the light microscope.

Experimental designs

For smut incidence and severity, the experimental design was Randomized Complete Block Design (RCBD) with five treatments (maize genotypes) and was replicated four times. Each block/replicate contained five experimental units (plots). The dimensions of each plot were 2m x 5m which gives an area of 10m² consisting of seven maize rows planted at a spacing of 75cm x 30cm. For yield loss quantification, the experimental design was a two-factor Randomized Complete Block Design (RCBD) with five maize genotypes and two inoculation statuses (inoculated and non-inoculated (control)) which were replicated five times. Each replicate contained 10 experimental units (plots). The dimensions of each plot were 2 m x 3.5m which gives an area of 7m² consisting of six rows planted at a spacing of 75cm x 30cm. In both designs, the distance from one plot to another was 0.5m and the distance from one replicate to the other was 1m. Inoculation of *U. maydis* was done to ensure the uniform presence of disease.

Inoculation of maize plants

Inoculations were performed in two growth stages of maize plants as follows: When the plants were 40-60 cm high, 2 mL inoculum (1×10⁶ teliospores mL⁻¹) was injected into the apical node of the plant using a hypodermic syringe (Tunçdemir, 1985). The ear inoculation method was used, thus for the ear silk of each emerging plant before pollination, 3 mL inoculum (1×10⁶ teliospores mL⁻¹) was injected into the ear of each plant through a hypodermic syringe (Patacky et al., 1995). Inoculations of apical nodes were performed on 9th February, and those of ears on 26th February and 3rd March 2022 from 1700 to 1900 hrs. The temperature being 24°C creating an ideal condition for successful *U. maydis* infection on maize plant (Aydoğdu and Boyraz, 2016). All tassels and ears of maize plants both inoculated and non-inoculated were covered with transparent polyethylene bags to prevent transmission of *U. maydis* spores through wind dispersion, but also to increase the success rate of fungus infection (Wang et al., 1988).

Data collection**Disease incidence**

Disease incidences on the maize cultivars were calculated based on the assessment scale of Aydoğdu and Boyraz (2016). Twenty maize plants were sampled from the inner rows of each plot for incidence determination. Incidence determination was done on five occasions thrice during early disease symptoms or signs of development and twice near the physiological maturity of a maize plant.

Data processing: was done as per (Aydoğdu and Boyraz, 2016)

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total plants assessed}} \times 100$$

Disease severity

The disease severity of the maize cultivars was rated according to Johnson and Christensen (1935). Twenty plants were sampled from inner rows in each plot during severity determination which was done on five occasions starting from 20 days after inoculations, then once weekly for the next four weeks. The diameter of smutty galls was measured by using a vernier calliper.

Data processing: disease severity was rated using a 0 to 5 scale; where

0: very small galls (< 2.5 cm in diameter)

1: small galls (2.5 to 5 cm in diameter)

2.5: medium galls (5 to 10 cm in diameter)

5: big galls (> 10 cm in diameter)

Kernel/cob

At harvest, all ears of inoculated and non-inoculated plots were collected separately. After all the ears were collected, they were husked and left to dry under the sun for three days. Then the mean number of kernel/cob was determined.

Data processing: was done per (Yanikoğlu et al., 1999)

$$\text{Kernel/cob} = \frac{\text{total number of kernels}}{\text{Total number of cobs}}$$

Grain weight

This was determined after the drying of husked grains under the sun for three days. The total weight for all harvested ears in each plot was measured by using an electronic weighing scale.

Data processing: was done per (Aydoğdu et al., 2015)

$$\text{Plot weight (kg)} = \text{total weight (both jar and grains)} - \text{weight of empty jar}$$

Yield

Afterward, the moisture contents of kernels for each treatment (inoculated and non-inoculated) were separately determined by keeping the kernels at 72 °C for 72 h, and the yield was adjusted for 15 % moisture content.

Data processing: was done according to Yanikoğlu et al. (1999)

$$\text{Adjusted Plot Yield} = \text{Plot weight} \times \frac{(100 - \text{moisture \%})}{85} \times \frac{(\text{kernel/cob})}{100}$$

$$\text{Yield (kg/ha)} = \text{Adjusted plot yield} \times \frac{10000}{\text{Plot area (m}^2\text{)}}$$

Yield loss

Yield loss from *U. maydis* infection was calculated by both using the formulae above and comparing inoculated plots with non-inoculated (controls).

Data processing: was done per (Aydoğdu et al., 2015)

$$\text{Yield loss (\%)} = \frac{\text{yield of control plot (kg/ha)} - \text{yield of inoculated plot (kg/ha)}}{\text{Yield of control plot (kg/ha)}} \times 100\%$$

Data analysis

Data were analysed using Excel (MS-2016) and GenStat statistical software (VSN International 16th edition). Analysis of variance (ANOVA) was used to test the responses of varieties to disease incidence and severity. Analysis of variance (ANOVA) was used also to test the responses of allocated treatments to different yield parameters and yield loss calculated. Mean separation was done using Tukey's Honest Significance test to rank varieties based on their responses to tested parameters ($\alpha=0.05$). Regression (R²) and simple correlation (r) between disease incidence and severity were also calculated to clarify the relationship between maize smut incidence and severity. But also Regression (R²) and simple correlation (r) between number of kernels and total grain weight, number of kernels and yield loss, and total grain weight with yield loss were calculated to clarify the relationship number of kernels, total grain weight, and yield loss.

3. RESULTS AND DISCUSSION

Following artificial inoculation through ear injection, about 3 weeks later swellings hereafter referred to as galls were observed on the inoculated plants. The galls' occurrence rate but also their size, shape, and texture varied according to the susceptibility level of a particular maize variety. Generally, galls varied from minute sizes of 0.1cm to 9cm in diameter.



Fig. 2: Pictures of smutty galls varied in sizes on maize cobs observed in the study.

Diseases incidence

The maize genotypes tested had significantly different ($p < 0.001$) responses on smut incidence. GEMBE was having a high average incidence (5.22%) as compared to other tested lines. Other selected genotypes (LUBANGO HYBRID, PIONEER PHB3253, SEEDCO SC419, and ZAMSEED ZMS402) had relatively similar responses statistically on disease incidence (Table 1).

Table 1: Smut incidence of maize genotypes

Genotype	Incidence (%)
GEMBE	5.220b
LUBANGO HYBRID	2.335a
PIONEER PHB3253	1.665a
SEEDCO SC 419	0.705a
ZAMSEED ZMS 402	0.660a
Grand mean	2.12
SE+/-	1.054
LSD ^{0.05}	1.851
CV%	49.8
<i>p-value</i>	<0.001

Results of this study show that none of the selected maize varieties tested in the field was found resistant to maize smut but only varied in tolerance level against the disease. Agrios (2005) stated that no single maize variety is known to be resistant to maize smut, but only some maize varieties could be tolerant to *U. maydis*. Variations in susceptibility levels among the selected maize varieties to maize smut resulted in varied disease incidences. (Mohan et al., 2013; Aydođdu and Boyraz 2016) reported similar findings.

Disease severity

There was a significant difference ($p < 0.001$) in smut disease severity among the genotypes. SEEDCO (SC 419) variety was least affected by the disease with very low severity (0.07cm) followed by ZAMSEED (0.27 cm) and PIONEER PHB3253 (0.61 cm) which statistically ranked relatively same. GEMBE was severely affected by smut (2.48 cm) followed by LUBANGO HYBRID (1.40cm) (Table 2).

Table 2: Smut severity of maize genotypes

Genotype	Severity (cm)
GEMBE	2.4837c
LUBANGO HYBRID	1.4025bc
PIONEER PHB3253	0.613ab
SEEDCO SC 419	0.070a
ZAMSEED ZMS 402	0.265ab
Grand mean	0.97
SE+/-	0.709
LSD ^{0.05}	0.878
CV%	73.3
<i>p-value</i>	<0.001

LUBANGO HYBRID was featured with higher cob sizes and average disease severity rates compared to SEEDCO SC419. This implies that the size of the smutty gall corresponds with the size of the particular maize variety cob. (Aydoğdu et al., 2015) reported similar findings. But also, in the study, the artificial inoculation of *U. maydis* was performed through ear injection in the silk channel. This explains that the differences in silk features such as aging prior to pollination among the selected maize genotypes resulted in variable reactions against maize smut. Since maize plants are less affected by smut disease in post-pollination stage than in pre-pollination stage according to Ferris and Walbot (2021). Husk leaves can affect silk properties and hence indirectly influence smut infection progress (Pataky and Richter, 2007). In the case of vulnerability of maize varieties to smut, the number of infected cobs is reduced with silk aging (Aydoğdu and Boyraz, 2018).

Variety response

For both interactions between the genotypes and scoring dates had no significant impacts in incidence ($p=0.611$) and in severity ($p=0.867$) (fig. 3). GEMBE was significantly affected by smut disease as compared to other varieties (fig. 3). The disease severity and incidence trend show that GEMBE was highly infected in March, followed by PIONEER PHB3253 in April while there was low infestation for SEEDCO SC419 scores in April (fig. 3). From the study, the selected maize genotypes showed variable reactions against maize smut. This phenomenon doesn't mean that these selected genotypes tested in Sengerema district, would express the same reactions to the smut disease in other maize-growing areas. Same results were observed by Aydoğdu and Boyraz (2016).

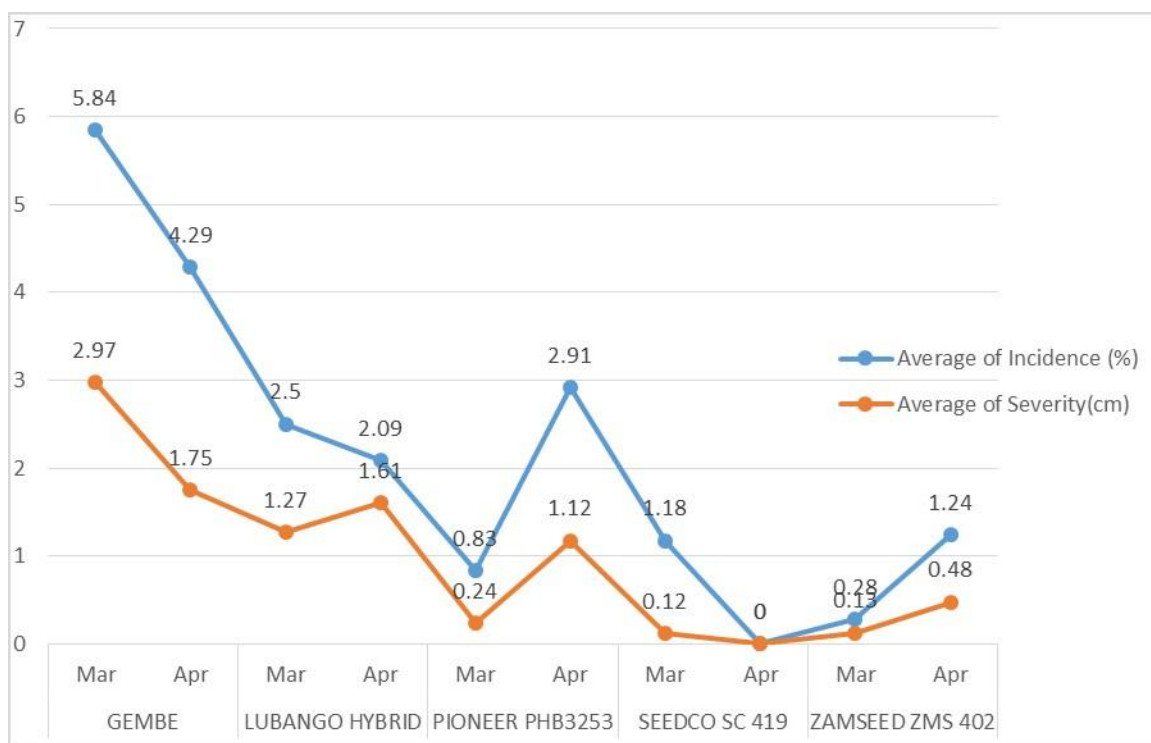


Fig. 3: Maize genotype response (incidence ($p=0.611$) and severity ($p=0.867$)) against smut disease

Since any maize variety or line seems to be susceptible to maize smut in every location and growing season (Agrios, 2005), but tolerance level to the *U. maydis* infection by a particular maize variety or line varies from one location to the other (Waligóra et al., 2014). In addition, (Szóke et al., 2021) stated that host tolerance against *U. maydis* might be influenced by climatic conditions and location. In all maize varieties tested in the experiment smut severity ranged from (0.07cm) to (2.48cm) in diameter both considered very small galls according to Johnson and Christensen (1935). Relatively little variation in severity degrees was observed among selected varieties due to uniformity in inoculum pressure.

The relationship between smut disease incidence and severity among selected varieties A highly significant difference ($p<0.001$) in the independent variable (incidence) with coefficient determination ($R^2=0.49$) indicated a moderately stronger relationship with severity (fig. 4). Thus smut severity rates increased with an increase in smut incidence levels in the experimental field (Moore et al., 2013). Generally, the incidence and severity of maize smut disease can be attributed to several factors including; variety, nature of the infection, inoculum pressure, time of infection, climatic conditions, quality of soil, and crop management practices (Aydoğdu and Boyraz, 2011; Aydoğdu and Boyraz, 2016).

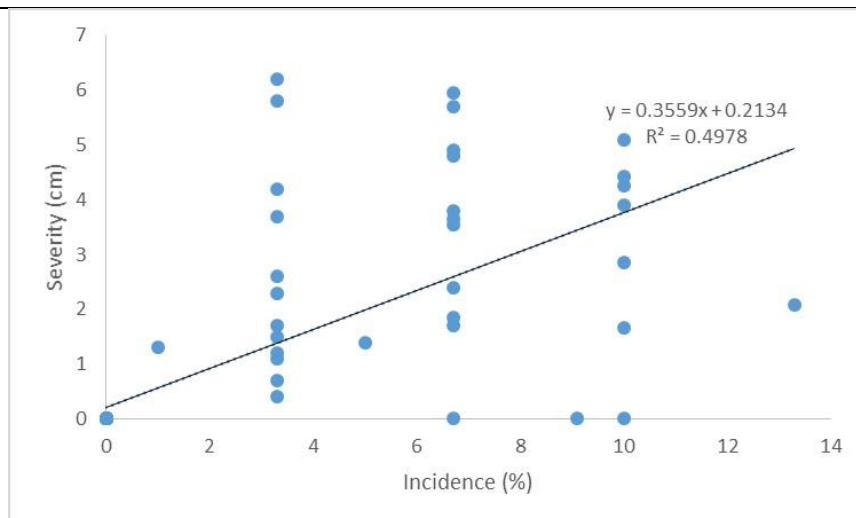


Fig. 4: Regression relationship between incidence (%) and severity (cm) ($p < 0.001$)

Number of kernels per cob

There was a significant difference in the number of kernels per cob between genotypes, inoculation statuses, and the interaction between genotype and inoculation statuses ($p < 0.001$). SEEDCO SC419 had the lowest mean number of kernels per cob (262.0) while LUBANGO HYBRID had the highest mean number of kernels per cob (434.4) (Fig. 5). The difference in the number of kernel/cob was mainly due to differences in morphological and physiological features such as the size of the cob, kernel depth, and length of the ear. A t-test to compare control treatment and inoculated treatment showed that control had the higher mean number of kernels per cob compared to inoculated treatment due to *U. maydis* infection effect (fig.6). Smutty tumors formed on the ear led to the formation of a small number of kernels but also reduced kernel quality in the smutted cobs resulting in a smaller kernel per cob ratio. Similar results were observed by Frommer et al. (2018).

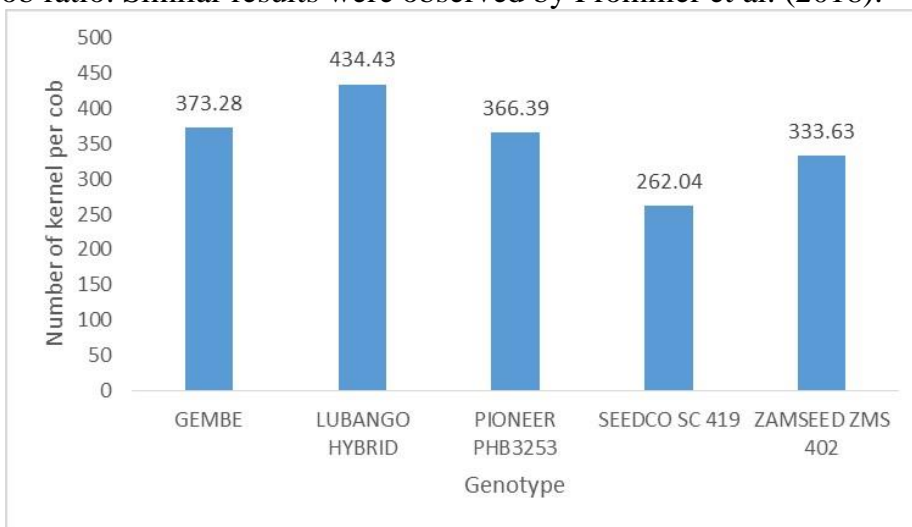


Fig. 5: Number of kernels per cob among the genotypes ($p < 0.001$)

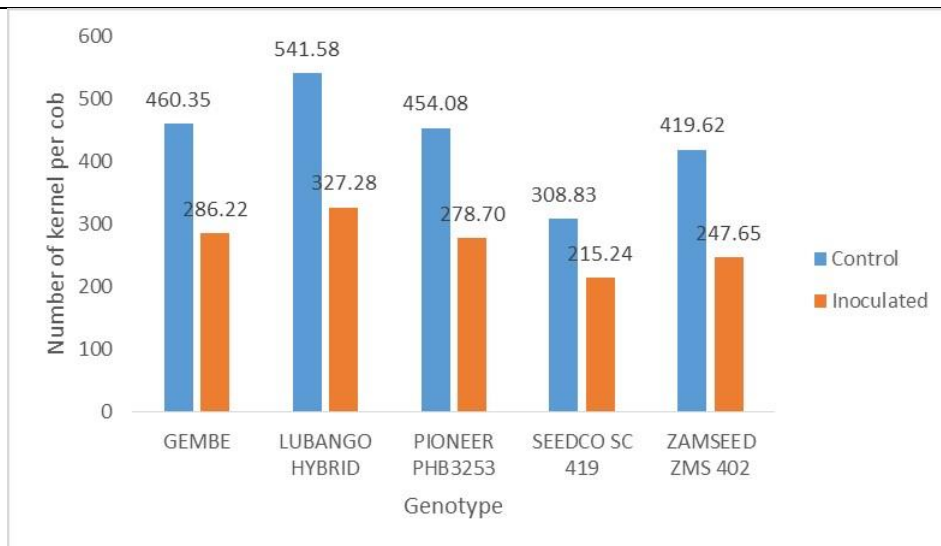


Fig. 6: Number of kernels per cob based on the interaction between inoculation statuses and genotype ($p < 0.001$).

Total grain weight (Plot weight)

The difference in mean total grain weight among genotypes, inoculation statuses, and the interaction between inoculation status and genotypes was highly significant ($p < 0.001$) (fig. 7). A t-test to compare the yield of inoculated and control treatment justified higher plot weight on control compared to inoculated plots (fig. 8). Impact of *U. maydis* not only reduced kernels number but also, quality and size due to contamination in infected cobs. These resulted in a significant reduction of total grain weight in inoculated treatment genotypes, similar findings were reported by Mohan et al. (2013). This scenario is due to the severity of maize smut on the ears of inoculated genotypes (Aydoğdu and Boyraz, 2018).

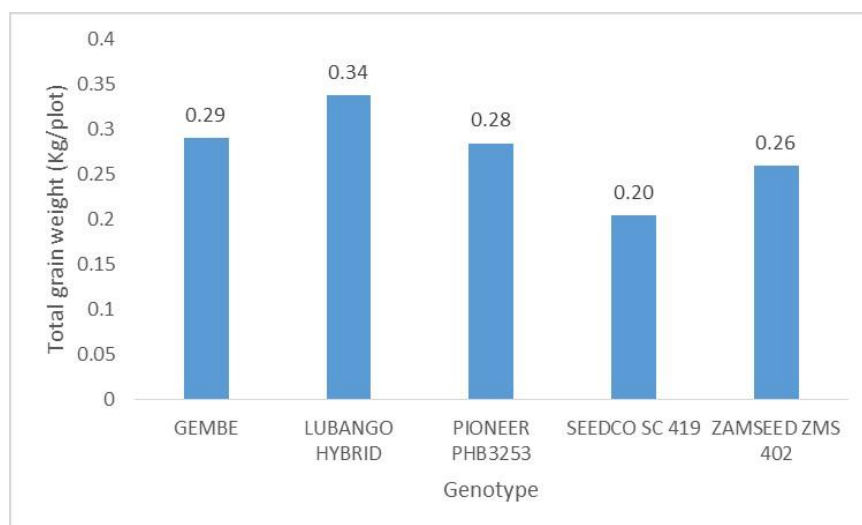


Fig. 7: Total grain weight based on genotypes ($p < 0.001$)

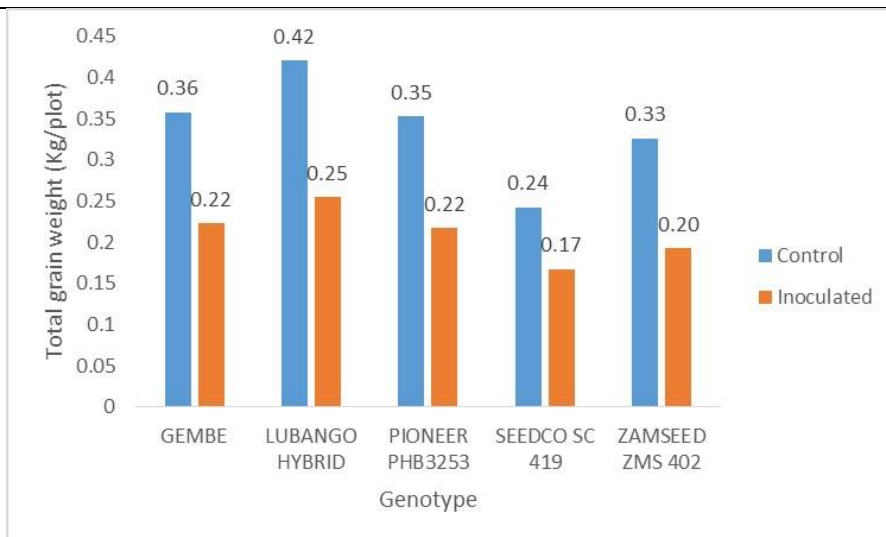


Fig. 8: Total grain weight based on the interaction between inoculation statuses and genotype ($p < 0.001$)

Yield

A significant difference in total yield between genotypes ($p < 0.001$) was observed (fig. 9). A significant difference ($p < 0.001$) was observed in interactions between inoculation statuses and genotypes (fig. 10). Hence this scenario explained the reduction in yield due to maize smut was significant. (Clough et al., 2011) stated in smut-infected plants the yield is 40% less compared to the healthy plants. Variations in the susceptibility levels among the tested genotypes resulted in varied yield reductions. Similar findings were described by Ferris and Walbot (2021).

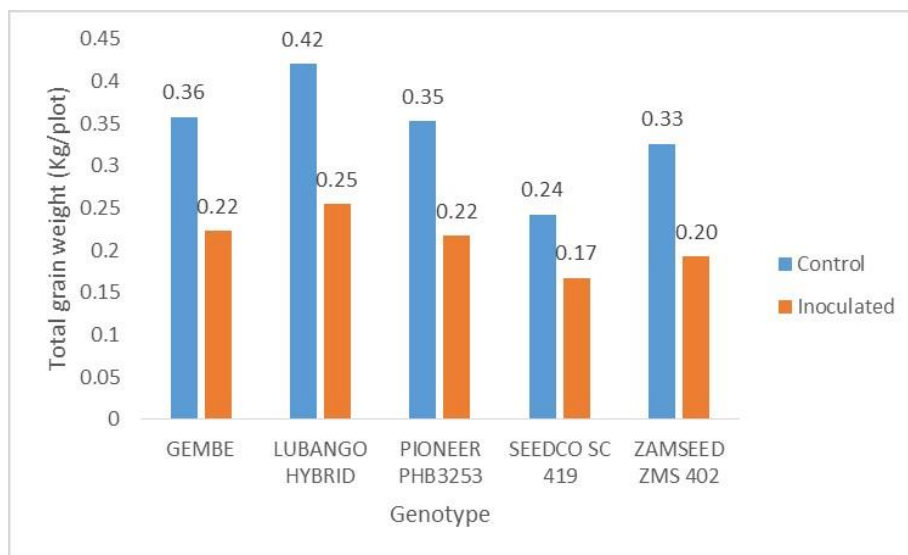


Fig. 9: Mean yield of maize genotypes ($p < 0.001$)

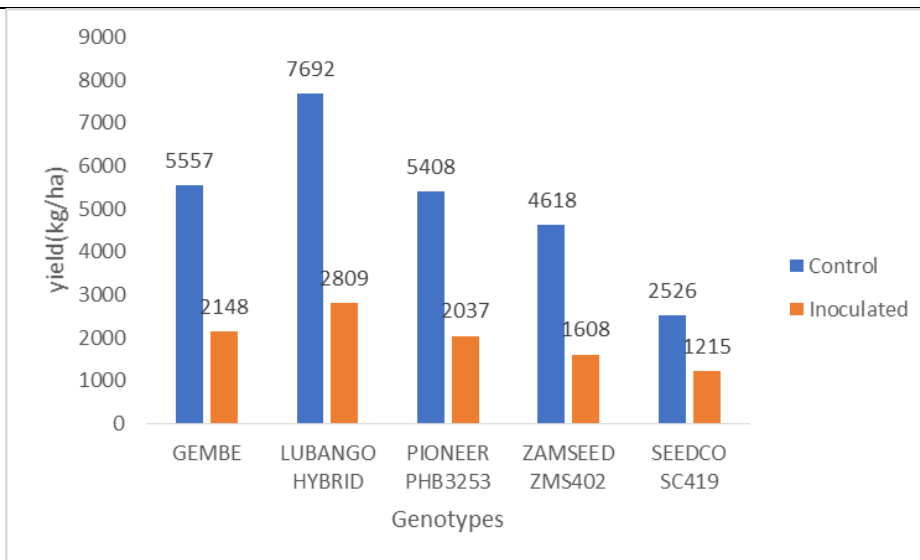


Fig 10: Mean yield of maize genotypes interacted with inoculation statuses ($p < 0.001$)

Yield loss

There was a significant difference in yield loss between genotypes ($p < 0.001$). Yield reductions due to *U. maydis* infection was not even among selected genotypes, due to differences in smut severity levels (Fig. 11). Galls on the maize plants varied in number, size, and shape according to the susceptibility degree of the maize varieties. (Aydoğdu et al., 2015) reported similar findings. In the study, smutty galls formed on the ears in selected maize genotypes led to significant yield losses. (Ur Rehman et al., 2021) reported the similar discoveries.

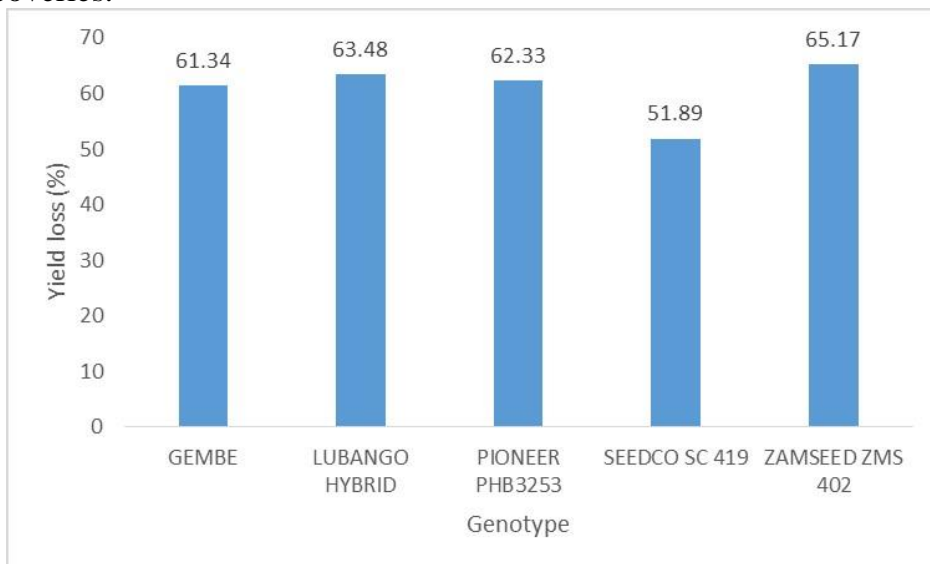


Fig. 11: The average yield loss per each genotype ($p < 0.001$)

Agrios (2005) stated that rates of yield losses due to maize smut disease increased according to an increase in severity levels respectively. Similar results were observed by

Clough et al. (2011). But also, the mean yield loss in LUBANGO HYBRID was higher compared to SEEDCO SC419 (fig.9). This can be explained by evaluating the variation in morphological features of these genotypes. Aydoğdu and Boyraz (2018) stated that maize smut -infected varieties possessing bigger ears had bigger galls on their ears than the ones on the other varieties. From the study average yield of all selected maize varieties decreased at a rate of 60.44% due to maize smut disease. (Agrios, 2005) explained that yield reductions caused by *U. maydis* infection ranged from 40 to 100%. The average yield reduction is considered to be high and was influenced by various factors such as climatic conditions and soil fertilizers application practices. The presence of longer periods of hot and dry conditions was observed in Sengerema district. In this regard, it could be inferred that environmental factors were more favourable to *U. maydis* development for successful infections. In the study conducted, following inoculation the average daily temperature for the ensuing three days was 24°C. This temperature provided optimum conditions for successful penetration of the pathogen to the host. Kahmann and Kämper, (2004) emphasized that when environmental factors are in favour of the pathogen in maize growing season, its infections appear at a higher level. Nitrogenous fertilizers, (UREA and DAP) were applied. These nitrogen-rich fertilizers can be explained as an influence in elucidating higher yield losses in tested genotypes. Excessive nitrogen available in the soil, increases the chance of smut infestation (Djawu, 2017).

Relationships between number of kernels per cob, grain weight, and yield loss

There was a high regression relationship between the number of kernels per cob and total grain weight per plot ($p < 0.001$, $R^2 = 0.9998$) (Fig. 12). There was a significant weak relationship between the number of kernels per cob to the yield loss for applied treatments ($p < 0.05$, $R^2 = 0.1654$) (Fig. 13). Between total grain weight and yield loss, there was a weak significant relationship ($p < 0.05$, $R^2 = 0.1622$) (Fig. 14).

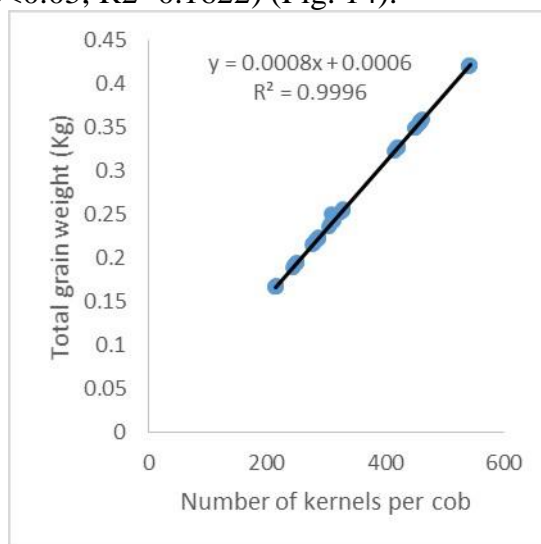


Fig 12: The regression relationship between number of kernels per cob and total grain weight ($p < 0.001$)

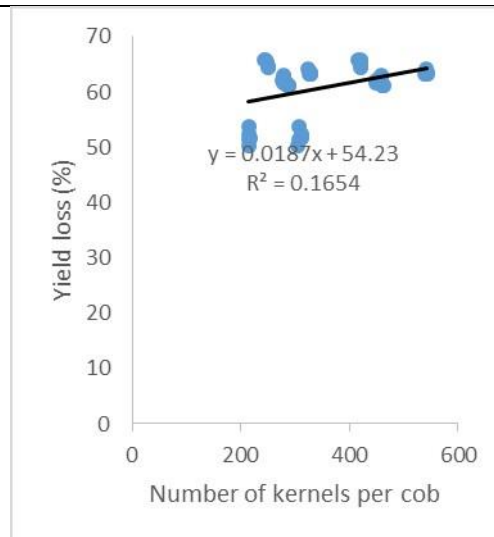


Fig. 13: The regression relationship between the number of kernels per cob and yield loss ($p < 0.05$)

Fig. 14: The regression relationship between total grain weight and yield loss ($p < 0.05$)

Since the measurement of total grain weight is based on the number of kernels of a particular variety weighed. Implying that the number of kernels per cob determines the total grain weight per plot at 99%. The number of kernels per cob and total grain weight relation with yield loss does not explain a lot of variation (relatively little influence) but is significant. The number of kernels per cob impacts yield loss due to maize smut with either presence or absence of galls. *U. maydis* infects individual maize kernels rather than infecting the whole ear (Ur Rehman et al., 2021). Yield reduction by *U. maydis* growth in a whitish appearance between the kernels on a healthy cobs (absence of smutty galls) takes place only if a single mating type is present according to Boyraz et al. (2018).

4. CONCLUSION

The five tested maize genotypes differ in their reaction to *U. maydis* with SEEDCO SC419 being more tolerant compared to the other varieties. Hence this genotype is more efficient in production and it can recommended to be grown by farmers in smut hot spot areas of Sengerema district. Since maize smut development can be influenced by climate and location. Further research is needed to assess the susceptibility of existing or preferable maize germplasm to determine which varieties or lines are more tolerable to *U. maydis* in other maize growing areas in Tanzania, also as to why tumor formation is different within tissue and between plants of the same growth stage to provide effective management of the disease.

Acknowledgment

Firstly, I thank God for enabling me to complete this task. I would like to express my sincere gratitude to Dr. Paul Jerry Njau of the Department of Crop Science and Horticulture, Sokoine University of Agriculture for his highly encouraging supervision in

every step of this article development. But also, I wish to thank all whom might have been involved in any way in making this work successful.

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