GENERATION MEAN ANALYSIS FOR *DIPLODIA* EAR ROT RESISTANCE IN TROPICAL MAIZE INBRED LINES

By

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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university

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DEDICATION

This thesis is gratefully dedicated to my parents, brother Ndatsinze Félix and sister-in-low Hategekimana Elise, sister Fausta Karaboneye and brother-in- low Ntakirutimana Michel, my love Mukamusoni Jacqueline and all my siblings.

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ABBREVIATIONS

ANOVA - Analysis of Variance

ASI - Anthesis Silking Interval

CAGR - Compound Annual Growth Rate

CAN - Calcium Ammonium Nitrate

CIMMYT - International Maize and Wheat Improvement Center

DAP - Diammonium Phosphate

DI - Disease Severity Index

DTMA - Drought Tolerant Maize for Africa

ELISA - Enzyme-linked immunosorbent assay

FAOSTAT - Food and Agriculture Organization corporate Statistics

GMA - Generation Mean Analysis

GLS - Gray Leaf Spot

IITA - International Institute of Tropical Agriculture

IRMA - Insect Resistant Maize for Africa

KALRO - Kenya Agricultural and Livestock Research Organization

MSV - Maize Streak Virus

QTL - Quantitative Trait Loci

USAD - United States Academic Decathlon

USA - United State of America

SAS - Statistical Analysis System

ABSTRACT

Diplodia ear rot caused by *Stenocarpella maydis* (Berk) Sutton is one of the most important fungal diseases of maize in the tropics. Breeding programs in Eastern and Southern Africa have developed maize inbred lines with tolerance or resistance to Diplodia ear rot. Utilization of tolerant/resistant inbred lines in commercial hybrids or for further breeding requires knowledge of the inheritance of resistance Diplodia ear rot in this germplasm. The objectives of this study were to determine the mode of gene action, estimate heritability and heterosis for Diplodia ear rot resistance and agronomic traits through generation means analysis. Five inbred lines from Kenya and Mexico were crossed to form six F_1 hybrids. Each F_1 hybrid was self-pollinated to form the F_2 and also backcrossed to each of the parental inbred lines. For each cross, six generations including the parental inbred lines (P_1 and P_2), F_1, F_2 and backcrosses (BC_1P_1 and BC_1P_2) were used to form six separate trials. Trials were arranged as a randomized complete block design (RCBD) replicated three times. Each plot consisted of 2 rows 4 m long, spaced 75 cm between rows and 20 cm within rows. The trials were evaluated at Kakamega, Alupe, and Kibos in western Kenya under artificial inoculation with Diplodia ear rot pathogen. Diplodia inoculum was raised at KARLO Kakamega Research Station. Inoculation of the top ear of each plant with Diplodia ear rot pathogen was done 18-20 days after mid-silk using the toothpick method. Agronomic traits (grain yield, days to anthesis, plant height, and ear aspect) and disease resistance parameters (Diplodia ear rot incidence and severity and weight of rotten ears) were recorded in the trials. Data were first subjected to analysis of variance and then generation mean analysis. Broad and narrow-sense heritability and heterosis were estimated for all the traits. Results showed that there were significant differences among generations for grain yield and other agronomic traits for all crosses. Combined analysis of variance revealed significant differences among generations for

Diplodia ear rot incidence, Diplodia ear rot severity and weight of rotten ears in some crosses.

Generation means for grain yield fitted an additive-dominance model in five crosses while generation means for other agronomic traits (days to anthesis, plant height and ear aspect) did not fit an additive-dominance model suggesting a more complex model including epistasis for these traits. The generation means for Diplodia ear rot severity and weight of rotten ears fitted an additive-dominance model in all crosses while a complex model was needed to fit the generation means for Diplodia ear rot incidence. The magnitude of dominance effects was much higher compared to additive effects suggesting greater importance of dominance gene effects relative to additive gene effects in control of inheritance of grain yield. There was preponderance of dominance effects over additive effects for the other agronomic traits. Presence of significant additive x additive [aa] and dominance x dominance [dd] epistatic effects was detected for ear aspect and plant height, respectively. There was variable importance of additive gene effects for Diplodia ear rot incidence and severity among the crosses. Five and four crosses showed significant additive effects for Diplodia ear rot incidence and Diplodia ear rot severity, respectively. All the significant additive effects for Diplodia ear rot incidence and severity were negative. Dominance in this set of crosses was in the direction of greater resistance. The results for weight of rotten ears showed that the dominance effects were positive and significant for five out of the six crosses. For most crosses, these results implied the importance and prevalence of additive mode of gene action for resistance to Diplodia ear rot. The prevalence of additive mode of gene action in the majority of crosses in study suggests that selection among inbred lines under artificial inoculation with Diplodia ear rot should be effective. Both Diplodia ear rot and severity reaction of the parental inbred lines should be a reliable indicator of disease reaction of their hybrids. The identification of some inbred lines like CML543 and LPSC7 with fairly good

levels of tolerance to Diplodia ear rot offers breeding programs in the region an opportunity to include these lines in their inbred line development programs. Some of the inbred lines used in this study like CML543, LPSC7 and VL06688 and other resistant inbred lines should be used for *S. maydis* resistance quantitative trait loci (QTL) validation and genome-wide association studies (GWAS) for detailed investigation of the genetics of Diplodia ear rot resistance in tropical maize for possible implementation of a marker-assisted breeding for this disease. Broad-sense and narrow-sense heritability estimates for grain yield were moderate to high for most of the crosses. Broad-sense heritability estimates for Diplodia ear rot incidence and severity were mostly low suggesting that the environment had a larger effect compared to the genotype. Relatively high narrow-sense heritability estimates for Diplodia ear rot incidence severity were obtained which suggested that resistance to Diplodia ear rot can be improved fairly quickly in this set of lines. Heterosis estimates were much larger for grain yield compared to other traits. Heterosis for the two disease parameters was negative in four out of the six crosses, which suggested that the F1 hybrids tended to have lower diseases ratings than some of the parents.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Maize (*Zea mays* L.) is the most widely distributed crop (FAOSTAT, 2010) and is an important staple food crop in many countries around the world. Maize is also used in animal feed and in many industrial applications (USDA, 2014). The dual purpose nature of the crop as food and cash crop has led to its increased utilization and diverse cultivation in the world (Ramadhani et al., 2002). Global production of maize has grown at a compound annual growth rate (CAGR) of 3.4 per cent over the last ten years, from 717 million metric tons in 2004-2005 to 967 million metric tons in 2013-2014 (USDA, 2014). The area under maize cultivation in the period has been increased at a CAGR of 2.2 per cent, from 146 million hectares in 2004-2005 to 168 million hectares in 2013-2014 (FAOSTAT, 2014; Runum and Pablo, 2014).

In Africa, maize is grown by small and large scale commercial farmers who cultivate the crop on an estimated area of 10 ha or less (DeVries and Toenniessen, 2001; Muhunyu, 2008). The cultivated land under maize only constitutes about 14% of the total arable land in Africa (FAOSTAT, 2012). In the year 2010, South Africa was the highest maize producer in Africa with average just below 13 million tons of maize on an annual basis while in the East African region Tanzania was the highest producer with approximately 4.5 million tons. The production in Kenya and Uganda was 3.2 million and 1.3 million tons respectively (FAOSTAT, 2010).

Consumption of maize in sub-Saharan Africa varies greatly from country to country, with maize flour and meal being two of the most popular products (Antonaci et al. 2014). In Kenya, maize has a per capita consumption of 98 kg/year, which takes to a national annual consumption of 3.32 million tons (Ministry of Agriculture, 2013). Though, the national

production of 3.2 million tons is far below the consumption (3.32 million tons), Kenya must import maize. This deficit is mainly occupied by cross border trade with the neighboring countries mainly from Uganda and Tanzania (Muhunyu, 2008). In East and Central Africa, maize has replaced other cereal food staples, particularly sorghum and millet, becoming a major source of cash for smallholder farmers (Shiferaw et al., 2011).

Despite the importance of maize as both food and cash crop, it is faced by a number of production constraints throughout sub-Saharan Africa. In Kenya, Low yields have been associated with both abiotic and biotic constraints. The most important abiotic factors that affecting maize production include low soil fertility and soil acidity (Edmeades et al., 1989). The large proportion of soils in the tropics are generally inherently infertile and most, especially those under subsistence agriculture have been mined of nutrients for years without replenishment with fertilizer (Smaling et al., 1997). The soil is eroded and degradation and desertification are evident. Therefore, under extremely low-input/ low risk systems where estimated average maize yields are just below 1.5 t ha^{-1} in sub-Saharan Africa (Bänziger and Diallo, 2004). The use of varieties with low production potential, poor agronomic practice. Unavailable and untimely input supply and drought are also other abiotic factors that affecting maize production in sub-Saharan Africa especially in Kenya (Lisuma et al., 2006; CIMMYT, 2012).

On the other hand, biotic stresses like foliar diseases, ear rot, and stalk rots caused by fungi and bacteria. Under favorable environmental conditions, these pathogens are capable of causing severe losses and deteriorate the quality of the produce (Lisuma et al., 2006). Others biotic stresses found in the zone include insect pests like army worms, cutworms, stalk borers, *Sitophilus* spp, and large grain borer (Davis and Pidigo, 1990). Others include weeds and striga especially *S. hermonthica* and can lose about 20 to 80% of their yields from striga

infestation (Kim et al., 2002). The common diseases that affect maize production in the zone include, leaf rust, maize streak virus disease (MSV) and northern leaf blight, fusarium, gibberella stalk and cob rots disease affecting all maize growing regions and invading the majority of maize varieties (CIMMYT, 2004).

1.2 Problem statement

One of the major cob diseases of maize is *Diplodia* ear rot (*Stenocarpella* ear rot) caused by pathogen namely, *Stenocarpella maydis.* This disease occur around the world, wherever maize is grown reducing yield and quality production (Dorrance ea al., 1998). *Diplodia* ear rot was most common ear rot disease in 1950s through 1960s throughout Corn Belt (Vincelli, 1979). High levels of ear rot infection and mycotoxins accumulation have been reported in pre-harvest maize in Europe, North and South America, and Asia (Vigier et al., 1997; Logrieco et al., 2002), South Africa (Rheeder et al., 1992), East Africa (Kedera et al., 1999; Kapindu et al., 1999; Bigirwa et al., 2007).

In Kenya, maize grain losses caused by ear rot especially *Diplodia* ear rot vary from insignificant to significant (13-70%) (Anon, 1986). Control of ear rot disease is important in maintaining both yield and quality production. Since the mid-1960s, the disease generally was much less important due to the presence of soybeans into crop rotations, and this may have helped reduce levels of infectious spores (Vinceli, 1979). The combination of good agronomic practices, crop sanitation, and timely harvesting have also been used in disease control, but with limited success (Munkvold, 2003). In addition, the use of resistant varieties is the most likely than other control measures, neither of which provides adequate control (Lengkeek, 1983; Byrnes and Carroll, 1986).

There have been reports of inbred lines with resistance to *Diplodia* ear rot in Africa (Rensburg et al., 2003) and other parts of the world (Vincelli, 2012; Das, 2014). Little is

known, about the genetic mechanisms of resistance in the resistant germplasm. Previous reports concerning gene action for *Diplodia* ear rot resistance are conflicting about the nature of resistance in maize germplasm. One study suggested that non-additive genes were important for resistance (Olatinwo et al., 1999) while other reports suggested that both general and specific additive genes effects were significant (Dorrance et al., 1998; Tembo et al., 2013). These contrasting results are most likely reflective of differences in germplasm used and locations under investigation in each case. Apparently, no maize hybrid currently in the market has a high level of resistance to *Stenocarpella* ear rot (Vincelli, 2012).

1.3 Justification

The recent work done by both CIMMYT Kenya and Mexico (CIMMYT, 2004; Wambugu, 2013) concentrated on screening for maize inbred lines with resistance to *Diplodia* ear rot and identification of the mode of gene action involved. Genetic improvement of both local and exotic maize germplasm for ear rot resistance would not only increase the frequency of genes for resistance to *Diplodia* ear rot but yield as well. Studies at CIMMYT in Mexico have shown that breeding for resistance to ear rots could increase yields by up to 25% (De Léon and Pandey, 1989). From different studies, it is apparent that there is a need for more studies on genetics of maize resistance to *Diplodia* ear rot since there is some uncertainty about the levels of resistance present in most of the sources used in the previous studies (Rossouw et al., 2002a; Tembo et al., 2013). This present study was designed to investigate genetic effects of resistance to *Diplodia* ear rot disease in six tropical maize crosses.

1.4 Objectives

The study was conducted to estimate genetic effects of Diplodia ear rot resistance and agronomic traits in inbred line and top cross hybrid of maize under artificial inoculation through generation mean analysis (GMA) over a range of environments.

The specific objectives were:

1. To determine gene action conditioning resistance to Diplodia ear rot
2. To determine the heritability of maize resistance to Diplodia ear rot
3. To estimate heterosis for Diplodia ear rot resistance and grain yield

1.5 Hypotheses

1. Gene action conditioning resistance to Diplodia ear rot in tropical maize can be elucidated through generation mean analysis using appropriate genetic models.
2. Diplodia ear rot resistance in tropical maize can be integrated into high heritability estimates
3. Relative importance of heterosis for Diplodia ear rot resistance can be elucidated using appropriate generations.

CHAPTER TWO: LITERATURE REVIEW

2.1 The major ear and stalk rots of maize

Stalk and ear rots are the most economically harmful of the maize diseases and occur everywhere maize is grown (Dorrance et al., 1998; CIMMYT, 2004). Although the damage caused by ear rots in terms of yield loss is significant, their most challenging aspect is the mycotoxins linked with several of the ear rot fungi (Munkvold, 2003; Matiello et al., 2015). These toxins have been related with a variety of human diseases ranging from nausea to neurological conditions to cancer (Richard and Payne, 2002; Snyman et al., 2011). Reports concerning the ability of *S. maydis* to produce toxins have been made. Chalmers et al. (1978) noted that in addition to loss of production, *Stenocarpella* spp. are known to produce mycotoxins (diplodiatoxin and diplodiol). Rabie et al. (1987) noted that regular and widespread occurrence of *Stenocarpella maydis* in South African maize may be of economic importance in the poultry industry but infected grain has been reported to cause mycotoxicosis when fed to cattle and sheep. However, Wolthers (1988) suggested that there is no evidence that this fungus produces toxin, although it can significantly reduce grain quality by causing the kernels to turn grey or brown under field conditions.

There are four major ear rot problems (*Fusarium* ear rot, *Aspergillus* ear rot, *Stenocarpella* ear rot *and Giberella* ear rot) and four major stalk rots (*Anthracnose, Giberrella, Fusarium and Diplodia*) affecting maize in Eastern Africa as well as in other tropical temperate agro ecologies zone (Bigirwa et al., 2007; Mukanga et al., 2010b). The causal agents of three of the four ear rot diseases named above have wide host ranges: *Fusarium verticillioides* and *Gibberella zeae* infect the seeds of rice, maize, and wheat as well as other grasses while *Aspergillus flavus* infects peanuts and cotton seeds among others (Richard and Payne, 2002). On the other hand, *Stenocarpella maydis, Fusarium verticillioides* and *Gibberella zeae* are

also the causes of important stalk rots. According to Hooker (1956), Rheeder et al. (1990) and Flett (1999), both three ear rots (*Fusarium* ear rot, *Aspergillus* ear rot *and Giberella* ear rot) and three stalk rot (*Stenocarpella, Fusarium* and *Gibberella*) share a causal organism. Little information exists on the possible correlations of reactions to ear rots and stalk rots in maize germplasm. It seems probable therefore that these generalist pathogens were able to form narrative interactions with the maize ear in a relatively rapid fashion in evolutionary terms coming either from phylogenetically closely related hosts or from other maize structures (Parker and Gilbert, 2004).

2.2 *Diplodia* ear rot of maize

The fungal disease that infect maize cobs such as *Fusarium verticillioides*, *Gibberella zea,* and *Stenocarpella maydis* are the major causes of maize ear rots and can be observed during all stage of maize (Bigirwa et al., 2007; Mukanga et al., 2010b). Infection of these fungi rarely cause severe yield losses over wide geographical areas (Vincelli, 1979; Smith and White, 1988), varying greatly between years depending on the prevailing pre-harvest environments (Matiello et al., 2015).

Diplodia ear rot (caused by *Stenocarpella maydis,* previously known as *Diplodia maydis*) is wide-spread in North and South America, Africa, Asia, Australia and Europe. When the incidence is high, it is of major economic importance as it can affect more than 50 percent of the field (Oerke, 2005). Diplodia ear rot is regarded as one of the more prevalent spoilage field fungi of maize in Kenya (Wambugu, 2013). The incidence of Diplodia ear rot has been reported to be variable where the pathogen population could be fairly large in some years causing substantial damage, yet other years damage could be very limited (Dorrance et al., 1998). The variability in Diplodia ear rot incidence can be attributed to a number of factors. Variability in host resistance, the use of cultural practices where no tillage is practiced, and

an increase in fungal inoculum or changes in the virulence of the fungus can affect expression of the disease (Dorrance et al., 1999). *Diplodia* ear rot is not as common as *Fusarium* or *Gibberella* ear rots but it can be just as critical. With abundant rainfall in the growing season, the severity of disease can be high in certain maize fields planted with susceptible hybrids (Tembo et al., 2013). Nwigwe (1974) and Lipps and Mills (2001) reported that *Diplodia* ear rot can cause between 1 to 37% loss in germination as well as being a serious pathogen of maturing plants. Chambers (1988) found that maize grain yield losses as high as 97% from *Diplodia* ear rot inoculations made 10 days after silking. Unfortunately, yield losses due to natural ear rot infections have not been estimated. Under most conditions, injuries caused by *Diplodia* ear rot is limited to the field, but if grain moisture is 20% or above, damage can be a dilemma in storage (Woloshuk and Wise, 2008).

2.2.1 Causal agent of *Diplodia* ear rot

Diplodia ear rot caused by *Stenocarpella maydis*, is a soil borne and seed transmitted disease (Smith and White, 1988). *Stenocarpella maydis* is the same fungus that causes Diplodia stalk rot (Rheeder et al., 1990; Flett, 1999). Another related fungus, *Stenocarpella macrospora*, has been found in at least one instance in the United States causing a similar ear rot during warm and humid weather (Vincelli, 1979; Latterell and Rossi, 1983). *Stenocarpella macrospora* also produces brown spots and streaks on leaves. The pycnidia-covered maize debris that remains on the soil surface will overwinter and provide a source of infection for the following year. Infection by *Diplodia* is enhanced by dry weather prior to silking, followed by wet conditions at and just after silking (Lipps and Mills, 2001). Ears are most susceptible to this disease during the first 21 days after silking. Earworm damage at the ear shank is often associated with the disease (Moremoholo, 2008)**.** Therefore, *Diplodia* ear rot can occasionally result in severe epidemics, causing rot on as many as 50% to 75% of the ears in a field (Vincelli and Hershman, 2013).

2.2.2 Symptom of *Diplodia* ear rot

Diplodia ear rot can occur as a thick white mold that usually starts at the base of the ear. The symptoms are seedling blight, and on the stalk the lower nodes turn brown and spongy several weeks after silking (Dorrance et al., 1998). Sub-epidermal pycnidia may come out clustered around the nodes. On the ear white fungal growth is found between seeds and pycnidia may be present on the seeds and cob (McGee, 1988). Husk of early-infected ears appear bleached or straw-colored and dry although the maize plant remains green and healthy (Flett, 1997). Infected ears are very light in weight and may be totally rotten. In some cases the mold may be noticed at the tip end of the ear (CIMMYT, 2004). A specific feature of *Diplodia* ear rot is the presence of raised black fruiting bodies of the fungus on moldy husks or kernels that usually form later in the season. If infection occurs early then the entire ear may be surrounded with mold. If infection occurs several weeks after silking, then only a portion of the ear may be affected. Later infections may result in only a fine web of fungal growth appearing on kernels (Lipps and Mills, 2001). *Stenocarpella maydis* over-winters as spores in pycnidia or mycelium on maize residue, cobs and on the maize kernels (Flett, 1990). Under moist and warm conditions, spores are extruded from pycnidia and dispersed by wind, rain and most likely insects (Olatinwo et al., 1990; Odriozola et al., 2005).

2.2.3 Disease initiation and development of *Diplodia* ear rot

The initiation of disease caused by biotic agents depends upon three main factors: the host, the environment and the pathogen. Environmental conditions play an important role in the development of ear rot diseases. Different environmental conditions favor the development of ear rots (Robertson, 2004). The incidence and severity of ear rots vary greatly from year to year and from field to field in a given year. The severity of disease development is dependent on the degree of interaction of these three main factors (Host, Environment and Pathogen) (Vincelli, 2012; Das, 2014).

The fungi that cause maize ear rots are often favored by late season humidity and rain following pollination. Delayed planting or conditions that slow grain drying in the field and delay harvest can lead to an increased incidence of ear rot diseases (Woloshuk and Wise, 2010). Fields with stalk rots may also be at a greater risk for developing ear rots. Plants have highly effective mechanisms for disease resistance that have contributed to survival under the selection pressure of evolution (Day, 1974). The fungus that causes the disease attacks maize and it survives between seasons in residue of maize stalks, ears, and fallen kernels. Thus, continuous maize production especially under conservation tillage allows the pathogen to build up to potentially destructive levels (Bissonnette, 2000).

Infection of maize plants occurs primarily through the crown, mesocotyl and roots or occasionally at the nodes between crown and ear (Flett, 1990). The pathogen then grows into the stalks. When plants are silking, spores may spray up to the ear leaf and then become deposited by rainwater around the ear shank to initiate infection (Flett et al., 2001). These spores can germinate and penetrate the ear shank, growing into the cob and outward into the cobs (Smith and White, 1988). The fungus does not invade the entire plant. Dry conditions early in the season and warm (28-30°C) coupled with a wet weather in three weeks after silking favor ear infection of *S. maydis* (Shurtleff, 1980; CIMMYT, 2004).

2.2.4 Impact and Economic importance of *Diplodia* ear rot disease

Direct yield loss due to *Diplodia* ear rot is caused by rotting of ears and kernels leading to reduced weight and nutritional content. Infection of kernels at the blister stage can result in reduced kernel size and grain filling (CIMMYT, 2004; Vincelli, 2012). Damage is most critical if infection occurs early (immediately following flowering), as the entire ear may rot and kernels may fail to develop fully. *Stenocarpella maydis* produces the mycotoxin diplodiatoxin and *S. macrospora* produces the mycotoxin diplodial, which are both harmful

to birds (Lipps and Millis, 2001). Livestock may refuse grain that is severely affected by *Stenocarpella* ear rot. In some cases up to 80% of ears can be affected by *Stenocarpella* ear rot, leading to considerable yield loss. Infected ears can weigh up to 35% less than healthy ears (Das, 2014). Rabie et al. (1987) reported that regular and widespread occurrence of *Stenocarpella maydis* in South African maize may be of economic importance in the poultry industry. Moremoholo et al. (2010) suggested that *Stenocarpella maydis* ear rot becomes of economic importance only in localized areas.

2.2.5 Management of *Diplodia* ear rot in maize

In plants, there are structural barriers to disease, preformed resistance factors and response factors. There are five principles of controlling maize diseases including exclusion, eradication, avoidance, protection and the use of resistant cultivars. However, a combination of crop sanitation, good agronomic practices and timely harvesting, has resulted in limited control of *Diplodia* ear rot (Tembo et al., 2013). Maize hybrids vary in their susceptibility to *Diplodia* ear rot. In areas where the disease is challenging, planting a resistant maize variety should be considered (OEPP/EPPO, 2006). The utilization of resistant genotypes, therefore, could be a more effective control method, because it can promote field sanity and consequently inoculum reduction (Moremoholo et al., 2010; Das, 2014). Early efforts to improve maize breeding material for resistance to *Diplodia* ear rot involved the elimination of experimental germplasm with pronounced susceptibility from breeding programs (Dorrance et al., 1998; Vincelli, 2012). Maize seed with insect protection traits and fungicides applied when foliar diseases are present at high levels help minimize stalk cannibalization during grain filling (Lipps and Mills, 2001). Management of infected crop debris (stalks, ears) following harvest reduces the amount of overwintering disease inoculum while storing grain at below 20% moisture content reduce storage rot caused by *Stenocarpella* species (Flett et al., 2001).

2.3 Susceptibility, tolerance and resistance of *Diplodia* ear rot in maize

Maize may be susceptible, tolerant, or resistant to different pathogens (Stuckey et al., 1993). The term susceptible indicates that, the maize without any internal improvement mechanism becomes diseased if the environment, time and pathogen are favorable (Vincelli, 1979; Tembo et al., 2013). The term tolerant implies that the maize may become diseased but modest damage occurs; it has some internally adjustable ability to stay with the disease (Dodds and Rathjen, 2010; Haggag, 2013). Resistance is less affected by external factors than other disease control measures. Resistant cultivars present the most feasible control of maize diseases and are widely used because they are also environmentally safe (Stuckey et al., 1993; Haggag et al., 2013). When new virulent pathogens appear, resistance may break down and new resistant gene(s) will be required to be incorporated into commercially acceptable maize cultivars (Elliot, 1958; Moore et al., 2011). However, maize disease resistance highlights some aspects of the subject that are currently of significant interest. Resistance of maize to *Diplodia* ear rot has been studied because of its potential to improve yields (Dorrance et al., 1998; Vincelli and Hershman, 2013).

Diplodia ear rot is best managed through the use of resistant hybrids. Crosses between two susceptible parents yielded offspring that were more susceptible than either parent (Hooker and White, 1976; Mukanga et al., 2010a). Rensburg et al. (2003) found that crosses with the most susceptible parents usually contributed susceptibility to the F_1 dependent on the year evaluated, while in crosses with resistant parents, the F_1 were closer to the susceptible parent in one year and to the resistant parent in another year. Vincelli (2012) tested the crosses with the most susceptible parent and found that all hybrids are susceptible to some degree; certain hybrids are most likely to susceptible for use in an infested field. Resistance to *Diplodia* ear rot is inherited independently from resistance to other ear rot pathogens (Hooker, 1956 as

cited by Darrance et al., 1998) and to other diseases caused by *S. maydis* (Hooker, 1956; Thompson et al., 1971 as cited by Dorrance et al., 1998).

2.4 Inoculation techniques of *Diplodia* ear rot

To identify resistant germplasm, it is necessary to use reliable artificial inoculation methods (Ullstrup, 1949; Klapproth and Hawk, 1991; Bensch et al., 1992; Bensch, 1995). Methods of *S. maydis* inoculation used in breeding programs should reproduce as closely as possible the infection under natural conditions (Kuhnem et al., 2012). Studies to induce maize ear rot by inoculations have been carried out for *Diplodia* ear rot (Raleigh, 1930; Wiser, 1956; Bensch et al., 1992; Moremoholo, 2008; Justino et al., 2011) and other ear diseases of maize (Ullstrup, 1970; Drepper and Renfro, 1990; Reid et al., 2002). The selected method of inoculation should also provide consistent data over the years, locations and genotypes, thus making it possible to make a clear distinction between susceptible and resistant genotypes (Ullstrup, 1970; Klapproth and Hawk, 1991; Bensch, 1995). Finally, these methods should be easy to apply.

There are several inoculation techniques for *Stenocarpella maydis* adopted by different researchers such as spray method, toothpick method and ground infected maize kernels applied in the whorl or on the silks (Nowell, 1992). The spray method of inoculation was first used by Burrill and Barrett in 1909 as cited by Koehler (1959) and was later developed more fully by Ullstrup (1949).

The toothpick method was first proposed and used by Young (1943). In this method, about 150 to 200 colonized toothpicks are prepared by removing inhibitory compounds such as tannis and phenolics through pasteurization in a period between two to six minutes (Young, 1943 as cited by Jeffers, 2002). Toothpicks are carefully washed in fresh tap water, dried and placed them in a glass jar with 45ml of potato dextrose gel broth such that it will deliver

sufficient liquid to moisten the toothpicks for good mycelial growth with a little addition liquid in the bottom of the jar. Immediately after the both is added, the jars of toothpicks are sterilized for 30 minutes and then allowed to cool and inoculated with the mycelium of *Stenocarpella maydis*. Three weeks of incubation at 25 to 30ºC, the fungus has colonized the toothpicks, and is ready for use. These colonized toothpicks are inserted into the shank of the ear at 14-21 days after silking (Young, 1943). It is important to hit the peduncle tissue for more reliable and uniform results (Jeffers, 2002). The toothpick is the most efficient, rapid and easy to use means of inoculation since the fungus grows uniformly over the toothpicks resulting in a similar amount of disease pressure being delivered to each plant (Gulya et al., 1980). Meanwhile *Stenocarpella maydis* normally enters the ear through the shank, this inoculation method delivers the inoculum in the location where the fungus passes to arrive in the ear.

For the ground infected maize kernel method the inoculation is applied in the whorl or on the silks of maize kernel. The diseased kernels 150g and 100ml of distilled water agar are autoclaved for 40 minutes on two consecutive days in a 375ml plastic box, then inoculated with a 10ml conidial suspension. The boxes are incubated at 25ºC, 12-hour photoperiod, for six to eight weeks and dried (Rheeder et al., 1990; Klapproth and Hawk, 1991; Flett, 1997). Three weeks after when sample is dried it will be milled and three to five grams of ground *Stenocarpella maydis* infested ears inoculated in the whorl three weeks prior to tasseling. If the environment is dry, water should be added to the whorl after adding the ground tissue (Jeffers, 2002). Inoculation in the whorl with ground grain is very easy to apply and does not injure the plant (Rheeder et al., 1990; Flett, 1997).

2.5 Mode of gene action for *Diplodia* ear rot resistance and agronomic traits in maize

The knowledge about the magnitude and behavior of genetic components for quantitative character being envisaged is very essential for a plant breeder since gene action and effects are key factors for understanding the inheritance of quantitative traits (Lamkey and Lee, 1993; Arora et al., 2010). Quantitative traits are usually considered to be controlled by multiple genes and are considerably influenced by interaction of environment (Hallauer and Miranda, 1981; Kearsey and Pooni, 1996). Various reliable biometrical techniques dealing with the genetic analysis of quantitative traits were developed (Mather and Jinks, 1982). Gene action is mainly studied through the use of mating designs like the diallel, North Carolina designs I, II, and III, and generation means.

.Generation mean analysis (GMA) was developed by Hayman (1958). Generation means analysis utilizes six population means P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 to estimate genetic effects (Hayman, 1958). It involves the estimation of various genetic effects including additive, dominance, additive x additive, additive x dominance and dominance x dominance of quantitatively inherited traits. Generation mean analysis has been widely used to study disease resistance and other agronomic traits in cereals (Boling and Grogan, 1965; Bernardo et al, 1992; Chungu et al., 1996; Frank and Hallauer, 1997; Hakizimana et al., 2004; Bucheyeki, 2012; Butrón et al., 2015).

Reports of gene action for ear rot resistance vary across studies. Mukanga et al. (2010a) reported that both additive and non-additive gene effects were important for ear rot resistance. Vincelli (1979) reported that resistance under artificial infection shows quantitative inheritance. Wiser et al. (1960) reported that susceptibility to percentage cob infection by *Diplodia* ear rot was controlled by dominance. In a diallel studies Rossouw et al., (2002a) and Tembo et al. (2013) reported that the resistance to *Diplodia* ear rot is

predominantly additively inherited. Dorrance et al. (1998) noted significance of additive and non-additive effects for *Diplodia* ear rot resistance in a study with temperate maize.

The inheritance of resistance to *Stenocarpella maydis* is complex and many types of inheritance mechanisms have been reported (Dorrance et al., 1998; Rossouw et al., 2002a). In a study on resistance to *Diplodia* (*Stenocarpella maydis*) ear rot, additive effects were found to be important (Villena, 1969). Hema et al. (2001) concluded that recessive genes controlled the inheritance anthesis-silking interval in maize, with prominent additive effects of genes. For grain yield and moisture content, Mehaljevic et al. (2005) noted that epistasis was of minor importance for both traits. Azizi et al. (2006) reported that preponderance of both additive and dominance effects were important for grain yield and other traits evaluated in this study but the dominance effects had a more pronounced for grain yield, kernel per row, cob weight and Anthesis. Ravikant et al. (2006) studied gene action for yield and yield component traits (grain yield per plant, anthesis and silking date, plant height, grains ear per row, shelling percentage and 100-grain weight) in maize using generation means analysis and found that most of the characters were controlled by additive effects.

Ravikant et al. (2006) further noted that shelling percentage, grain yield per plant and some other traits were governed by duplicate epistatic genetic effects. Sofi and Rather (2006) reported preponderance of non-additive gene action for the expression of most of the traits studied except for days to 50% husk browning (maturity) and 100-grain weight which exhibited the prevalence of additive type of gene action. Sher et al. (2012) reported that additive gene action was important in the inheritance of days to 50% tasselling and days to 50% silking, and prevalence of non-additive gene action for days to 50% husk browning and grain yield.

Other studies using generation means analysis are summarized in Table 2.1.

Table 2. 1: Studies one gene action for various traits in maize using generation mean analysis

Authors	Traits studied	Gene action identified	Germplasm used
Gamble (1962a and1962b)	Plant height, ear length ear weight Kernel row number, ear diameter and seed weight	The additive X additive and additive X dominance gene effects were relatively more important than the dominance X dominance effects. Epistatic effect, dominance x domina epistatic effects	Six inbred lines were used as parents in this study of which Hy, WF9, and Oh4, 87,Bl4, and B36, are of more recent origin
Kassem et al. (1978a and 1978b)	Silking, tasseling, ear height, ear length grain yield plant height and ear diameter	Additive effects more important, additive x dominance effects were predominant Additive x additive and Dominance x dominance effects played a major role in the inheritance.	A cross between H99 with susceptible inbred line A619
Hallauer and Miranda (1988)	Ear height, number of ear per plant, corn rows number, corn weight, corn depth, cob diameter, ear length and ear diameter	Additive x dominance effects were predominant over non-additive gene action for grain per row	B14, B37, B73, and B84
Frank and Hallauer (1997)	Twin-ear shoots (the total number of shoots found at the top three ear nodes on each plant after flowering); the total number of ears	Penetrance and expressivity have positive additive effect and negative dominance effects for penetrance that were significant to the epistatic effects	B79 and Mo17
Atanaw et al. (2006)	Days to 50% tasseling and days to 50% silking. days to 50% husk browning and grain yield	Additive gene action was important in the inheritance	HYD.SEL 13, and CI-5

…… continued

Table 2.1: (Continued)……

Authors	Traits studied	Gene action identified	Germplasm used
Bernardo et al. (1992)	Percentage of plants infected with head smut on each plot prior to harvest	Dominance and epistatic effects were minor compared with additive effects.	A632 x A188 and LH74 x LMZ66
Zvonimir et al. (2008)	The sample size of 80 plants for uniform generations and 240 plants for segregating generations.	The additive, dominance and heterosis. The obtained gene effect showed that heterosis, the dominance effects appeared to be prevailing in most crosses for yield.	Os36-16 (SSS-B14), Os3-48 (Iodent), B84 (SSS-B73), Va99 (OH43), Os163-9 (from a single cross), Os645Kr (SSS-B37), Os6-2 (Lancaster Sure Crop), and Os86-39 (Wf9).
Iqbal et al. (2010)	Leaf area and plant height on plant basis.	Significant dominance effects were indicated in all crosses for both traits and these effects were much higher in magnitude than their corresponding additive effects. Epistasis played a considerable role in controlling leaf area and plant height trait	Four flint white kernel maize inbred lines: Pop.9804X Pop.9801 Pop.9804 X FRW-4 Pop.9805 X Pop.9801 Pop.9805 X FRW-4
Rodrigo et al. (2012)	Lesion length (cm) of anthracnose stalks rot.	Dominance, additive x dominant and additive x additive, Results indicated contrasting modes of inheritance. Heterosis was also founded was widely differed between populations, which can be attributed to the genetic background of the parental resistant lines	Three inbred lines (Das21, Das64, and Das86) were used
Haq et al. (2010)	Number of grains per row, 100-grain weight and grain yield per plant.	Genetic and epistatic effects leading the inheritance of grain yield and yield components, the result showed the preponderance of non-additive effects for all the characters.	FR-37, NYP-8, NYP-8-1, NCQPM-1, NCQPM-2, NCQPM-4

2.6. Heritability in maize

Heritability estimates provide reliable information about the extent to which a particular genetic trait will be transmitted to the subsequent generations and could help significantly in making selection (Nyquist, 1991; Falconer and Mackay, 1996). Estimation of heritability is essential for breeders to predict the genetic potential of breeding materials, identify effective promising combinations in hybridization and to determine effective methods of selection there from (Hanson, 1963; Ceballos et al., 1991). The higher heritability, the simpler and less time consuming the selection procedure and the greater will be the genetic improvement (Allard, 1960; Milligan et al., 1990; Soher et al., 2013). Heritability can be estimated from generation means and variances (Kearsey and Pooni, 1996; Holland et al., 2003).

Heritability is used in both a broad and narrow sense. Broad sense heritability (H) is the ratio of total genetic variance to the phenotypic variance, while narrow sense heritability (h^2) is the ratio of additive genetic variance to the phenotypic variance (Nyquist, 1991; Falconer and Mackay, 1996). The latter is more important to plant breeders because the effectiveness of selection depends on the additive portion of the genetic variance in relation to the total variance (Falconer and Mackay, 1996). High values of narrow sense heritability indicate that additive gene action is more involved in controlling particular traits especially under weak dominance effects (Jawaharlal et al., 2011). A low heritability value can indicate several things: that a significant proportion of the trait variation is due to the environment or experimental error; that a large number of genes govern the trait and that relative differences among genotypic values depend on the environment (genotype by environment interaction) (Atlin et al., 2000; Holland et al., 2003). Heritability is high if the genetic variation in a progeny is large in relation to the environmental variation. According to Stanfield (1991) heritability value (<0.2) is classified as low, medium (0.2–0.5) and high (>0.5). There are two

ways to report heritability: family-basis heritability, and plot-basis heritability, and their interpretation are quite different (Holland et al., 2003). Heritability estimates can be influenced by parent materials and environment interactions (Holland et al., 2003).

Heritability estimates for maize ear rot disease traits have been made in several studies. In a study by Rossouw et al. (2002a), high broad sense and narrow sense heritability estimates were reported for rotten ears, rotten kernels by mass and disease severity index in maize artificially inoculated with *Stenocarpella maydis* in tropical maize. Tembo et al. (2013) reported low narrow sense heritability for *S. maydis* (0.18) and *Fusarium graminearum* (0.35) disease severity across locations in tropical maize. In a study on *Fusarium* ear rot, Horne et al. (2016) reported high heritability (entry mean basis) estimates of 0.65 and 0.77 for cycle 0 and cycle 1, respectively, and moderate heritability estimates of 0.52 for cycle 2 of recurrent selection for *Fusarium* ear rot percentage in temperate maize. In another study with temperate maize, Bolduan et al. (2009), reported low (0.42) and high (0.80) heritability estimates for *Gibberella* ear rot rating under natural conditions and artificial inoculation with *F. graminearum,* respectively. In the same study Bolduan et al. (2009), also reported low (0.32) and moderate (0.65) heritability estimates for *Fusarium* ear rot rating under natural conditions and artificial inoculation with *F. verticillioides,* respectively.

2.7. Heterosis in maize

The term heterosis was coined by Shull (1908). Heterosis is defined as the superiority of an F_1 hybrids over either one of its parents in terms of yield or some other characters. Heterosis, when defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1996) is termed mid-parent heterosis. When a hybrid performs better than either of the parents, the phenomenon is termed high-parent heterosis.

Expression of heterosis in the F_1 hybrid progeny is the ultimate aim of plant breeders in hybrid development programme. Many researchers in the world have described development of hybrid cultivars based on heterosis in maize (Hallauer and Miranda, 1981). Studies on the genetic basis of heterosis for polygenic traits in various crops have revealed that heterosis is the result of a partial to complete dominance, over dominance and epistasis or a combination of all these (Schnell and Cockerham, 1992; Stuber, 1994; Kearsey and Pooni, 1996; Lamkey and Edwards, 1999).

Heterosis can be positive or negative. The interpretation of heterosis depends on the nature of trait under study. For example, positive heterosis is preferred in yield studies because it shows inclination towards higher yield (Duvick, 2001). On the contrary, negative heterosis is desired in disease resistance as it indicates that the breeding materials lean towards resistance. In crosses between broad-based maize populations, Rezende and Souza (2000) reported 7.7% heterosis for grain yield. Muraya et al. (2006) reported that heterosis was more important in grain yield, yield components, plant height, number of leaves plant^{-1} and leaf area indices than for the other traits studied.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Germplasm used in this study

Five inbred lines developed at CIMMYT maize breeding programs in Kenya, Zimbabwe and Mexico were used in this study (Table 3.1). Selection of these inbred lines was based on top cross performance, adaptation to mid-altitude agro-ecology of Eastern and Southern Africa and widely used in breeding. Three out of the five inbred lines used were tolerant to ear rots while the other two are susceptible to ear rots to generate F_1, F_2, BC_1P_1, and BC_1P_2.

Table 3.1: List of maize inbred lines and pedigree to be used in this study

Line	Code	Name	Pedigree	Origin	Characteristics
1	P1	CL-RCW31	CL-RCW31	Mexico	Good combiner, tolerant to ear rots
2	P2	CML543	CKL05003	Kenya	Good combiner, tolerant northern corn leaf bright GLS and to ear rots
3	P3	LPSC7	La Posta Seq C7-F103-2-1-1-1xMIRTC5Bco F80-4-2-1-1-1-3-1-B-B-B	Mexico	Good combiner, tolerant to ear rots
4	P4	CML442	CML 442	Zimbabwe	Good combiner, tolerant to northern corn leaf blight, susceptible to ear rots
5	P5	VL06688	[CML312/CML445//[TUXPSEQ]C1F2/P49- SR]F2-45-3-2-1-BBB]-1-2-1-1-2-BBB-B-B-B-B	Zimbabwe	Good combiner, tolerant to northern corn leaf blight, susceptible to ear rots

3.2 Development of F_1, F_2 and back cross (BC_1P_1 and BC_1P_2) generations

Five inbred lines were crossed to form six F_1 hybrids (Table 3.2) in Kenya at KALRO Kiboko during 2012/2013 growing season. These hybrids were previously screened for their reaction to *Diplodia* ear rot disease (Wambugu, 2013).

During the long rains season of 2014, the five inbred line and six F_1 hybrids were planted in the nursery at KALRO-Kiboko Research Station (37.72^{o}E, 2.21^{o}S, 975 masl) to produce F_2, BC_1P_1 and BC_1P_2 generations (Figure 3.1). Each of the F_1 hybrids was self-pollinated to produce the F_2 generation. Each of the F_1 hybrids was backcrossed to each of the inbred line parents to generate BC_1P_1 (F_1 crossed to the tolerant parent (P_1)) and BC_1P_2 (F1 crossed to the susceptible parent (P_2)). To make the backcrosses, the F_1s were used as the females.

Table 3.2: List of F_1 hybrids and their pedigrees

Cross	Pedigree	Origin
1	CL-RCW31/ CML442	Kenya
2	CKL05003/CML442	Kenya
3	CKL05003/CL-RCW31	Kenya
4	CKL05003/La Posta Seq C7-F103-2-1-1-1xMIRTC5 Bco F80-4-2-1-1-1-3-1-B-B	Kenya
5	La Posta Seq C7-F103-2-1-1-1xMIRTC5 Bco F80-4-2-1-1-1-3-1-B-B/CML442	Kenya
6	La Posta Seq C7-F103-2-1-1-1xMIRTC5 Bco F80-4-2-1-1-1-3-1-B-B/VL06688	Kenya

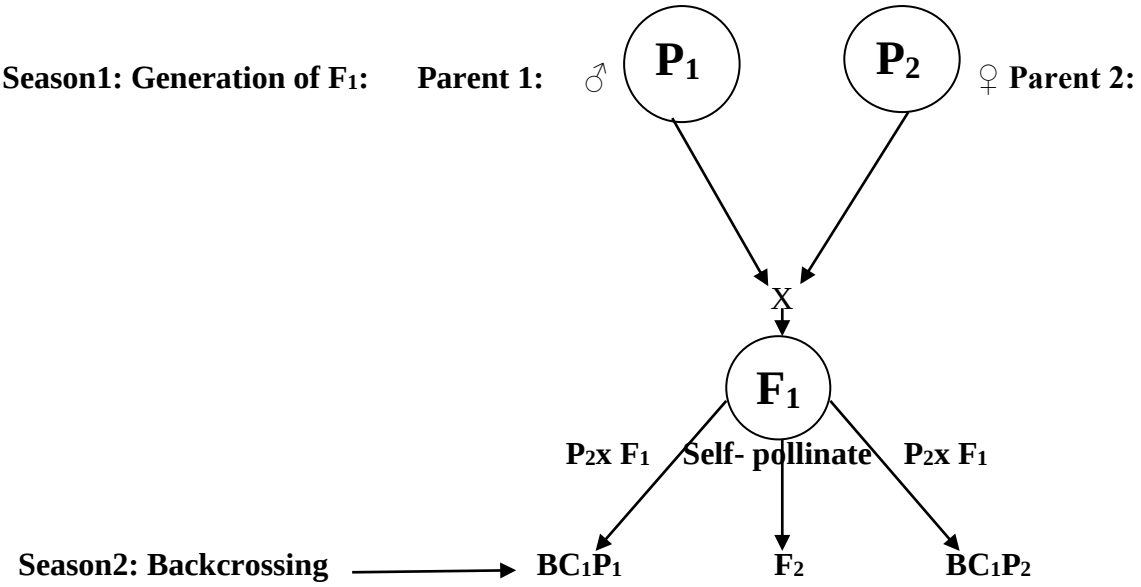

Figure 3.1: Diagrammatic representation of the procedure used to develop different generations of maize used in this study.

3.3 Field evaluation of six generations

The six generations (two parents P_1 and P_2, F_1, F_2, BC_1P_1 and BC_1P_2) for each of the six crosses were evaluated in 2014/2015 short rain season. The six generations for each cross was composed into an individual trial. The trial was arranged as a randomized complete block design (RCBD) with three replications. The experimental unit was a 4m two row plot with a spacing of 75cm between rows and 25cm between hills. During planting, two seeds were placed in each hill and later thinned to one per hill while compensating for any that failed to germinate two weeks after emerging so as to achieve a population density of 53,333 plants per hectare. Because of shading effects, an inbred line CML202 was used to border the inbred line parents (P_1 and P_2) in the trial. At planting DAP basal fertilizer was applied at a rate of 80 Kg P_2O_5 and 31 Kg N/ha while CAN was used for top-dress six weeks after planting at a rate of 80Kg N/ha. Carbofuran (2-3,-dihydro-2, 2-dimethyl-7-benzofuranol methylcarbamate) was applied at a rate of 2 g per hill at planting to control cutworms while Bulldock, 0.05 GR (Beta-cyfluthrin) was applied 30 days after emerging to control stalk borers.

3.4 Description of locations used for the study

The nurseries for making crosses were planted in April 2014 at KALRO Kiboko research station (37°72'E, 2°21'S, 975 masl) located in Makueni County in Eastern province of Kenya. The station receives between 545 and 629mm of rainfall with bimodal distribution (April-May and October- January). This is a hot dry region with a mean annual temperature of 22.6°C, mean annual maximum of 28.6°C and mean annual minimum of 16.5°C. The soil type at Kiboko is well drained, very deep, dark reddish brown to dark red, friable sandy clay to clay (Acri-Rhodic Ferrassols) developed from undifferentiated basement system rocks, predominantly banded gneisses (CIMMYT, 2013).

The trials were planted in September 2014 at three locations sites (Kakamega, Alupe, and Kibos) representing some of the major maize growing agro-ecological zones in Kenya. The site at KALRO Kakamega (34° 49' E, 0°16'N, 1534 masl) is in the upper midland (UML) agro-ecological zone, with average annual rainfall of 1850 mm with a bimodal distribution (March-July and September-November). The soil type at Kakamega is classified as Eutric Nitisol and is a clay loam. The site at Kibos (02.324°S, 34.48°E, 1193 masl) is in lower midland (LM2) agro-ecological zone, with an average annual rainfall of 865mm with a bimodal distribution (March-July and September-November). The soil type at Kibos is classified as Eutric Cambisol and is a sandy loam. The site at KALRO-Alupe (0.30°N, 34.07°E, 1145 masl) is located in the lower midland (LM1) agro-ecological zone, with average annual rainfall of 1400mm with a bimodal distribution (March-July and September-November). The soil type at Alupe is classified as Orthic Ferralsol and is a sandy clay loam.

3.5 Culturing of Diplodia *(Stenocarpella maydis)* and inoculation procedures

The Diplodia ear rot pathogens were initially isolated from infected maize cobs obtained from KALRO-Kakamega maize trial fields during the main planting season of April-August 2014. *Diplodia* infected maize cobs were identified using the CIMMYT guide for field

identification manual (CIMMYT, 2004). Media was prepared by measuring 39g of potato dextrose agar and placing it in 1 litre of acidified water (10ml of lactic acid in 1 litre of water). The solution was autoclaved at 121˚C for 15 minutes. Then, 20ml of the media was dispensed in sterilized glass plates and left for some time to solidify. Infected grains were first sterilized by soaking in 5% sodium hypochlorite (NaOCl) (Reckitt Benkiser East Africa Limited, Nairobi, Kenya) solution for five minutes and then after rinsed three times in distilled water. The seeds were subsequently blotted on sterilized filter paper to dry and then five seeds were plated on potato dextrose agar (DPA) (Becton Dickinson, Sparks, MD, USA) plates. The fungal growth on plates was sub-cultured and incubated at 25˚C for four days and was ready for transfer to toothpicks after 5–7 days. White soft mycelial growth indicated the presence of *S. maydis,* the causal fungus of *Diplodia* ear rot.

Toothpicks were used as the inoculation tool because they can trap culture media and are sharp to be used to injure and inoculate maize cobs. The toothpicks were initially sterilized by boiling in water for 20 min and then air-dried. They were then placed upright in vases measuring 7 cm in diameter and 4 cm in height, containing 250–350 ml of potato dextrose agar to coat the toothpicks, autoclaved at 121^{o}C for 15 minutes and left to cool at room temperature. Each vase held approximately 1000–1500 toothpicks. In order for the fungal culture coated toothpicks to be used as carrier for inoculum, culture plugs from pure cultures of *S. maydis* were placed in each bottle containing the sterile toothpicks and allowed to colonize them for 10 days. After the toothpicks were fully colonized, they were air-dried before inoculating the test genotypes. Inoculation was performed by inserting a colonized toothpick in the middle of a developing maize cob at approximately 10 days after mid-silking stage (Chambers, 1988). Only the top ear on each plant was inoculated. Care was taken not push the toothpick up to the cob. The toothpick was left in the cob until harvest (Plate 3.1).

Plate 3.1: Inoculation with *Diplodia* pathogen for maize silks using toothpick inoculation method

3.6 Assessment of agronomic traits in six generations

The agronomic traits recorded were number of days to 50% anthesis (AD) in a plot. Plant height (PH) measured as the distance in centimeters from ground to the top of tassel (using five plants per plot sampled at random). At all locations, trials were hand harvested. Ear aspect was scored on a scale of one to five where one was scored for clean, uniform, large and well filled ears with preferred texture in area while five was for rotten, variable, small and partially filled ears with the undesirable texture in the area. Field weight (FW) of the cobs with grain and percent grain moisture content was recorded. To obtain grain yield in tons per hectare and adjusted to 12.5% moisture content using the following formula (Carangal et al., 1971):

$$Grain\ Yield = \frac{(100 - M.C)X\ FEW\ X\ Shelling\ Co - effecient\ X10{,}000}{(100 - 12.5)X\ Plot\ Area}$$

where, *MC* = Percent moisture in grains at harvest, *FEW* = Fresh ear weight (kg) at harvest

Shelling Co-efficient = Shelling percentage / 100.

3.7 Assessment of *Diplodia* ear rot severity

The top ears which were inoculated in each plot were assessed to quantify disease incidence and disease severity of *Stenocarpella maydis.* The incidence of *Stenocarpella* in ears was evaluated on the basis of presence of white mycelia and dark pycnidia or symptoms on the grain. Healthy ears were visually separated from the infected ones. The incidence was expressed in percentage of infected ears (Ullstrup, 1949).

The incidence of *Diplodia* ear rot disease for each plot was calculated:

$$\text{Diplodia ear rot incidence (DEI)}(\%) = \left[\frac{\text{Number of rotten ears}}{\text{Total number of ears assessed}}\right] x100$$

To assess the severity of *Stenocarpella maydis* in the ears, infected ears were classified using the procedure of Ullstrup (1949). The procedure was applied to the number of ears in each category of severity in order to calculate the degree of severity (%) for each treatment.

The severity of *Diplodia* ear rot in each plot was expressed as a Diplodia ear rot severity (DES):

$$\text{Diplodia ear rot severity (DES)}(\%) = \left[\frac{0.25n1 + 0.5n2 + 0.75n3 + n4}{N}\right] x100$$

where, DES is Diplodia ear rot severity, n= number of ears in classes n_1 to n_4 (n_1 number of ears for 1 to 25% of kernels on the cob are rotten, n_2 number of ears for 26 to 50% of kernels on the cob are rotten, n_3 number of ears for 51 to 75% of kernels on the cob are rotten and n4 number of ears for 76 to 100% of the kernels on the cob are rotten) and N is the total number of ears harvested per plot.

Weight of rotten ears (WER) was taken as the Field weight (FW) for rotten cobs (weight of the cob with rotten grain) and percent of grain moisture content recorded from each plot.

3.8 Statistical analysis

Square root transformation was performed on *Diplodia* ear rot disease severity data to normalize distribution before analysis of variance. Data were then subjected to combined analysis of variance across environments following a general linear model. The statistical model for analysis of data across environments was:

$Y_{rge} = \mu + \alpha_g + \beta_e + \rho_r(\beta_e) + \alpha_g\beta_e + \varepsilon_{rge}$

where, Y_{rge} is the measured trait of genotype *g* in replicate *r* at environment *e*, μ is the grand mean, α_g and β_e are the genotype and environment main effects, $\rho_r(\beta_e)$ is the replicate effect nested within an environment, $\alpha_g\beta_e$ is the interaction between main effects, and ε_{rge} is the random experimental error.

Genotypes were considered fixed while environments and blocks were considered random effects. The outline of the ANOVA across environments is shown in Table 3.3. The frequency distribution, mean and variances each of trait for each generation was computed using the SAS (SAS Institute, 2011).

Table 3.3: Analysis of variance for generations combined across environments

Source of variation	df	Expected mean squares
Env (E)	e-1	-
Reps (Env)	e(r-1)	-
Genotype	g-1	$\delta^2_e+r\delta^2_{ge}+re\delta^2_g$
Genotype*Env	(g-1)(e-1)	$\delta^2_e+r\delta^2_{ge}$
Error	e(g-1)(r-1)	δ^2_e
Total	reg-1	

3.9 Generation means analysis for resistance to *Diplodia* ear rot resistance

Generation means analysis was performed to determine the mode of gene action for agronomic traits and disease parameters under artificial inoculation with Diplodia ear rot. Two types of analyses steps were carried out. First, the differences among the means of the six generations, P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2, for each trait of the six crosses were analyzed by the joint scaling test (Cavalli 1952; Mather and Jinks 1982) to test the fitness of the additive-dominance model to the generation means. The mid-parent (m), additive effect (a) and dominance effect (d) were estimated by weighted least squares method. The scaling tests (A, B and C) are:

$A = 2\,\overline{BC_1P_1} - \overline{F_1} - \overline{P_1}$

$B = 2\,\overline{BC_1P_2} - \overline{F_1} - \overline{P_2}$

$C = 4\,\overline{F_2} - 2\,\overline{F_1} - \overline{P_1} - \overline{P_2}$ where, A, B and C are the values of the each scaling test,

The variance of the tests are:

$V_A = 4V_{BC_1P_1} + V_{P_1} + V_{F_1}$

$V_B = 4V_{BC_1P_2} + V_{P_2} + V_{F_1}$ and

$V_C = 16V_{F_2} + 4V_{F_1} + V_{P_1} + V_{P_2}$ where, V_A, V_B, V_C are their respective variances,

The significance of each scaling test was determined using a T-test. The T-test for each scaling test is given as:

$$\pm t = \frac{Deviation}{standard\ error} = \frac{Deviation\ (Values\ of\ A\ or\ B\ or\ C)}{\sqrt{Variation\ of\ deviation}}$$

$\pm t_A = \frac{A}{\sqrt{V_A}}$ and $t_B = \frac{B}{\sqrt{V_B}}$ and $t_c = \frac{C}{\sqrt{V_C}}$ where t_A, t_B and t_C are T-test for scaling test A, B and C. Significance of a scaling test implies that the additive-dominance model is inadequate.

Secondly, we used the results of the scaling test to fit either the three-parameter or six-parameter genetic model developed by Hayman (1958) using the notation of Gamble (1962a). The model is:

$Y = m + \propto d + \beta a + \propto^2 aa + 2 \propto \beta ad + \beta^2 dd$

where, Y = observed mean for a particular generation, m = the mean effect, $\propto$ and β = coefficients of the following pooled genetic effects: a = additive, d = dominance, aa = additive x additive, ad = additive x dominance, dd = dominance x dominance. The coefficients used to fit the model are given in Table 3.4.

Table 3.4: Coefficients for different generations in generation means analysis

	Genetic effect					
Generation	m	a	d	aa	ad	dd
P_1	1	1	0	1	0	0
P_1	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	0.5	0	0	0.25
BC_1P_1	1	0.5	0.5	0.25	0.25	0.25
BC_1P_2	1	-0.5	0.5	0.25	-0.25	0.25

The genetic effects are estimated as:

$$[\mathrm{m}] = \overline{F_2}$$

$$[\mathrm{a}] = \overline{BC_1P_1} - \overline{BC_1P_2}$$

$$[\mathrm{d}] = \overline{F_1} - 4\,\overline{F_2} + 2\,\overline{BC_1P_1} - 2\,\overline{BC_1P_2} - \frac{1}{2}\overline{P_1} - \frac{1}{2}\overline{P_2}$$

$$[aa] = 2\overline{BC_1P_1} + 2\,\overline{BC_1P_2} - 4\overline{F_2}$$

$$[ad] = \overline{P_2} - \overline{P_1} + 2\,\overline{BC_1P_1} - 2\,\overline{BC_1P_2}$$

$$[dd] = \overline{P_2} + \overline{P_1} + 2\overline{F_1} + 4\overline{F_2} - 4\,\overline{BC_1P_1} - 4\,\overline{BC_1P_2}$$

The genetic parameters in this model and their respective standard errors were estimated using least squares regression on transformed data.

3.10 Heritability of resistance to *Diplodia* ear rot in maize

Broad-sense heritability for traits across environments was calculated using the variance components according to Hallauer et al. (2010) as:

$H = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_{ge}}{e} + \frac{\sigma^2_e}{re}}$ where, σ^2_g is the genotypic variance, σ^2_{ge} is the genotype by environment interaction variance, σ^2_e is the error variance, *e* is the number of environments, and *r* is the number of replications for a single environment.

Narrow-sense heritability across environments was calculated directly from the weighted least squares (WLS) estimates according to Kearsery and Pooni (1996) as:

$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_E}$ where, σ^2_a is the additive variance, σ^2_E is the environmental variance resulting from differences between individuals within families.

3.11 Estimation of heterosis for *Diplodia* ear rot resistance in maize

Heterosis was computed as the mean performance of the F_1 over that of either mid-parent or high-parent using adjusted means and expressed as percent (Hallauer and Miranda, 1981; Fehr, 1993).

Mid-parent heterosis was calculated as:

$MPH = \frac{(F_1 - MP)}{MP} x100$ where, F_1 is the mean F_1 hybrid performance and $MP = (P_1 + P_2)/2$ where P_1 and P_2 are the means of the two parents. High-parent heterosis was calculated as $HPH = \frac{(F_1 - HP)}{HP} x100$ where HP is mean of the best parent.

CHAPTER FOUR: RESULTS

4.1 Environment effects for maize resistance to *Diplodia* ear rot

4.1.1 Analysis of variance for agronomic traits and Diplodia ear rot disease parameters

This study was conducted at three locations (Kibos, Alupe and Kakamega) in western Kenya under artificial inoculation with *Stenocarpella maydis*, the causal pathogen of *Diplodia* ear rot. Combined analysis of variance showed significant (P<0.05) differences among environments for grain yield and other agronomic traits for all crosses (Tables 4.1 to 4.6) except for ear aspect for cross CML543 x CLRCW31 (Table 4.3). Results from analysis of variance showed that there were highly significant (P< 0.01 and P< 0.001) differences among generations derived from all crosses for grain yield and other agronomic traits (Tables 4.1 to 4.6).

Table 4.1: Mean squares for grain yield, agronomic traits and disease parameters for the cross CLRCW31 x CML442 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of		**Grain**	**Agronomic traits**			**Disease parameters**		
Variation	**df**	**yield**	AD†	EA	PH	DEI	DES	WER
		(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Env	2	9.30***	885.63***	4.18***	1208.80***	535.28	1085.77*	4.96***
Rep(Env)	6	0.20	2.07	0.09	63.89	302.43	334.66	0.06
Generation	5	21.67 ***	39.23***	2.59***	2802.69***	503.62*	279.89	0.52*
Env*Generation	10	0.80 **	3.56	0.29	164.91***	309.64	299.91	0.26
Error	30	0.21	3.90	0.15	37.22	202.18	247.92	0.17

* Significant at P < 0.05 probability level.
** Significant at P < 0.01 probability level.
*** Significant at P < 0.001 probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

Table 4.2: Mean squares for grain yield, agronomic traits and disease parameters for the cross CML543 x CML442 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of		**Grain**	**Agronomic traits**			**Disease parameters**		
Variation	**df**	**yield**	AD†	EA	PH	DEI	DES	WER
		(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Env	2	1.86**	734.74***	0.62*	450.02***	2273.51***	1945.40***	6.79***
Rep(Env)	6	0.16	2.20	0.06	91.96*	73.01	111.63	0.24
Generation	5	25.76***	97.41***	3.14***	3699.80***	483.21*	490.02**	1.86***
Env*Generation	10	0.65	3.05	0.46**	83.80*	384.21*	266.40*	0.67**
Error	30	0.35	2.38	0.14	37.41	169.08	119.30	0.17

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

Significant ($P < 0.05$) differences among environments for Diplodia ear rot incidence were recorded for crosses CML543 x CML442, CML543 x CLRCW31, LPSC7 x CML442 and LPSC7 x VL06688 (Tables 4.2, 4.3, 4.5 and 4.6). Significant ($P < 0.05$) differences among environments for Diplodia ear rot severity were recorded for crosses CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, and LPSC7 x CML442 (Tables 4.1, 4.2, 4.3, and 4.5). Significant ($P < 0.05$) differences among environments for weight of rotten ears were observed for crosses CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, and CML543 x LPSC7 (Tables 4.1, 4.2, 4.3, and 4.4).

Table 4.3: Mean squares for grain yield, agronomic traits disease parameters for the cross CML543xCLRCW31 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of Variation	**df**	**Grain yield** (t ha^{-1})	**Agronomic traits**			**Disease parameters**		
			AD† (days)	EA (1-5)	PH (cm)	DEI (%)	DES (%)	WER (kg ha^{-1})
Env	2	3.35**	881.41***	0.12	2234.72***	2282.29***	2649.12***	4.53***
Rep(Env)	6	0.34	1.33	0.05	185.65**	152.21	129.78	0.17**
Generation	5	21.62***	36.95***	1.06***	3013.06***	101.29	103.72	1.13***
Env*Generation	10	1.44*	1.81*	0.16	166.94**	48.04	70.31	0.21**
Error	30	0.61	0.87	0.10	52.32	75.01	85.42	0.04

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

Table 4.4: Mean squares for grain yield, agronomic traits and disease parameters for the cross CML543 x LPSC7 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of Variation	**df**	**Grain yield** (t ha^{-1})	**Agronomic traits**			**Disease parameters**		
			AD† (days)	EA (1-5)	PH (cm)	DEI (%)	DES (%)	WER (kg ha^{-1})
Env	2	2.51*	1032.07***	4.68***	2172.69***	19.29	57.23	0.34*
Rep(Env)	6	0.75	3.87**	0.04	32.41	46.91	28.96	0.08
Generation	5	13.33***	60.06***	1.07***	6152.96***	189.87*	122.83	0.31**
Env*Generation	10	0.54	1.27	0.74***	210.46	83.33	66.33	0.14
Error	30	0.60	0.98	0.10	166.30	63.07	81.93	0.07

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

Combined analysis of variance revealed significant ($P < 0.05$) differences among generations for Diplodia ear rot incidence for five out of the six crosses (CLRCW31 x CML442, CML543 x CML442, CML543 x LPSC7, LPSC7 x CML442 and LPSC7 x VL06688; Tables 4.2, 4.3, 4.5 and 4.6). For Diplodia ear rot severity, differences among generations were only significant ($P < 0.05$) for cross CML543 x CML442 (Table 4.2). There were significant ($P < 0.05$) differences among generations for weight of rotten ears for all crosses except LPSC7 x VL06688 (Table 4.6). The generation x environment interaction was significant ($P < 0.05$) for all three disease parameters (*Diplodia* ear rot incidence, *Diplodia* ear rot severity and weight of rotten ears) in only cross CML543 x CML442 (Table 4.2) while weight of rotten ears in cross was significant in cross CML543 x CLRCW31 (Table 4.3). This implies that there were differences among the generations in reaction to inoculation with *Stenocarpella maydis* at different locations for these two crosses.

Table 4.5: Mean squares for grain yield, agronomic traits and disease parameters for the cross LPSC7 x CML442 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of Variation	**df**	**Grain yield** (t ha^{-1})	**Agronomic traits**			**Disease parameters**		
			AD† (days)	EA (1-5)	PH (cm)	DEI (%)	DES (%)	WER (kg ha^{-1})
Env	2	1.56*	623.69***	1.23***	1464.35***	2313.58**	3241.05***	0.59
Rep(Env)	6	0.44	3.59*	0.43**	106.94	542.98*	678.62*	0.54
Generation	5	7.74***	80.06***	3.33***	3646.02***	600.19*	591.18	2.98***
Env*Generation	10	0.36	1.37	0.31**	198.24*	315.16	285.62	0.25
Error	30	0.30	1.17	0.09	73.61	219.84	251.77	0.31

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

Table 4.6: Mean squares for grain yield, agronomic traits and disease parameters for the cross LPSC7 x VL06688 inoculated with *Diplodia* ear rot across three environments in 2014.

Source of Variation	df	Grain yield	Agronomic traits			Disease parameters		
			AD†	EA	PH	DEI	DES	WER
		(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Env	2	3.64***	726.06***	2.95***	1318.06***	588.76*	268.97	0.14
Rep(Env)	6	0.06	1.26	0.39*	114.82*	172.01	107.60	0.41
Generation	5	4.83***	134.84***	0.82**	3674.44***	585.80**	598.87**	0.73
Env*Generation	10	0.37**	3.10**	0.20	207.50**	136.40	70.23	0.24
Error	30	0.11	1.02	0.12	40.93	144.94	121.99	0.31

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.

4.1.2 Generation means for grain yield, agronomic traits and Diplodia ear rot disease parameters

The performance of the six generations under *Diplodia* infection varied significantly (P<0.05) for grain yield in all the crosses (Tables 4.7 to 4.12). As expected the F_1 had the highest yield among the generations. In two crosses (CLRCW31x CML442 and CML543 x LPSC7) the rank order for grain yield was first generation (F_1) followed by backcross to parent one (BC_1P_1) next was second generation (F_2) while backcross to parent two (BC_1P_2) was less yield than other hybrid (Tables 4.7 and 4.10). In two crosses (CML543 x CLRCW31 and LPSC7 x CML442), the rank order for grain yield was $F_1 > BC_1P_1 > BC_1P_2 > F_2$ (Tables 4.9 and 4.11). Only one cross (CML543 x CML442) had a rank order where $F_1 > F_2 > BC_1P_1 > BC_1P_2$ (Table 4.8). The highest F_1 grain yield was recorded for cross CML543 x CML442 (6.15 t ha^{-1}, Table 4.8) followed by cross CML543 x CLRCW31 (5.90 t ha^{-1}, Table 4.9) while the least grain yield was recorded for cross LPSC7 x CML442 (3.24 tha^{-1}, Table 4.11).

Table 4.7: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross CLRCW31 x CML442 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean±SE	σ^2	Mean± SE	σ^2	Mean±SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2
P_1	0.98±0.13	0.15	74.67±2.03	37.25	3.22±0.28	0.69	173.89±2.98	79.86	33.92±5.97	320.77	32.91±5.90	313.47	0.56±0.09	0.08
P_2	0.91±0.12	0.14	72.22±2.45	54.19	3.83±0.24	0.50	158.33±1.44	18.75	43.19±1.58	22.56	40.01±0.91	7.42	0.66±0.18	0.28
F_1	5.09±0.41	1.54	69.00±2.59	60.25	2.22±0.17	0.26	201.67±5.95	318.75	28.20±8.06	583.94	27.63±8.19	603.49	1.15±0.29	0.77
F_2	2.78±0.29	0.77	69.89±1.76	27.86	2.94±0.13	0.15	198.33±1.18	12.50	29.12±4.86	212.59	28.32±5.07	230.96	1.10±0.26	0.59
BC_1P_1	3.13±0.32	0.90	71.89±1.92	33.36	2.72±0.19	0.32	196.11±4.31	167.36	24.79±6.18	343.44	23.89±6.43	371.69	0.73±0.17	0.26
BC_1P_2	2.30±0.29	0.77	69.89±1.80	29.11	2.89±0.11	0.11	198.89±3.31	98.61	41.41±1.58	22.57	32.31±5.77	299.99	0.89±0.17	0.27
Mean	2.50		70.00		2.40		178.00		27.10		20.50		0.80	
LSD	0.30		1.00		0.30		6.20		11.60		8.70		0.50	
CV	13.10		1.40		14.50		3.60		44.50		44.20		71.20	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.

P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

Table 4.8: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross CML543 x CML442 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2
P_1	2.17± 0.32	0.91	77.11±2.03	37.11	2.61±0.18	0.30	175.11±4.72	200.11	24.55±4.84	210.97	23.26±5.22	244.83	0.57±0.12	0.13
P_2	1.13± 0.11	0.11	70.33±1.68	25.50	3.61±0.14	0.17	166.67±1.44	18.75	41.60±5.32	255.04	38.15±5.94	317.36	0.67±0.04	0.01
F_1	6.15± 0.20	0.37	67.44±1.92	33.28	1.83±0.14	0.19	220.56±2.12	40.28	22.98±5.09	233.20	22.37±5.05	229.25	1.67±0.35	1.11
F_2	3.60± 0.17	0.26	70.67±2.40	51.75	2.50±0.14	0.19	206.11±1.62	23.61	24.99±3.97	141.69	23.01±3.99	143.26	0.95±0.17	0.27
BC_1P_1	3.57± 0.31	0.86	71.67±1.64	24.25	2.33±0.12	0.13	203.33±1.18	12.50	31.86±6.27	353.63	22.21±4.55	186.15	1.35±0.28	0.72
BC_1P_2	3.08± 0.14	0.18	69.22±1.71	26.19	2.83±0.19	0.31	198.33±3.82	131.25	35.16±7.77	542.91	35.65±5.05	229.61	1.52±0.35	1.10
Mean	3.30		71.00		2.60		195.00		30.20		27.40		1.10	
LSD	0.60		1.50		0.40		5.90		12.50		10.50		0.40	
CV	18.00		2.20		14.20		3.10		43.10		39.80		36.80	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.
P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

Inbred line CML543 (parent 1 in three of the six crosses used in this study) was the highest yielding parent. The difference between parents (P_1-P_2) was largest and positive (1.24 t ha^{-1}) for parents CML543 (P_1) and CLRCW31 (P_2) in cross CML543 x CLRCW31 (Table 4.9) and smallest (-0.01 t ha^{-1}) for parents LPSC7 (P_1) and VL06688 (P_2) in cross LPSC7 x VL06688 (Table 4.12). The difference between back-crosses (BC_1P_1 - BC_1P_2) was largest and positive (0.93 t ha^{-1}) for cross CML543 x LPSC7 with CML543 as parent 1 (Table 4.10) and smallest (-0.22 t ha^{-1}) for cross LPSC7 x VL06688 with LPSC7 as parent 2 (Table 4.12).

Under artificial inoculation with *Stenocarpella maydis,* significant differences among the generations were recorded. The days to anthesis ranged from 67 days (F_1 for cross CML543 x CML442) to 78 days (parent LPSC7) (Tables 4.8 and 4.10). The F_1 hybrids flowered significantly earlier than either parent in all crosses except in cross LPSC7 x VL06688 in which the days to anthesis for the F_1 hybrid was not significantly different from that of parent 2 (Table 4.12). The F_1 hybrids flowered significantly earlier than either backcross (BC_1P_1 or BC_1P_2) in four of the six crosses (Tables 4.8, 4.9, 4.10 and 4.11). With the exception of cross CLRCW31 x CML442 (Table 4.7), all F_1 hybrids flowered significantly earlier than the F_2 generation. The backcross to parent 2 (BC_1P_2) reached 50% pollen shed earlier than backcross to parent 1 (BC_1P_1) in five of the six crosses (Tables 4.7, 4.8, 4.9, 4.11 and 4.12).

There were significant differences (P<0.05) among generations for plant height in all crosses (Tables 4.7 to 4.12). The F_1 had the tallest plants in all crosses except cross CML543 x CLRCW31 where the F_2 had slightly taller plants than the F_1 although these two generations were not significantly different (215 vs. 214 cm) (Table 4.9). The parents (P_1 and P_2) had the shortest plants among the six generations. Ear aspect varied among generations in all crosses with the F_1 having the best ear aspect compared to the rest of the generations (Tables 4.7 to 4.12).

Table 4.9: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross CML543 x CLRCW31 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean± SE	σ^2	Mean± SE	σ^2	Mean ± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2
P_1	2.52± 0.21	0.40	75.78±2.06	38.19	2.22±0.12	0.12	176.67±4.41	175.00	23.34±3.72	124.78	22.90±3.76	127.33	0.46±0.07	0.05
P_2	1.28± 0.13	0.15	73.67±2.18	42.75	2.56±0.13	0.15	175.56±3.38	102.78	27.26±4.73	201.16	26.47±4.65	194.42	0.53±0.14	0.17
F_1	5.90± 0.47	1.97	69.67±1.86	31.00	1.72±0.12	0.13	214.44±6.37	365.28	23.45±4.66	195.33	21.99±4.55	186.52	1.42±0.28	0.69
F_2	3.33± 0.31	0.85	72.22±2.08	38.94	2.72±0.09	0.07	207.78±4.87	213.19	27.58±5.62	283.86	26.17±5.73	294.94	0.90±0.20	0.34
BC_1P_1	4.05± 0.41	1.48	72.89±1.64	45.86	2.33±0.08	0.06	215.56±4.60	190.28	18.33±2.87	74.23	17.40±3.84	132.70	0.73±0.14	0.18
BC_1P_2	3.62± 0.19	0.32	71.89±1.83	30.11	2.22±0.09	0.07	205.00±2.50	56.25	24.11±4.04	146.71	24.10±5.08	231.90	1.00±0.17	0.27
Mean	3.50		73.00		2.30		199.00		24.00		23.2		0.90	
LSD	0.80		0.90		0.30		7.00		8.30		8.90		8.80	
CV	22.60		1.30		13.50		3.60		36.10		39.80		24.90	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.
P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

Table 4.10: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross CML543 x LPSC7 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean± SE	σ^2	Mean± SE	σ^2	Mean± SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean± SE	σ^2
P_1	1.54±0.21	0.40	76.56±2.04	37.53	2.44±0.13	0.15	175.56±2.82	71.53	17.06±2.01	36.47	16.62±2.08	38.96	0.63±0.04	0.04
P_2	1.42±0.26	0.61	78.00±2.53	57.50	2.89±0.33	0.99	153.33±4.64	193.75	27.71±2.17	42.48	25.81±2.09	39.14	0.36±0.05	0.02
F_1	4.55±0.31	0.86	70.89±2.17	42.36	1.83±0.25	0.56	223.33±3.33	100.00	22.44±3.49	109.31	21.67±3.29	97.19	0.85±0.10	0.08
F_2	3.02±0.35	1.08	73.56±2.15	41.53	2.33±0.22	0.44	208.33±5.71	293.75	27.58±1.34	16.21	20.38±2.79	69.93	0.76±0.11	0.10
BC_1P_1	3.69±0.36	1.15	73.44±2.12	40.28	2.17±0.08	0.06	212.78±5.66	288.19	17.70±1.19	12.77	16.06±1.17	12.32	0.86±0.10	0.10
BC_1P_2	2.76±0.05	0.02	73.00±2.29	47.00	2.33±0.19	0.31	197.78±7.51	506.94	22.82±4.26	163.44	22.46±4.33	168.66	0.74±0.16	0.23
Mean	2.80		74.00		2.30		195.00		22.60		20.50		0.70	
LSD	0.80		1.00		0.30		12.40		7.70		8.70		0.30	
CV	27.40		1.30		13.80		6.60		35.20		44.20		37.00	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.

P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

Inbred line parent CML442 had the poorer ear aspect in all the three crosses where it was used as parent 2 (Table 4.7, 4.8, and 4.11) while CML543 had the better ear aspect in all the three crosses where it was used as parent 1 (Table 4.8, 4.9, and 4.10).

The generation means for the three *Diplodia* ear rot disease parameters (*Diplodia* ear rot incidence, *Diplodia* ear rot severity and weight of rotten ears) differed among six generations. All values of *Diplodia* ear rot incidence were significantly different from zero (Tables 4.7 to 4.12). In one cross (CML543 x CML442), the F_1 had the lowest incidence of Diplodia ear rot among the six generations (Table 4.8). In three crosses (CLRCW31 x CML442, CML543 x CLRCW31 and LPSC7 x VL06688), the BC_1P_1 generation had the lowest incidence of Diplodia ear rot among the six generations (Tables 4.7, 4.9 and 4.12). However, the rank order of *Diplodia* ear rot incidence in the other generations was different in each of the three crosses. In cross CML543 x CLRCW31, the rank order in terms of increasing *Diplodia* ear rot incidence was backcross to parent one (BC_1P_1) was less affected followed by parents one (P_1) then first generation (F_1) followed by backcross to parent two (BC_1P_2) and next was parent two (P_2) while second generation (F_2) was most affected by Diplodia ear rot incidence (Table 4.9). In cross LPSC7 x VL06688, the rank order in increasing incidence was $BC_1P_1 > F_1 > P_1 > F_2 > BC_1P_2 > P_2$ (Table 4.12). The difference in *Diplodia* ear rot incidence between means of segregating and non-segregating generations was largest (35.1% vs. 31.8%) in cross CLRCW31 x CML442 (Table 4.7). In two crosses (CML543 x LPSC7 and LPSC7 x CML442), the inbred line parents recorded the lowest *Diplodia* ear rot incidence among all the generations. Inbred lines CML543 and LPSC7 had the lowest incidence (17.1 and 21.7%, respectively) of *Diplodia* ear rot (Tables 4.10 and 4.11). On the other hand inbred line CML442 tended to have the higher incidence of Diplodia ear rot in all the crosses in which it was involved. All values of *Diplodia* ear rot severity were significantly different from zero (Tables 4.7 to 4.12).

Table 4.11: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross LPSC7 x CML442 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean ± SE	σ^2	Mean± SE	σ^2	Mean ±SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean ±SE	σ^2
P_1	1.40± 0.17	0.26	76.44±1.79	28.78	3.00±0.20	0.38	147.78±3.13	88.19	21.71±4.11	151.79	21.71±4.11	151.79	0.33±0.05	0.02
P_2	0.64± 0.04	0.01	70.56±1.73	27.03	3.89±0.11	0.11	158.33±3.82	131.25	44.46±8.23	608.82	43.07±8.28	617.51	0.64±0.17	0.26
F_1	3.24± 0.08	0.06	67.89±1.65	24.61	2.22±0.21	0.21	195.00±4.41	175.00	37.08±4.74	202.14	35.32±4.98	223.51	2.01±0.28	0.69
F_2	2.14± 0.29	0.74	69.33±1.70	26.00	2.67±0.12	0.13	188.33±6.01	325.00	36.37±8.13	594.12	35.41±8.26	614.4	1.11±0.24	0.51
BC_1P_1	2.65± 0.22	0.45	71.44±2.03	37.03	2.28±0.15	0.19	193.33±4.56	187.50	26.46±5.93	316.02	25.52±5.97	320.95	0.93±0.13	0.15
BC_1P_2	2.37± 0.29	0.78	69.56±1.54	21.28	2.86±0.13	0.15	187.78±2.65	63.19	35.41±6.07	331.07	38.74±8.77	692.21	1.20±0.21	0.39
Mean	2.10		71.00		2.80		178.00		33.60		33.30		1.00	
LSD	0.50		1.00		0.30		8.30		14.30		15.30		0.50	
CV	26.50		1,50		10.40		4.80		44.20		47.70		53.40	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.

P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

Table 4.12: Mean (± standard error) and variance of six generations for grain yield, agronomic traits and disease severity scores for the cross LPSC7 x VL06688 inoculated with *Diplodia* ear rot across three locations in 2014.

Generation	Grain yield (t ha^{-1})		Agronomic traits						Disease parameters					
			Days to anthesis (days)		Ear aspect (1-5†)		Plant height (cm)		Diplodia ear rot incidence (%)		Diplodia ear rot severity (%)		Weight of rotten ears (Kg ha^{-1})	
	Mean ± SE	σ^2	Mean± SE	σ^2	Mean ±SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean ± SE	σ^2	Mean ±SE	σ^2
P_1	1.61± 0.28	0.68	77.33±2.36	50.25	2.61±0.22	0.42	147.78±1.69	25.69	24.08±5.38	260.79	16.62±0.45	1.85	0.42±0.05	0.02
P_2	1.62± 0.27	0.66	67.33±1.84	30.50	2.78±0.19	0.32	159.44±2.12	40.28	38.38±4.15	155.30	34.94±2.83	72.19	0.60±0.09	0.08
F_1	3.30± 0.09	0.08	67.67± 1.68	25.25	1.94±0.18	0.28	199.44±3.17	90.28	17.15±1.42	18.19	16.59 ±1.68	25.35	0.61±0.05	0.02
F_2	2.50± 0.12	0.13	69.56±1.99	35.53	2.17±0.17	0.25	190.00±3.63	118.75	29.21±5.37	259.51	27.67±5.38	260.58	0.92±0.10	0.09
BC_1P_1	2.87± 0.16	0.24	70.33±1.62	23.50	2.28±0.19	0.32	190.56±6.99	440.28	20.28±4.30	166.42	19.07±4.43	176.44	1.13±0.40	1.46
BC_1P_2	3.09± 0.05	0.02	67.11±1.67	25.11	2.33±0.12	0.13	182.78±3.55	113.19	33.35±3.80	129.99	32.20±4.17	156.79	1.04±0.13	0.14
Mean	2.50		70.00		2.40		178.00		27.10		24.51		0.80	
LSD	0.30		1.00		0.30		6.20		11.60		10.63		0.50	
CV	13.10		1.40		14.50		3.60		44.50		44.11		71.2	

†Ear aspect rating on a scale of 1 to 5, where 1 = nice uniform cobs with the preferred texture and 5 = cobs with the undesirable texture.

P_1 = parental inbred line 1; P2 = parental inbred line 2; F_1 = hybrid between parent 1 and parent 2; F_2 = Selfed generation of F_1 hybrid; BC_1P_1 = Backcross to parent1; BC_1P_2 = Backcross to parent 2; σ^2 = Variance; SE= standard error.

In five crosses (CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, CML543 x LPSC7 and LPSC7 x VL06688), the BC_1P_1 generation had the lowest incidence of Diplodia ear rot severity among the six generations (Tables 4.7, 4.8, 4.9, 4.10, and 4.12). However, the rank order of Diplodia ear rot severity in the other generations was different in each of the five crosses. For example in cross CLRCW31 x CML442, the rank order in terms of increasing *Diplodia* ear rot severity was backcross to parent one(BC_1P_1) followed by first generation (F_1) next was second generation (F_2) followed by BC_1P_2 and next was P_1 while P_2 was the most affected (Table 4.7). For cross LPSC7 x VL06688, the rank order in terms of increasing *Diplodia* ear rot severity was $BC_1P_1 > P_1 > F_1 > F_2 > BC_1P_2 > P_2$ (Table 4.12). Three of these crosses (CLRCW31 x CML442, CML543 x CLRCW31 and LPSC7 x VL06688) also had the lowest *Diplodia* ear rot incidence in the BC_1P_1 generation. The difference in *Diplodia* ear rot severity between means of segregating and non-segregating generations was largest (33.5% vs. 28.2%) in cross CLRCW31 x CML442 (Table 4.7). An inbred line parent recorded the lowest *Diplodia* ear rot severity among all the generations in only cross LPSC7 x CML442 (Table 4.11). Inbred lines CML543 and LPSC7 recorded the lowest severity (16.6%) of *Diplodia* ear rot among all the inbred lines used in this study (Tables 4.10 and 4.12). On the other hand inbred line CML442 tended recorded the higher severity of Diplodia ear rot in all the crosses in which it was involved (Tables 4.7, 4.8 and 4.11).

All values of weight of rotten ears were significantly different from zero (Tables 4.7 to 4.12). The F_1 had the highest weight of rotten ears in four of the six crosses (CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, and LPSC7 x CML442) (Tables 4.7, 4.8, 4.9 and 4.11). Among the segregating generations, the BC_1P_1 generation had the highest weight of rotten ears in two crosses (Tables 4.10 and 4.12) while the BC_1P_2 generation had the highest weight of

rotten ears in three crosses (CML543 x CML442, CML543 x CLRCW31 and LPSC7 x CML442) (Tables 4.8, 4.9, and 4.11). The second generation (F_2) had the highest weight of rotten ears in only one cross CLRCW31 x CML442 (Table 4.7). In all cases, the inbred lines recorded the lowest weight of rotten ears among the generations because of their inherent lower grain yield compared to the other generations (Tables 4.7 to 4.12). Among the inbred lines, LPSC7 recorded the lowest weight of rotten ears (0.33 t ha^{-1}) and had the lower weight of rotten ears in all crosses where it was a parent (Tables 4.10, 4.11, and 4.12) while inbred line CML442 recorded highest weight of rotten ears (Tables 4.7, 4.8 and 4.11). In two crosses (CML543 x LPSC7 and LPSC7 x CML442) the backcross to inbred line LPSC7 gave the lower weight of rotten ears compared to the backcross to the second parent (Tables 4.10 and 4.11) while in cross LPSC7 x VL06688, the backcross to LPSC7 had a slightly higher weight of rotten ears compared to the backcross to VL06688 (Table 4.12).

4.2 Genetic effects for agronomic traits and *Diplodia* ear rot disease resistance

Results of the joint scaling test (A, B and C) for the six traits in the six crosses are presented in Table 4.13. The joint scaling test revealed that the generation means for grain yield fitted an additive-dominance model in five crosses except for cross LPSC7 x VL06688 in which a more complex model was required (Table 4.13). The generation means for other agronomic traits (days to anthesis, plant height and ear aspect) did not fit an additive-dominance model suggesting a more complex model including epistasis was required for these traits (Table 4.13). The generation means for two *Diplodia* ear rot disease resistance parameters (*Diplodia* ear rot severity and weight of rotten ears) fitted an additive-dominance model in all crosses (Table 4.13). Different genetic models were necessary to fit the generation mean data for *Diplodia* ear rot incidence in this study.

Table 4.13: Joint scaling test (A, B, and C) for grain yield, agronomic traits and disease resistance parameters for six maize crosses inoculated with *Diplodia* ear rot across three locations in 2014.

		Scaling test		
Cross	**Traits**	**A**	**B**	**C**
CLRCW31 x CML442	Grain yield	0.95ns	0.25ns	-0.95ns
	Days to anthesis	73.45***	2.56ns	-5.33ns
	Plant height	166.11***	32.22ns	57.76ns
	Ear aspect	3.53*	-0.61ns	0.27ns
	Diplodia ear rot incidence	38.56ns	-21.81ns	-17.03ns
	Diplodia ear rot severity	36.46ns	-19.86ns	-14.90ns
	Weight of rotten ears	0.61ns	-0.35ns	0.88ns
CML543 x CLRCW31	Grain yield	1.90ns	0.92ns	-2.28ns
	Days to anthesis	74.73***	2.44ns	0.09ns
	Plant height	176.12***	41.12ns	50.01ns
	Ear aspect	2.39**	0.38ns	2.66*
	Diplodia ear rot incidence	25.30ns	-14.05ns	12.82ns
	Diplodia ear rot severity	24.69ns	-13.66ns	11.33ns
	Weight of rotten ears	0.50ns	-0.49ns	-0.23ns
CML543 x CML442	Grain yield	1.65ns	-0.14ns	-1.20ns
	Days to anthesis	73.72***	5.57ns	0.36ns
	Plant height	170.89***	19.43ns	41.54ns
	Ear aspect	3.11**	-0.78ns	0.12ns
	Diplodia ear rot incidence	33.08ns	-0.86ns	-12.15ns
	Diplodia ear rot severity	30.71ns	-16.10ns	-14.11ns
	Weight of rotten ears	0.62ns	0.36ns	-0.78ns
CML543 x LPSC7	Grain yield	1.48ns	1.41ns	0.02ns
	Days to anthesis	77.28***	-2.01ns	-2.10ns
	Plant height	164.45***	48.90ns	57.77ns
	Ear aspect	2.67*	-0.38ns	0.33ns
	Diplodia ear rot incidence	22.39ns	-14.75ns	20.67ns
	Diplodia ear rot severity	21.22ns	-15.36ns	-4.25ns
	Weight of rotten ears	0.50ns	0.51ns	0.35ns
LPSC7 x CML442	Grain yield	1.02ns	1.42ns	0.04ns
	Days to anthesis	73.50***	4.43ns	-5.46ns
	Plant height	153.06***	33.33ns	57.21ns
	Ear aspect	3.45***	-1.55*	-0.65ns
	Diplodia ear rot incidence	33.09ns	-28.62ns	5.15ns
	Diplodia ear rot severity	32.39ns	-27.35ns	6.22ns
	Weight of rotten ears	0.49ns	-0.79ns	-0.55ns
LPSC7 x VL06688	Grain yield	1.62ns	0.82*	0.17ns
	Days to anthesis	72.33***	5.66ns	-1.76ns
	Plant height	153.61**	22.24ns	53.90ns
	Ear aspect	2.70*	-0.16ns	-0.59ns
	Diplodia ear rot incidence	31.23ns	-19.49ns	11.04ns
	Diplodia ear rot severity	21.22*	-10.84ns	4.79ns
	Weight of rotten ears	0.51ns	1.05ns	1.44ns

* Significant at $P < 0.05$ probability level.

** Significant at $P < 0.01$ probability level; *** Significant at $P < 0.001$ probability level; ns, Not significant,

A = test for significance of additive x additive epistatic effects; B = test for significance of additive x dominance epistatic effects; C = test for significance of dominance x dominance epistatic effects.

For five crosses (CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, CML543 x LPSC7 and LPSC7 x CML442) an additive-dominance model was sufficient while for cross LPSC7 x VL06688 a model with epistatic effects was required (Table 4.13). The results of the analysis of genetic effects using a three-parameter model showed that the mid-parents effects (m) were highly significant ($P < 0.01$ or $P < 0.001$) for grain yield, agronomic traits and *Diplodia* ear rot resistance parameters in all six crosses used in this study (Tables 4.14 to 4.19). For grain yield, additive effects were significant and positive in three crosses (CML543 x CML442, CML543 x CLRCW31 and LPSC7 x CML442) (Table 4.15, 4.16, and 4.18).

Table 4.14: Additive, dominance and epistatic effects for yield, agronomic and disease parameters based on the three and six parameter models for the cross CLRCW31 x CML442 inoculated with *Diplodia* ear rot across three locations in 2014.

Parameter	Grain yield (t ha^{-1})	Agronomic traits			Disease parameters		
		AD[†] (days)	EA (1-5)	PH (cm)	DEI (%)	DES (%)	WER (kg ha^{-1})
Three parameter model							
m[§]	0.85±0.18***	73.21±1.32***	3.52±0.12***	171.01±2.75***	37.99±3.37***	35.09±3.66***	0.62±0.13***
a	0.19±0.18	1.38±1.30	-0.28±0.12*	5.67±2.71*	-7.04±3.33*	-4.52±3.60	-0.74±0.13
d	4.04±0.34***	-4.68±2.45	-1.31±0.23***	40.46±5.09***	-10.92±6.25	-10.20±6.77	0.55±0.24*
Six parameter model							
m		69.44± 10.48	4.08±0.96	169.44±17.77***			
a		1.22± 1.50	-0.31±0.14	7.78±2.54**			
d		2.22± 25.18	-2.69±2.31	83.33±42.70*			
aa		4.00± 10.37	0.56±0.95	-3.33±17.59			
ad		1.56± 6.70	0.28±0.61	-21.11±11.35			
dd		-2.67±15.56	0.83±1.43	-51.11±26.38*			

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
[†]AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
[§]m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

Table 4.15: Additive, dominance and epistatic effects for yield, agronomic and disease parameters based on the three and six parameter models for the cross CML543 x CML442 inoculated with Diplodia ear rot across three locations in 2014.

Parameter	Grain yield	Agronomic traits			Disease parameters		
		AD†	EA	PH	DEI	DES	WER
	(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Three parameter model							
m§	1.48± 0.15***	73.70±1.19***	3.14±0.10***	173.31±1.94***	34.01±3.61***	30.85 ± 3.16***	0.67± 0.16***
a	0.52± 0.15**	3.20± 1.18**	-0.50±0.10***	4.38± 1.91*	-7.48±3.56*	-8.65 ± 3.12**	-0.07± 0.16
d	4.33± 0.28***	6.30± 2.21**	-1.25±0.18***	52.09± 3.59***	9.16± 6.68	-8.18 ±5.86	1.09± 0.30**
Six parameter model							
m		74.61± 9.48***	2.78± 0.76**	192.00± 13.91***			
a		3.39± 1.35*	-0.50±0.11***	4.22± 1.99*			
d		-8.61± 22.78	-0.17± 1.84	27.89± 33.43			
aa		-0.89± 9.38	0.33± 0.76	-21.11± 13.77			
ad		-1.89± 6.06	0.00± 0.49	1.56± 8.89			
dd		1.44± 14.07	-0.78± 1.13	0.67± 20.65			

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
§m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

The additive effects for days to anthesis were positive and significant for three crosses (Tables 4.15, 4.18 and 4.19). The additive effects for ear aspect were negative and significant for three crosses (CLRCW31 x CML442, CML543 x CML442 and LPSC7 x CML442) (Tables 4.14, 4.15 and 4.18). For plant height, additive effects were significant and positive in three crosses (CLRCW31 x CML442, CML543 x CML442, and CML543 x LPSC7) (Table 4.14, 4.15, and 4.17). The dominance effects were significant and positive for grain yield for all crosses (Tables 4.14 to 4.19). The dominance effects for other agronomic traits (days to anthesis, ear aspect and

plant height) were significant for all crosses except for cross CLRCW31 x CML442 in which the dominance effect for days to anthesis was not significant (Table 4.14). The dominance effects for ear aspect were negative in all crosses used in this study (Tables 4.14 to 4.19). The genetic effects results using the three-parameter model showed that the additive effects were significant and negative for *Diplodia* ear rot incidence in five crosses (Tables 4.14, 4.15, 4.17, 4.18 and 4.19) and non-significant for cross CML543 x CLRCW31 (Table 4.16).

Table 4.16: Additive, dominance and epistatic effects for yield, agronomic and disease parameters based on the three and six parameter models for the cross CML543 x CLRCW31 inoculated with *Diplodia* ear rot across three locations in 2014.

Parameter	**Grain**	**Agronomic traits**			**Disease parameters**		
	yield	AD†	EA	PH	DEI	DES	WER
	($t\ ha^{-1}$)	(days)	(1-5)	(cm)	(%)	(%)	($kg\ ha^{-1}$)
Three parameter model							
m§	1.81± 0.20***	74.77±1.28***	2.52±0.09***	181.11±3.25***	24.93±2.76***	24.45 ±2.94***	0.47± 0.12**
a	0.58± 0.20**	1.04± 1.26	-0.11±0.08	2.56± 3.20	-2.72±2.73	-2.77± 2.90	-0.08± 0.11
d	3.92± 0.37***	-5.01± 2.36*	-0.54± 0.16**	43.33± 6.02***	-2.22± 5.12	-2.93 ±5.44	0.89± 0.21***
Six parameter model							
m		74.06±10.15***	4.17±0.53***	166.11±22.37***			
a		1.06± 1.45	-0.17± 0.08*	0.56± 3.20			
d		-2.94± 24.38	-3.33± 1.27*	118.33± 53.76*			
aa		0.67± 10.04	-1.78± 0.52**	10.00± 22.14			
ad		-0.11± 6.48	0.56± 0.34	20.00± 14.29			
dd		-1.44± 15.06	0.89± 0.79	-70.00± 33.21*			

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
§m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

The additive effects for *Diplodia* ear rot severity were significant and negative in four crosses namely CML543 x CML442, CML543 x LPSC7, LPSC7 x CML442, LPSC7 x VL06688 (Tables 4.15, 4.17, 4.18 and 4.19). The additive effects for weight of rotten ears were significant and positive in only one cross CML543 x LPSC7 (Table 4.17).The dominance effects for *Diplodia* ear rot incidence were significant and positive in only cross LPSC7 x VL06688 (Table 4.19). The dominance effects for weight of rotten ears were significant and positive in five crosses namely CLRCW31 x CML442, CML543 x CML442, CML543 x CLRCW31, CML543 x LPSC7 and LPSC7 x CML442 (Tables 4.14, 4.15, 4.16, 4.17 and 4.18).

Table 4.17: Additive, dominance and epistatic effects for grain yield, agronomic and disease parameters based on the three and six parameter models for the cross CML543 x LPSC7 inoculated with *Diplodia* ear rot across three locations in 2014.

Parameter	**Grain**	**Agronomic traits**			**Disease parameters**		
	yield	AD[†]	EA	PH	DEI	DES	WER
	(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Three parameter model							
m[§]	1.53± 0.18***	77.01±1.39***	2.68±0.14***	168.82±3.51***	22.49±1.74***	20.58±1.77***	0.53±0.07***
a	0.24± 0.18	-0.49± 0.72	-0.21±0.13	11.89± 3.46**	-5.29±1.71**	-4.96± 1.74**	0.13± 0.06*
d	3.12± 0.33***	-6.65± 2.57**	-0.82± 0.25**	63.27± 6.49***	0.15± 3.22	-0.19 ±3.27	0.40± 0.12**
Six parameter model							
m		78.61± 10.99***	3.00± 1.07**	176.67± 25.69***			
a		-72± 1.57	-0.22± 0.15	11.11± 3.67**			
d		-12.50± 26.41	-1.50± 2.57	80.00± 61.73			
aa		-1.33± 10.88	-0.33± 1.06	-12.22± 25.42			
ad		2.33± 7.02	0.11± 0.68	7.78± 16.41			
dd		4.78± 16.32	0.33± 1.59	-33.33± 38.13			

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
[†]AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
[§]m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

Table 4.18: Additive, dominance and epistatic effects for yield, agronomic and disease parameters based on the three and six parameter models for the cross LPSC7 x CML442 inoculated with *Diplodia* ear rot across three locations in 2014.

Parameter	Grain yield ($t\ ha^{-1}$)	Agronomic traits			Disease parameters		
		AD[†] (days)	EA (1-5)	PH (cm)	DEI (%)	DES (%)	WER ($kg\ ha^{-1}$)
Three parameter model							
m[§]	1.11±0.13***	73.29±1.09***	3.36±0.10***	158.63±3.20***	32.26±4.01***	32.17±4.35***	0.43± 0.12**
a	0.36±0.13**	2.73± 1.08*	-0.47±0.10***	-3.11± 3.15	-10.89±3.95**	-11.19±4.28*	-0.18± 0.12
d	2.31±0.25***	-5.82± 2.03**	-1.31± 0.19***	47.52± 5.92***	3.17± 7.42	2.70 ±8.05	1.47± 0.23***
Six parameter model							
m		68.83± 8.65***	3.84± 1.78***	144.17±20.98***			
a		2.94± 1.23*	-0.44±0.11**	-5.28± 3.00*			
d		2.94± 20.78	-3.09± 1.87	125.83± 50.42*			
aa		4.67± 8.56	-0.40± 0.77	8.89± 20.77			
ad		-2.11± 5.52	-0.27± 0.50	21.67± 13.40			

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
[†]AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
[§]m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

Results of the analysis of genetic effects using a six-parameter model showed that additive x additive and dominance x dominance epistatic interactions were significant for grain yield for cross LPSC7 x VL06688 (Table 4.19). Significant dominance x dominance epistatic interactions were detected for plant height in crosses CLRCW31 x CML442 (Table 4.14) and CML543 x CLRCW31 (Table 4.16). In addition, plant height exhibited significant additive x dominance epistatic interactions for cross LPSC7 x VL06688 (Table 4.19). No significant epistatic interactions were detected for *Diplodia* ear rot severity for cross LPSC7 x VL06688 (Table 4.19).

Table 4.19: Additive, dominance and epistatic effects for yield, agronomic and disease parameters based on the three and six parameter models for the cross LPSC7 x VL06688 inoculated with *Diplodia* ear rot across three locations in 2014.

Parameter	Grain	Agronomic traits			Disease parameters		
	yield	AD†	EA	PH	DEI	DES	WER
	(t ha^{-1})	(days)	(1-5)	(cm)	(%)	(%)	(kg ha^{-1})
Three parameter model							
m§	1.74± 0.13***	71.98±1.18***	2.67±0.11***	157.58±2.84***	32.43±2.72***	20.58± 1.76***	0.67± 0.13***
a	-0.05± 0.12	4.64± 1.16**	-0.08±0.11	-3.11± 2.80	-8.33±2.68**	-4.96± 1.74**	-0.05± 0.12
d	1.81± 0.23***	-5.02± 2.18*	-0.77± 0.21**	49.80± 5.25***	-12.87± 5.03*	-0.19±3.27	0.27± 0.23
Six parameter model							
m	-0.32± 0.91	75.67± 9.29***	2.14± 0.88*	166.94± 19.39***		25.69±13.91	
a	-0.00± 0.13	5.00± 1.33**	-0.08±0.13	-5.83± 2.77*		-4.60±1.99*	
d	7.64± 2.18**	-16.44± 22.32	0.31± 2.12	59.72± 46.59		-17.23±33.42	
aa	1.93± 0.90*	-3.33± 9.19	0.56± 0.87	-13.33± 19.19		-4.47± 13.76	
ad	-0.45± 0.58	-3.56± 5.93	0.06± 0.56	27.22± 12.39*		-3.61± 8.88	
dd	-4.02± 1.34**	-8.44± 13.79	-0.50± 1.31	-27.22±28.78		13.22±20.64	

* Significant at $P < 0.05$ probability level.
** Significant at $P < 0.01$ probability level.
*** Significant at $P < 0.001$ probability level.
†AD, days to anthesis; EA, ear aspect; PH, plant height; DEI, *Diplodia* ear rot incidence; DES, *Diplodia* ear rot severity; WER, weight of rotten ears.
§m, mid-parent effect; a, additive effect; d, dominance effect; aa, additive x additive epistatic effect; ad, additive x dominance epistatic effect; dd, dominance x dominance epistatic effect.

4.3 Heritability of agronomic trait and Diplodia ear rot resistance parameters

Heritability estimates for grain yield, agronomic traits and disease severity parameters for six maize crosses across three locations are presented in Table 4.20. Broad-sense heritability (H) estimates for grain yield were high and ranged from 0.88 in cross CML543 x LPSC7 to 0.95 in crosses CLRCW31 x CML442 and CML543 x CML442 (Table 20). The estimate of narrow-sense heritability for grain yield varied among crosses and was lowest in cross LPSC7 x VL06688 (0.36) and highest in cross LPSC7 x CML442 (0.92). For agronomic traits, both days

to anthesis and plant height recorded the highest broad-sense heritability estimate (0.96) while ear aspect had the lowest broad-sense heritability (0.27) for these crosses (Table 4.20). Plant height also had the highest narrow-sense heritability estimate (0.91) among the agronomic traits.

Broad-sense heritability estimates for *Diplodia* ear rot incidence ranged from 0.13 in cross CML543 x CML442 to 0.51 in cross LPSC7 x VL06688 (Table 4.20). Narrow-sense heritability estimates for *Diplodia* ear rot incidence parameters ranged from 0.54 in cross CLRCW31 x CML442 to 0.79 in cross CML543 x CML442 (Table 4.20). For *Diplodia* ear rot severity broad-sense heritability estimates ranged from 0 in cross CLRCW31 x CML442 to 0.60 in cross LPSC7 x VL06688 while narrow-sense heritability ranged from 0.61 in cross CML543 x CML442 to 0.85 in LPSC7 x VL06688 (Table 4.20). Broad-sense heritability estimate for weight of rotten ears was lowest (0.32) in cross CLRCW31 x CML442 and highest (0.76) in cross CML543 x CML442 x CLRCW31 (Table 4.20). On the hand the highest estimate of narrow-sense heritability (0.98) for weight of rotten ears was recorded for cross LPSC7 x VL06688.

4.4 Heterosis for agronomic traits and *Diplodia* ear rot resistance parameters

Results of mid-parent (MPH) and high-parent (HPH) heterosis estimates are presented in Table 4.21. Among the agronomic traits, grain yield had the highest estimates of both MPH and HPH in this study. The highest MPH and HPH estimates for grain yield (439 and 419%, respectively) were recorded for cross CLRCW31 x CML442 (Table 4.21). The cross that showed the least MPH and HPH for grain yield (104 and 105%, respectively) was LPSC7 x VL06688. Both MPH and HPH estimates for days to anthesis and ear aspect were negative (Table 4.21) suggesting that the F_1 hybrids flowered earlier and also had better ear aspect compared to their parents.

Table 4.20: Broad-sense and narrow-sense heritability for grain yield, agronomic traits and disease resistance parameters for six maize crosses inoculated with *Diplodia* ear rot across three environments in 2014.

	CROSSES											
Parameter	CLRCW31xCML442		CML543 xCML442		CML543 x CLRCW31		CML543 x LPSC7		LPSC7 x CML442		LPSC7 x VL06688	
	H	h^2	H	h^2	H	h^2	H	h^2	H	h^2	H	h^2
Grain yield	0.95	0.73	0.95	0.69	0.89	0.68	0.88	0.65	0.89	0.92	0.89	0.36
Days to anthesis	0.77	0.55	0.92	0.61	0.91	0.67	0.95	0.66	0.96	0.69	0.96	0.58
Ear aspect	0.80	0.47	0.79	0.67	0.74	0.49	0.27	0.40	0.86	0.59	0.59	0.57
Plant height	0.92	0.66	0.96	0.63	0.91	0.54	0.92	0.87	0.91	0.66	0.92	0.91
Diplodia ear rot incidence	0.21	0.54	0.13	0.79	0.14	0.56	0.38	0.74	0.27	0.67	0.51	0.63
Diplodia ear rot severity	0.00	0.69	0.12	0.61	0.08	0.68	0.20	0.76	0.28	0.75	0.60	0.85
Weight of rotten ears	0.32	0.59	0.54	0.81	0.76	0.60	0.39	0.88	0.75	0.63	0.33	0.98

H = Broad -sense heritability; h^2 = Narrow-sense heritability

Table 4.21: Mid-parent and high-parent heterosis (%) for grain yield, agronomic traits and disease resistance parameters for six maize crosses inoculated with *Diplodia* ear rot across three environments in 2014.

	CROSSES											
Parameter	CLRCW31xCML442		CML543xCML442		CML543xCLRCW31		CML543xLPSC7		LPSC7xCML442		LPSC7xVL06688	
	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
Grain yield	438.62	419.39	272.73	183.41	210.53	134.13	207.43	195.46	217.65	131.43	104.33	104.97
Days to anthesis	-6.05	-4.46	-8.52	-4.11	-6.77	-5.43	-8.27	-9.12	-7.63	-3.78	-6.44	-12.49
Ear aspect	-37.02	-31.06	-41.16	-29.89	-28.03	-22.52	-31.33	-25.00	-35.56	-26.00	-28.02	-25.67
Plant height	21.41	15.98	29.07	25.96	21.76	21.38	35.81	27.21	27.41	31.95	29.84	34.96
Diplodia ear rot incidence	-26.86	-16.86	-30.52	-6.40	-7.31	0.47	0.25	31.54	12.08	70.80	-30.61	-10.01
Diplodia ear rot severity	-24.22	-16.04	-27.15	-3.83	-10.92	-3.97	2.15	30.39	9.05	62.69	-19.16	3.19
Weight of rotten ears	88.53	105.36	169.36	192.98	186.87	208.70	71.72	34.92	314.43	509.09	19.61	45.24

MPH = Mid-parent heterosis; HPH = High-parent heterosis

For plant height, both MPH and HPH estimates were positive (Table 4.21) implying that the F_1 hybrids had taller plants compared to their inbred line parents. Both MPH and HPH estimates for *Diplodia* ear rot incidence were negative in three crosses CLRCW31 x CML442, CML543 x CML442 and LPSC7 x VL06688 (Table 4.21) which implied that the F_1 hybrids performed better (had less *Diplodia* ear rot incidence) than their parental inbred lines. The highest desirable MPH for *Diplodia* ear rot incidence was recorded for cross LPSC7 x VL06688 (Table 4.21). In two crosses (CML543 x LPSC7 and LPSC7 x CML442), the F_1 hybrids had higher *Diplodia* ear rot incidence than the parental inbred lines. Both MPH and HPH estimates for *Diplodia* ear rot severity were desirable (negative) in three crosses CLRCW31 x CML442, CML543 x CML442 and CML543 x CLRCW31 (Table 4.21) which implied that the F_1 hybrids performed better (showed less *Diplodia* ear rot severity) than their parental inbred lines. Cross LPSC7 x VL06688 showed negative MPH but positive HPH for *Diplodia* ear rot severity. For weight of rotten ears, both MPH and HPH estimates were positive (Table 4.21) suggesting that the F_1 hybrids tended to have more damaged ears compared to their parental inbred lines. The highest MPH and HPH estimates for weight of rotten ears (314 and 509%) were recorded for cross LPSC7 x CML442 (Table 4.2).

CHAPTER FIVE: DISCUSSION

The present study revealed highly significant differences among generations for grain yield and agronomic traits in all the six crosses. These results indicated differential performance of the generations under inoculation with *Stenocarpella maydis*. The results showed that the F1s had higher grain yield, flowered earlier and in most cases had taller plants. These results in this study showed highly significant generation x environment interaction for grain yield and other agronomic traits in most of the crosses. This indicated that the generations performed differently at the locations in western Kenya. These locations had different climatic and soil conditions and this might have led to the variable performance of the different generations. Similar results have been reported in other studies on agronomic traits in maize (Darrah and Hallauer, 1972; Melchinger et al., 1986; Betrán et al., 2003; Malvar et al., 2008; Makumbi et al., 2015; Chen et al., 2015).

Significant differences were observed among generations for Diplodia ear rot incidence and weight of rotten ears in five crosses, and for Diplodia ear rot severity in one cross. There was no significant generation x environment interaction for all three disease parameters (Diplodia ear rot incidence, Diplodia ear rot severity and weight of rotten ears) in all crosses with the exception of cross CML543 x CML442, and for weight of rotten ears in cross CML543 x CLRCW31(Table 4.2 and 4.3). This suggested that the different generations expressed the same reaction to the *Stenocarpella maydis* inoculum used and that the environmental conditions at the different locations did not affect disease development. Presence and absence of significant genotype by environment interaction for disease ratings have been reported in other studies on maize under artificial disease pressure (Carson and Hooker, 1981; Gendloff et al., 1986; Treat et al., 1990; Bernardo et al., 1992; Coates and White, 1998; Mukanga et al., 2010a; Tembo et al., 2013; Butrón et al., 2015).

5.1 Generation means for grain yield, agronomic traits and disease resistance parameters

Results of this study showed that the parental inbred lines designated as P1 had higher grain yield, better ear aspect, and matured later compared to P2s in all crosses, except for cross LPSC7 x VL06688 where P2 and slightly higher but non-significant grain yield compared to P1. Inbred CML543 line had the highest grain yield among the parents used. This inbred line is a recent CIMMYT maize line (CML) release for Eastern and Southern Africa and it is known for it good general combining ability for grain yield, resistance to major foliar diseases (northern corn leaf blight and gray leaf spot), and tolerance to ear rots. Inbred line LPSC7 appeared to be well-adapted to the mid-altitude of East Africa despite being of tropical lowland Mexico origin based on its grain yield. In this study the F1 mean for grain yield was two to six times larger than the mid-parent and the best parent suggesting presence of both mid-parent and high-parent heterosis. These results are in agreement with those from studies by Gamble (1962a), Darrah and Hallauer (1972), Moreno-Gonzalez and Dudley (1981) and Malvar et al. (2008) in temperate maize and Makumbi et al. (2011) in tropical maize. In all crosses the F1 had higher grain yield compared to both the F2 and backcross generations to either parent, a result similar to that reported by Mihaljevic et al (2005). There was expression of heterosis for plant height between the F1 and mid-parent, a result which corroborates findings by Darrah and Hallauer (1972) and Moreno-Gonzalez and Dudley (1981).

The results of this study showed that Diplodia ear rot incidence and Diplodia ear severity were significantly different among the parental inbred lines in four out of the six crosses (Tables 4.8, 4.10, 4.11 and 4.12). Based on this, these four crosses (CML543 x CML442, CML543 x LPSC7, LPSC7 x CML442 and LPSC7 x VL06688) were the best to study inheritance of Diplodia ear rot resistance. It is interesting to note that these inbred lines in

these crosses were chosen for this study based on general tolerance (CML543 and LPSC7) or susceptibility (CML442 and VL06688) to ear rots based on a previous assay for Diplodia ear rot resistance (Wambugu, 2013). Hence the choice of the inbred lines for this study was justified. Inbred CML543 line had the lowest Diplodia ear rot incidence and severity (Table 4.10) among all parents in this study. Inbred line CML442 tended to have the highest incidence and severity of Diplodia ear rot among the parents used. This inbred line is one of the most widely used parents in commercial hybrids in East Africa, suggesting that there is a possibility that some of the hybrids in which it is a parent are susceptible to Diplodia ear rots.

In this study there was variation in reaction of F1 hybrids to inoculation with *Stenocarpella maydis* compared to parental lines. In two crosses (CLRCW31 x CML442 and CML543 x CML442) the F1 had lower Diplodia ear rot incidence and severity compared to both parents (P1 and P2), which suggested negative heterosis for these parameters. Negative heterosis for disease ratings means that the F1 will be more resistant than the parental lines and is therefore useful. In five out of the six crosses the F1 had lower Diplodia ear rot incidence and severity compared to the mid-parent value, another indicator of heterosis. This result is in agreement with a study on Gibberella ear rot severity (Martin et al., 2012; Butrón et al., 2015) and Fusarium ear rot (Butrón et al., 2015). In two crosses (CML543 x CLRCW31 and CML543 x LPSC7), the F1 had lower Diplodia ear rot incidence and severity than P2 but had higher rating for the two disease parameters than P1 in cross CML543 x LPSC7 and equal Diplodia ear rot incidence with P1 in cross CML543 x CLRCW31 (Tables 4.9 and 4.10). For all crosses the F1 regressed towards the more resistant parent of the two parents. However, very few differences between F1 and P1 were significant. This result is in contrast to that of Wiser et al (1960) who reported that F1 values were closer to those of the more susceptible parent in temperate maize inbred lines evaluated for Diplodia ear rot resistance. These differences could be attributed to the different germplasm used in the two studies.

In five out of the six crosses the BC1P1 showed more resistance when measured as Diplodia ear rot incidence and severity compared to the F1, and was closer to the more resistant parent of the two. This result is in agreement with the findings of Wiser et al (1960) in temperate maize. Kappelman and Thompson (1966) and Chungu et al (1996) used generation mean analysis to study inheritance of resistance to Diplodia stalk rot and *Fusarium graminearum*, respectively, and reported that backcrosses were skewed to their recurrent parents. From their study, Chungu et al (1996) concluded that several loci controlled resistance to *Fusarium graminearum.*

5.2 Inheritance of grain yield, agronomic traits and disease resistance parameters

The simple additive-dominance model was sufficient to fit generation means for grain yield in all crosses except one cross (LPSC7 x VL06688) where the model showed significant lack of fit. This suggested the importance of both additive and dominance gene action in control of grain yield for the majority of the crosses. Significant lack of fit for grain yield in the cross LPSC7 x VL06688 suggested presence of epistatic effects in genetic control of grain yield. There was variable importance of additive gene action for grain yield among the crosses. Only three crosses (CML543 x CML442, CML543 x LPSC7, and LPSC7 x CML442) out of the six showed significant additive effects (Tables 4.15, 4.16 and 4.18) for grain yield. The magnitude of the additive gene effects for grain yield in this study was small, suggesting that they were of minor importance in controlling inheritance of grain yield. Similar results were also reported for grain yield in studies with temperate maize (Gamble, 1962a; Darrah and Hallauer, 1972; Moreno-Gonzalez and Dudley, 1981; Malvar et al., 2008; Chen et al., 2015). In this study the dominance gene effect estimates for grain yield were positive and highly significant for all crosses (Tables 4.15 to 4.19). The magnitude of dominance estimates was much higher compared to additive estimates suggesting greater importance of dominance gene effects relative to additive gene effects in control of grain yield. These results agree with

those of studies in tropical maize (Ceballos et al., 1998; Pérez-Velásquez et al., 2008) and temperate maize (Gamble, 1962a; Darrah and Hallauer, 1972; Moreno-Gonzalez and Dudley, 1981; Malvar et al., 2008; Chen et al., 2015) in which greater importance of dominance gene effects in control of grain yield variation was reported.

A six-parameter model was used to assess epistatic effects for grain yield in one cross (LPSC7 x VL06688) that showed significant lack of fit for the additive-dominance model. The results revealed no significant epistatic effects for grain yield. It is interesting to note that dominance x dominance [dd] effects were positive while additive x additive [aa] effects were negative. Studies on epistasis for grain yield in maize have generated differing results, a phenomenon that can be attributed to differences in germplasm. The lack of significant dominance x dominance epistatic effects observed in this study is similar to results by Chen et al (2015) who also did not find significant dominance x dominance effects in temperate maize in China. However Chen et al (2015) reported significant additive x additive epistatic effects in one cross but Wolf and Hallauer (1997) did not report significant additive x additive epistasis for grain yield through a triple testcross design. Ceballos et al. (1998) reported presence and absence of epistasis for grain yield in two and three tropical maize crosses evaluated under acid soils, respectively. In other studies with temperate maize (Gamble, 1962a, Darrah and Hallauer, 1972; Moreno-Gonzalez and Dudley, 1981; Malvar et al., 2008; Hauck et al., 2014) significant and non-significant epistatic effects for grain yield were reported. In a study using testcross generation means in temperate maize (Lamkey et al., 1995), significant additive x additive epistatic effects for grain yield were reported. Significant additive x additive epistasis for grain yield was also reported in a study by Melchinger et al (1986) in European maize.

The simple additive-dominance model showed significant lack of fit for days to anthesis, ear aspect and plant height for all crosses, suggesting a role for epistatic effects in the inheritance of these traits. For most crosses, the models indicated importance of both additive and dominance modes of gene action for these agronomic traits. Results showed highly significant dominance effects for ear aspect and plant height in all crosses and significant dominance effects for days to anthesis in five out of six crosses. Plant height showed the greatest magnitude of dominance effects compared to additive effects suggesting that dominance gene action plays a bigger role in the inheritance of plant height. This result is in agreement with several studies in temperate and tropical maize (Gamble, 1962b; Darrah and Hallauer, 1972; Moreno-Gonzalez and Dudley, 1981; Pérez-Velásquez et al., 2008). The negative estimates of dominance for days to anthesis and ear aspect suggests presence of dominance of alleles that enhance earlier maturity and desirable ear aspect, respectively.

Additive gene effects were significant for the three agronomic traits (days to anthesis, plant height and ear aspect) in three crosses, suggesting that additive gene action plays a role to the inheritance of these traits in these particular crosses. The magnitude of the additive effects was smaller compared to the mean effects, suggesting that additive effects play a smaller role in the inheritance of these traits. Similar results of the prevalence of dominance effects over additive effects were also reported for plant height in several studies (Gamble, 1962b; Darrah and Hallauer, 1972; Moreno-Gonzalez and Dudley, 1981; Pérez-Velásquez et al., 2008). All significant additive effects for plant height and days to anthesis were positive. In contrast, Pérez-Velásquez et al. (2008) reported both positive and negative significant additive effects for plant height in maize evaluated under acid and non-acid soils. On the other hand all significant additive effects for ear aspect were negative.

The results in this study revealed a negative significant additive x additive [aa] effect for ear aspect in one cross (CML543 x CLRCW31). The negative [aa] effects suggest a diminishing effect on ear aspect because of this type of gene effect. This study also revealed significant dominance x dominance [dd] effects in two crosses and significant additive x dominance [ad] effect in one cross for plant height. The significant dominance x dominance [dd] effects for plant height were negative in this study, a result similar to those reported by Moreno-Gonzalez and Dudley (1981) and Gamble (1962b) in temperate maize with the exception of a few crosses, and Pérez-Velásquez et al (2008) and Iqbal et al (2010)in tropical maize. The significant negative [dd] effects in this study were greater than the additive and dominance effects in magnitude suggesting a larger contribution to inheritance of plant height in these crosses. The negative [dd] effects suggest a diminishing effect on plant height because of this type of gene effect. The significant additive x dominance [ad] effect for plant height in this study was negative, which is contrary to findings by Pérez-Velásquez et al. (2008) in tropical maize under acid and non-acid soils.

Simple additive-dominance model fitting of generation means for the two disease parameters Diplodia ear rot incidence and severity did not exhibit significant lack of fit in all crosses except in one cross (LPSC7 x VL06688) that showed significant lack of fit for Diplodia ear rot severity hence the use of a six-parameter model for that cross (Table 4.19). There was variable importance of additive gene effects for Diplodia ear rot incidence and severity among the crosses used in this study. Five crosses (CLRCW31 x CML442, CML543 x CML442, CML543 x LPSC7, LPSC7 x CML442, and LPSC7 x VL06688) showed significant additive effects for Diplodia ear rot incidence. On the other hand four crosses (CML543 x CML442, CML543 x LPSC7, LPSC7 x CML442, and LPSC7 x VL06688) showed significant additive effects for Diplodia ear rot severity. All the significant additive effects for Diplodia ear rot incidence and severity were negative. Significant dominance

effect was recorded for Diplodia ear rot severity for only cross LPSC7 x VL06688. For most crosses, these results indicated the importance and prevalence of additive mode of gene action for resistance to Diplodia ear rot. A negative sign was associated with dominance effects, suggesting that dominance was in the direction of greater resistance. The results for weight of rotten ears showed that the additive effects were negative but non-significant in all crosses except in cross CML543 x LPSC7 where the additive effect was positive and significant. The dominance effects for weight of rotten ears were positive and significant for five out of the six crosses.

Studies with Diplodia ear and stalk rots and two of the other ear rots of maize (Gibberella and Fusarium ear rot) have reported different magnitudes of importance of additive and dominance gene effects. The results obtained in this study are in agreement with previous studies by Rossouw et al (2002b) for *S. maydis* incidence and Tembo et al (2013) for *S. maydis* disease severity in which preponderance of additive effects in genetic control of resistance to *S. maydis* in tropical maize was reported when using diallel studies. Dorrance et al (1998) reported both significant additive and non-additive effects for Diplodia ear rot disease rating in temperate maize using diallel analysis. These results are also in agreement with a study by Russell (1961) who found additive gene effects to be more important than dominance effects for Diplodia stalk rot in temperate maize. Kappelman and Thompson (1966) found both additive and dominance gene effects to be important for Diplodia stalk rot resistance in temperate maize. For *F. graminearum*, Odiemah and Manniger (1982) and Chungu et al (1996) found predominance of additive effects over dominance effects in a diallel and a generation means study, respectively. In a study with combined ear rot disease complex (*S. maydis, F. verticillioides*, and *A. flavus*) Mukanga et al (2010a) reported the importance of both additive and non-additive gene effects in tropical maize through a diallel study.

However, these results are contrary to those of Das et al (1984) and Olatinwo et al (1999) who reported greater importance of dominance gene effects for resistance to Diplodia ear rot in Asia using diallel analysis and to *Stenocarpella macrospora* in West Africa using generation mean analysis, respectively. In the study by Olatinwo et al (1999), results of diallel analysis indicated the importance of both additive and non-additive effects for *S. macrospora* ear rot ratings in West Africa. In a quantitative trait loci (QTL) mapping study, Tembo et al. (2014) reported negative additive effects associated with QTL for *S. maydis* resistance. In a study with Fusarium ear rot, Boling and Grogan (1965) found significant additive and additive x dominance effects. Gendloff et al. (1986) working with Fusarium ear rot reported significant additive gene action in five of eight crosses and occasionally significant dominance, additive x additive and additive x dominance effects, depending on a particular cross. In another study with Fusarium ear rot, Nakam and Pataky (1996) reported the importance of both additive and dominant gene action using two temperate maize crosses. In a study with Gibberella ear rot, Martin et al. (2011) found significant additive effects for Gibberella ear rot severity in all the five crosses of European maize they evaluated and significant dominance effect in only one cross. Working with both Gibberella and Fusarium ear rots, Butrón et al (2015), also found significant additive and dominance effects for ear rot severity of the two diseases in temperate maize. This study did not reveal any significant epistatic effects for Diplodia ear rot severity (Table 4.19). Significant epistatic interactions (additive x additive, additive x dominance and dominance x dominance) were found for Gibberella ear rot severity in a study by Butrón et al (2015). In the same study Butrón et al (2015) found significant additive x dominance and dominance x dominance epistatic effects for Fusarium ear rot severity. The significant dominance effects for weight of rotten ears in this study agree with results reported by Rossouw et al (2002b) for % of rotten ears which is a

related trait. Rossouw et al (2002b) also reported that additive effects were equally important for % of rotten ears.

The variation in importance of additive, dominance and epistatic gene effects reported in these studies can be attributed to differences in germplasm used, species and race of pathogen and the environment. For example studies conducted in sub-Saharan Africa have used different germplasm which could explain the differences. Germplasm resistant to Diplodia ear rot has been reported in Africa (van Rensburg and Ferreira, 1997; van Rensburg et al., 2003; Moremoholo et al., 2010; Tembo et al., 2013). In this study we used inbred lines available from the CIMMYT maize program in Kenya. The mode of gene action may be different in all these germplasm pools. The use of QTL mapping is a good option to study the contribution of both additive [a] and dominance [d] gene effects to Diplodia ear rot resistance in addition to generation mean analysis. Some of the inbred lines used in this study like CML543, LPSC7 and VL06688 plus others reported elsewhere (van Rensburg et al., 2003; Moremoholo et al., 2010; Tembo et al., 2013) could be used to develop populations for QTL validation studies and genome-wide association studies (GWAS) to further investigate the genetics of Diplodia ear rot resistance in tropical maize as a step towards implementation of marker-assisted breeding. Linkage mapping provides detailed information on the effects individual markers linked to QTL or single genes (Martin et al., 2011) which are not possible in generation mean analysis.

Knowledge of the mode of gene is important in designing a breeding strategy for a disease. The detection of significant additive effects in the majority of crosses in study suggests that selection among inbred lines under artificial inoculation should be effective. Both Diplodia ear rot and severity reaction of the parental inbred lines should be a reliable indicator of disease reaction of their hybrids. The identification of some inbred lines like CML543 and

LPSC7 with fairly good levels of tolerance to Diplodia ear rot offers breeding programs in the region an opportunity to include these lines in their inbred line development programs.

5.3 Heritability Estimates

Heritability estimates (broad-sense and narrow-sense) for grain yield were moderate to high for most of the crosses except cross LPSC7 x VL06688 in which narrow-sense heritability was very low (0.36). Broad-sense heritability estimates for plant height were very high (0.91-0.96). The very high broad-sense heritability estimates for both grain yield and plant height are probably biased upwards. Broad-sense heritability is higher for grain yield, this implying a greater genetic role of these parameters compared to the non-genetic one quantity. High broad-sense heritability estimates for grain yield in maize have been reported in other studies (Singh et al., 1989; Alika, 1994; Unay et al., 2004; Sumathi et al., 2005). The narrow-sense heritability estimates for grain yield suggest that early generation selection would be effective to improve grain yield. Broad-sense heritability estimates for Diplodia ear rot incidence (range 0.13-0.38) and Diplodia ear rot severity (range 0.00-0.28) were mostly low with the exception of one cross LPSC7 x VL06688 showing a moderate estimates.

The low estimates of broad-sense heritability for these two traits suggest that the environment had a larger effect compared to the genotype. Eller et al (2010) also reported low broad-sense heritability for Fusarium ear rot. However Bolduan (2009) reported high broad-sense heritability for Gibberella ear rot and moderate broad-sense heritability for Fusarium ear rot under artificial inoculation. Narrow-sense heritability for both Diplodia ear rot incidence and severity ranged from moderate to high depending on the cross. The relatively high narrow-sense heritability estimates for Diplodia ear rot incidence severity suggest that resistance to Diplodia ear rot can be improved fairly quickly in this set of lines (Dudley and Moll, 1969).

5.4 Heterosis Estimates

Heterosis for grain yield was largest for the cross CLRCW31 x CML442, a cross between a line adapted to lowland tropics of Mexico and a line adapted to the mid-altitude region of East Africa. This agrees with what has been reported that heterosis is usually more in crosses between unrelated lines compared to crosses between related lines. Heterosis was much larger for yield than other traits. This result corroborates findings by Moreno-Gonzalez and Dudley (1981) and Chen et al (2015) using generation mean analysis in temperate maize. Heterosis for the two disease parameters was negative in four out of the six crosses, which suggested that the F1 hybrids tended to have lower diseases ratings than some of the parents. Mid-parent heterosis for resistance was found for Gibberella ear rot severity (Martin et al., 2011). The heterosis values for the two disease parameters were low. Low heterosis estimates have also been reported in other cereal disease (Miedaner et al., 2002; Oettler et al., 2004).

CHAPTER SIX: CONCLUSIONS AND RECOMMMENDATIONS

6.1 Conclusion

Generation mean analysis leads to important conclusions about the inheritance of different traits in question in a particular study. The major objectives of this study were to investigate the inheritance of agronomic traits and resistance to Diplodia ear rot in tropical maize under artificial inoculation. The generation mean analysis was carried out using six crosses developed from five elite maize inbred lines.

The magnitude of the additive gene effects for grain yield was small, suggesting that they were of minor importance in controlling inheritance of grain yield. The magnitude of dominance estimates was much higher compared to additive estimates suggesting that dominance gene effects were of greater importance compared to additive gene effects for control of grain yield in this set of inbred lines. The greater importance of dominance for grain yield indicated that selection among inbred line for yield should be done after several generations to be effective and reliable indicator of disease reaction of their hybrid. For plant height, days to anthesis and ear aspect, both additive and dominance modes of gene action were important. Significant epistatic effects for ear aspect were important for a few of the crosses used in this study. Additive effects were important for both Diplodia ear rot incidence and Diplodia ear rot severity. For most crosses, these results indicated the importance and prevalence of additive mode of gene action for resistance to Diplodia ear rot but epistatic effects appeared to be of minor importance. The detection of significant additive effects for Diplodia ear rot incidence and severity in the majority of crosses in study suggests that selection among inbred lines for Diplodia ear rot resistance should be done in early generations. This selection should be effective and a reliable indicator of disease reaction of their hybrids.

The study showed that there was high broad and narrow sense heritability for grain yield, days to anthesis, plant height, and ear aspect, in all six crosses. This indicates the suitability of those parameter in improvement of those traits in future breeding. The lower levels of broad sense heritability for weight of rotten ears, disease incidence and severity showed that these traits were significantly influenced by environmental effects and characters are less heritable because of high number of genes are involved in the control of those characters.

Meanwhile, all of first generations (F_1's) hybrids were lower than the mid-parents or high-parents for days to anthesis, ear aspect, ear rot incidence and ear rot severity in most crosses, thus, these result led to negative heterosis values. It can therefore be concluded that with negative values contributes to *Diplodia* disease resistance while the crosses with positive better-parent inclined and leaned to susceptibility class.

The variation of these gene effects reported in this study can be attributed to differences in germplasm used, species and race of pathogen and the environment. The estimate of high broad-sense heritability indicate that resistance to Diplodia ear rot is heritable and that phenotypic selection in replicated plots should be effective in improving traits with more durable effect. While high narrow-sense heritability estimates for Diplodia ear rot incidence severity suggest that resistance to Diplodia ear rot can be improved fairly quickly in this set of lines. Heterosis for the two disease parameters was negative in four out of the six crosses, which suggested that the F1 hybrids tended to have lower diseases ratings than some of the parents.

6.2 Recommendation

Based on these findings from this study, it is recommended that:

1. Concentration of promising genes effects reported in this study through generation mean analysis should be recommended to the researchers to improve resistant hybrids to Diplodia ear rot disease.
2. Inbred lines used in this study like CML543, LPSC7 and VL06688 should be used for further investigation of Diplodia ear rot resistance in tropical maize before implementation of a marker-assisted breeding for Diplodia ear rot resistance.
3. Inbred lines like CML543 and LPSC7 that were identified in this study with fairly good levels of tolerance to Diplodia ear rot should be recommended breeding programs in the region to develop Diplodia ear rot resistant inbred lines.

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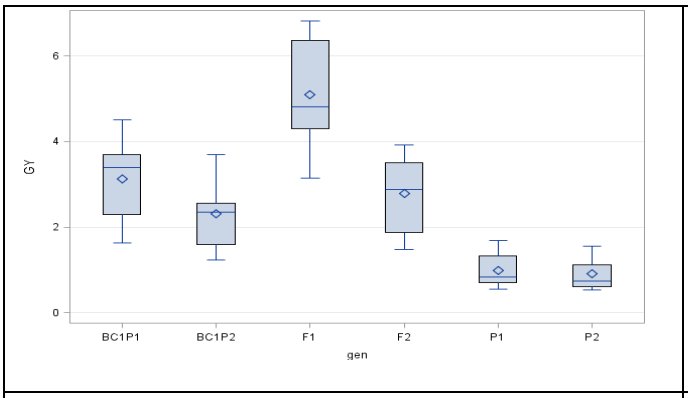
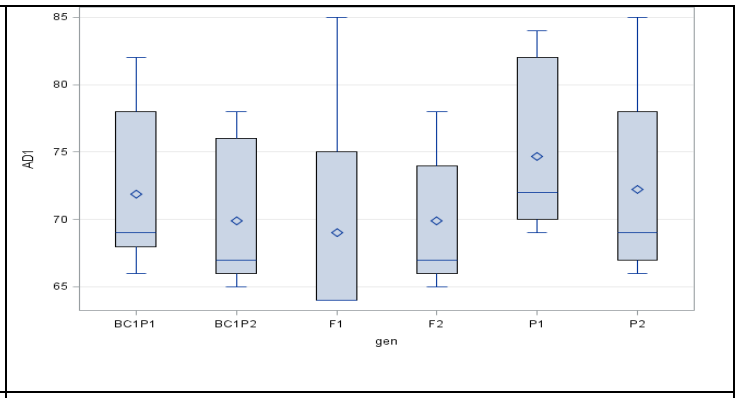
APPENDICES

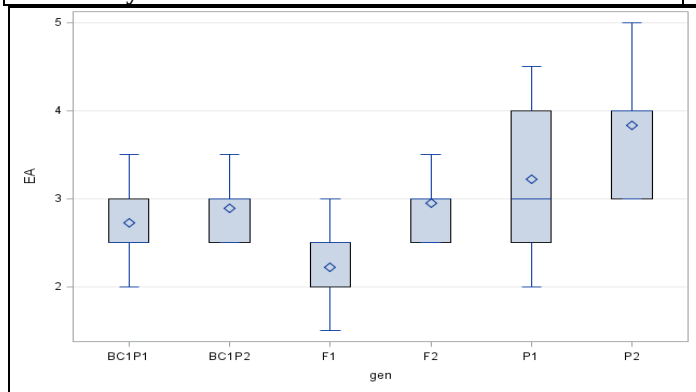
Appendix 1: Distribution of six generation means for agronomic traits and disease severity scores for the cross CLRCW31 x CML442 evaluated under artificial *Diplodia* ear rot inoculation at Kibos, Kakamega and Alupe in 2014**.**



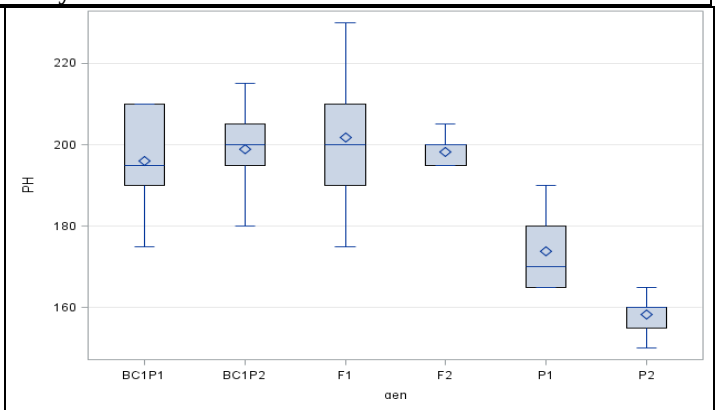
a. Grain yield



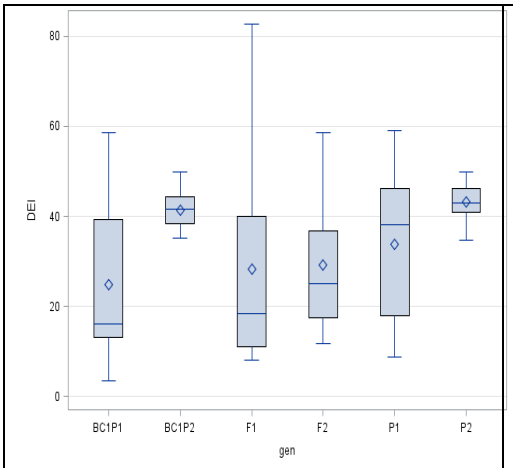
b. Days to anthesis



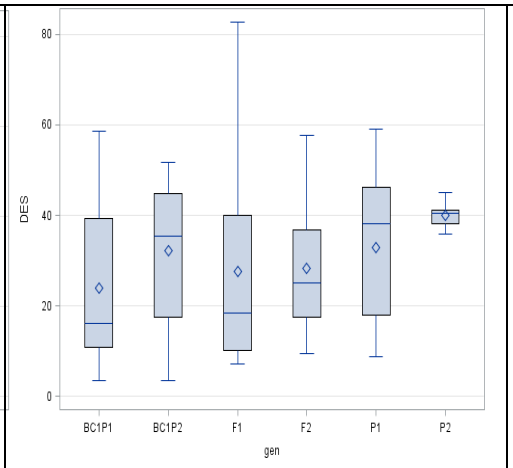
c. Ear aspect



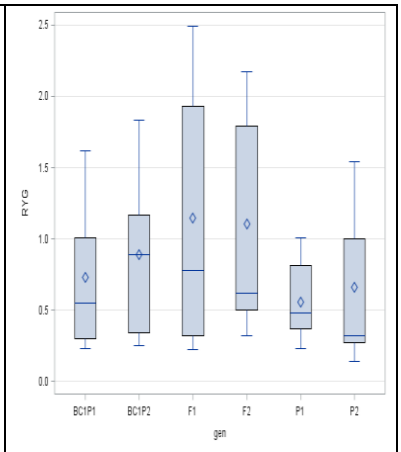
d. Plant height



a. Disease incidence

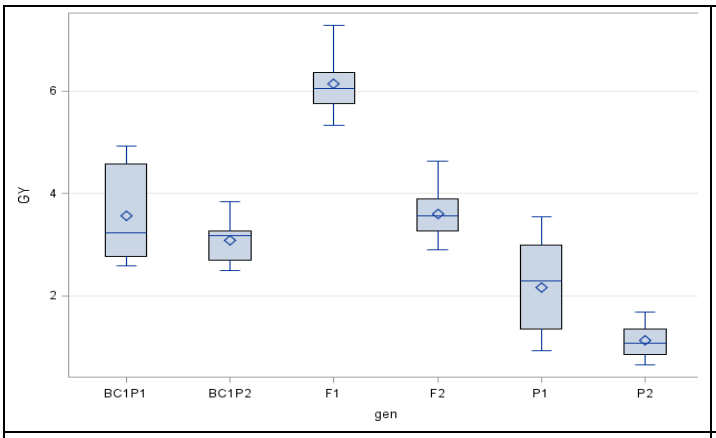


b. Disease severity

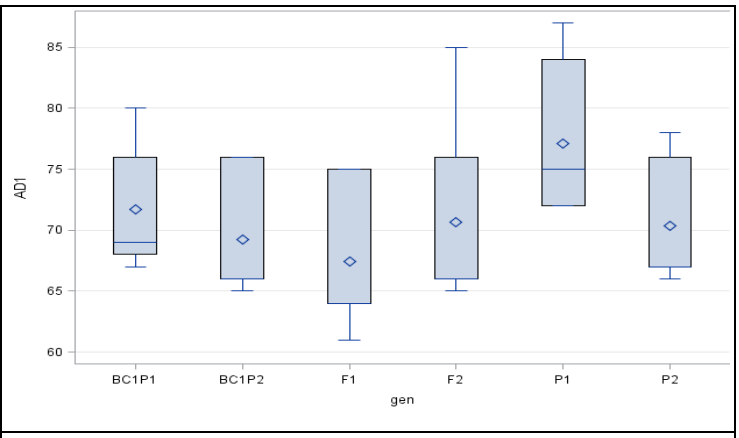


c. Weight of rotten ears

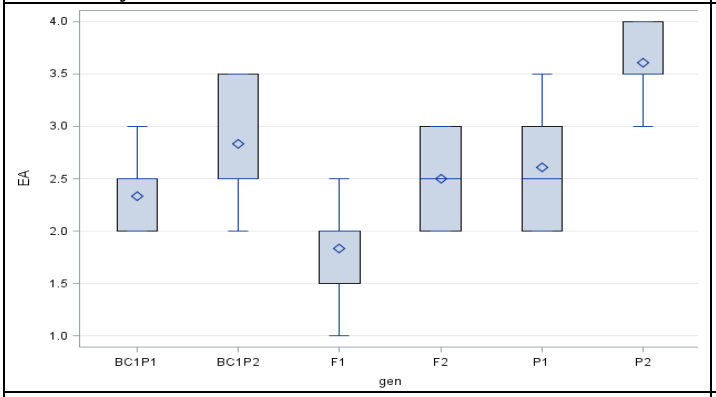
Appendix 2: Distribution of six generation means for agronomic traits and disease severity scores for the cross CML543 x CML442 evaluated under artificial *Diplodia* ear rot inoculation at Kibos, Kakamega and Alupe in 2014.



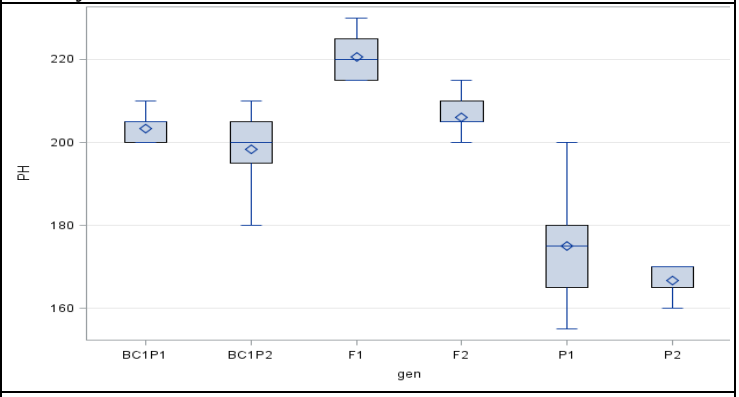
a. Grain yield



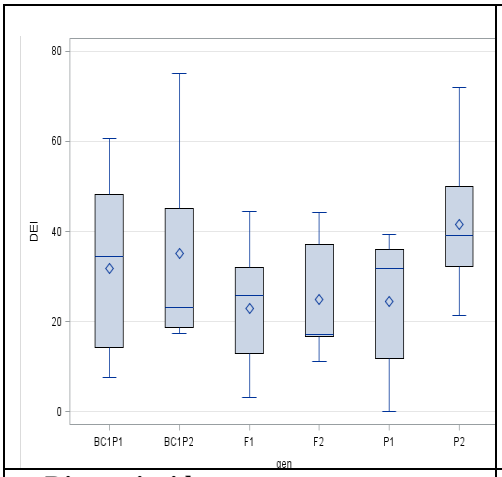
b. Days to anhtesis



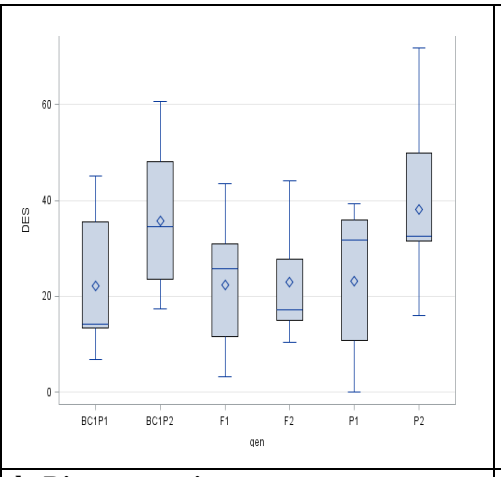
c. Ear aspect



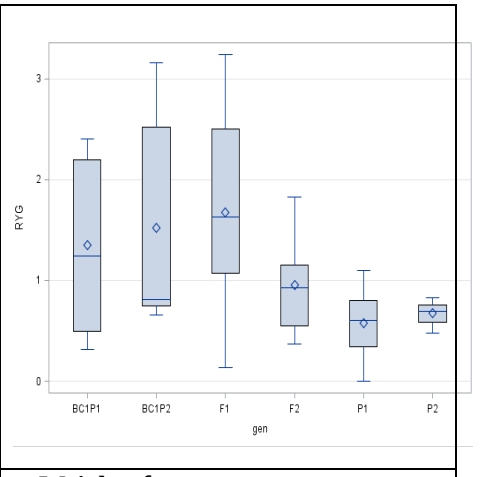
d. Plant height



a. Disease incidence

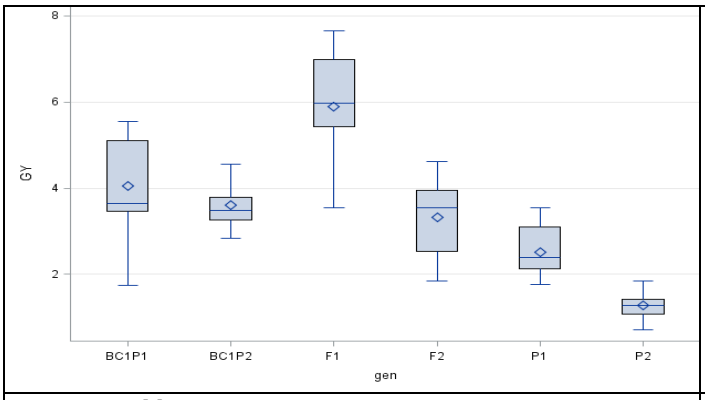


b. Disease severity

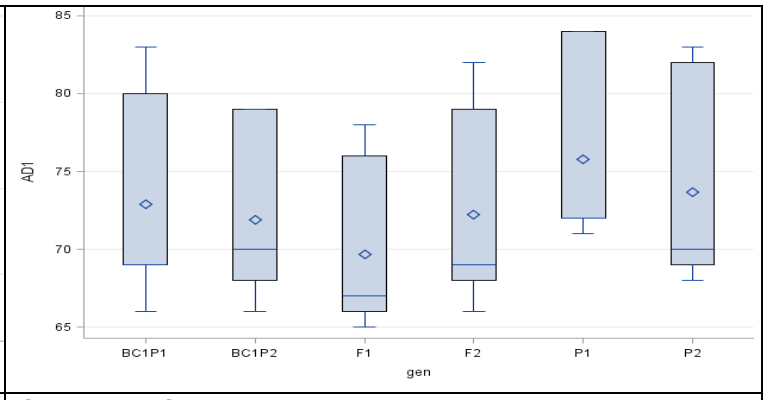


c .Weight of rotten ears

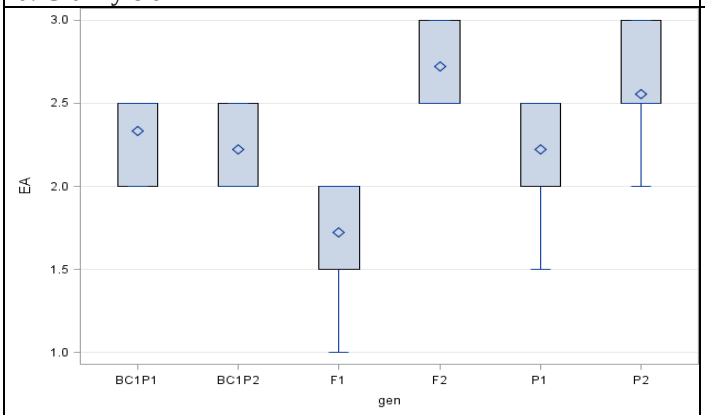
Appendix 3: Distribution of six generation means for agronomic traits and disease severity scores for the cross CML543 x CLRCW31 evaluated under artificial *Diplodia* ear rot inoculation at Kibos, Kakamega and Alupe in 2014.



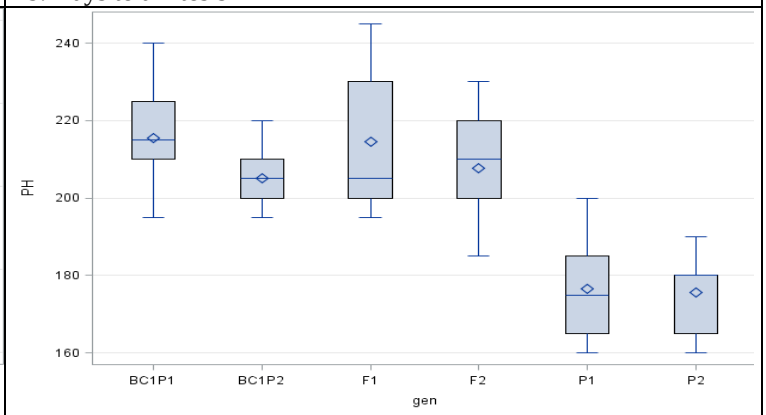
a. Grain yield



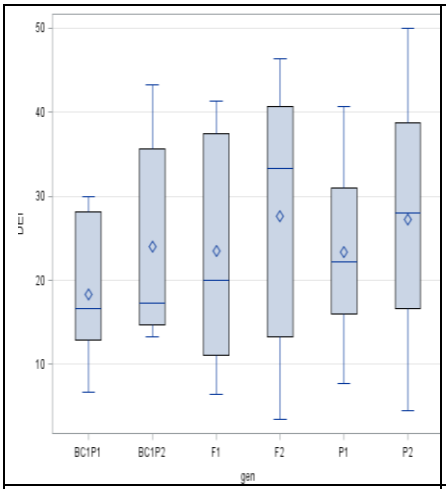
b. Days to anhtesis



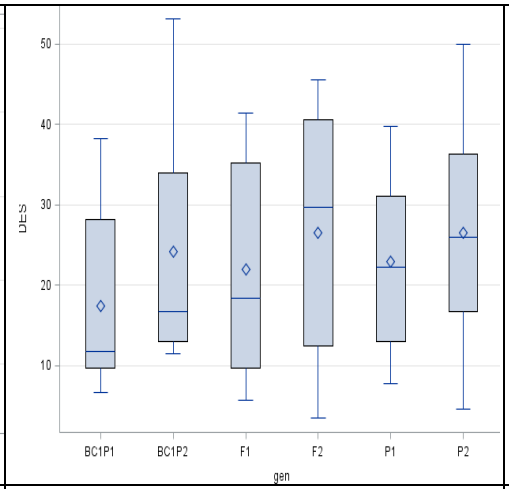
c. Ear aspect



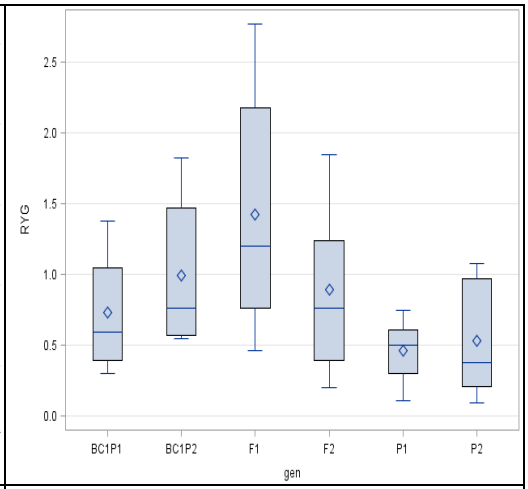
d. Plant height



a. Disease incidence

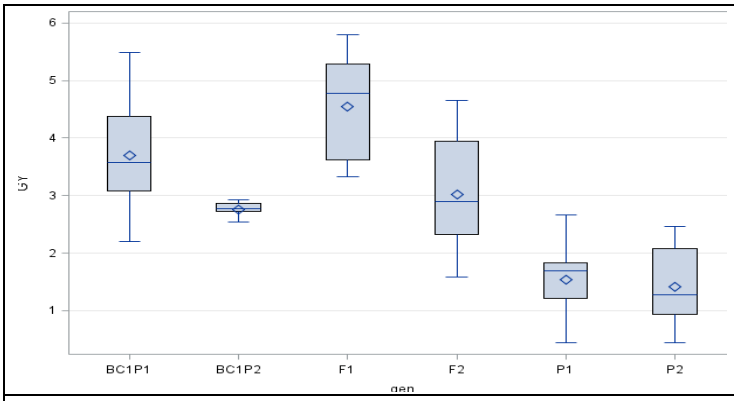


b. Disease severity

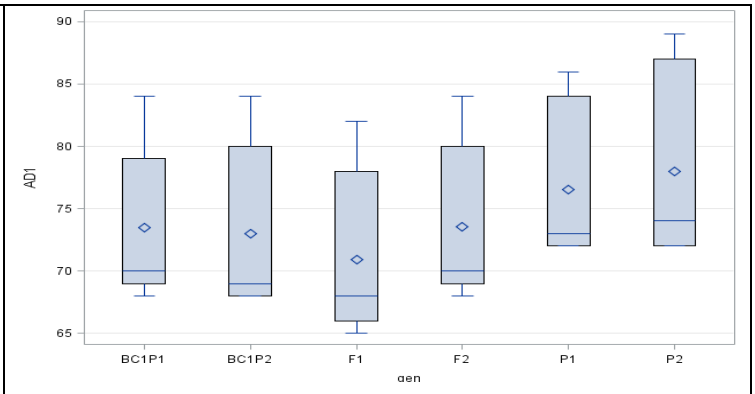


c. Weight of rotten ears

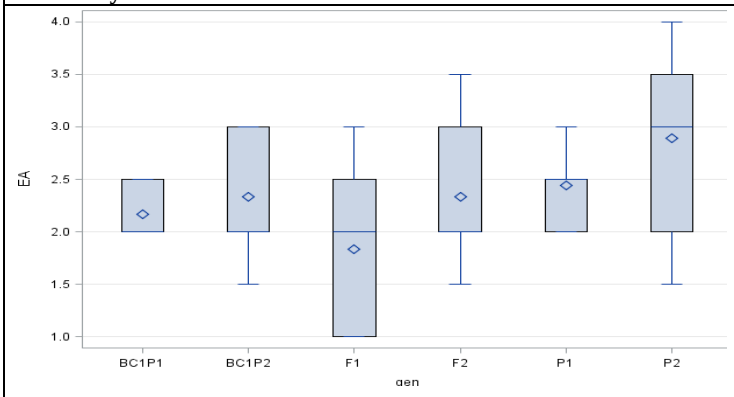
Appendix 4: Distribution of six generation means agronomic traits and diseases score severity of six generations for the cross CML543 x LPSC7 evaluated under artificial *Diplodia* ear rot inoculation across three environments in 2014.



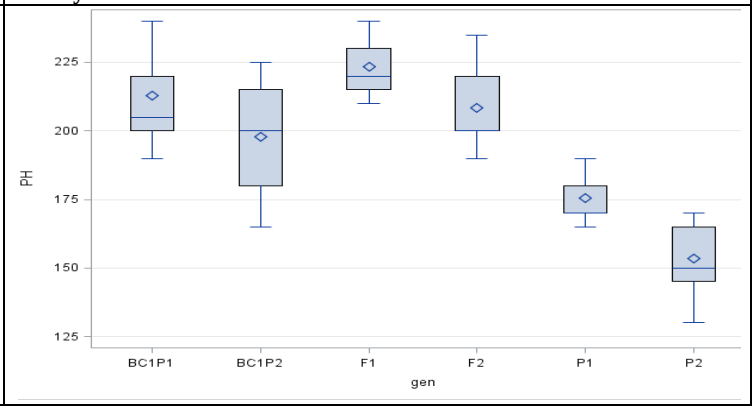
a .Grain yield



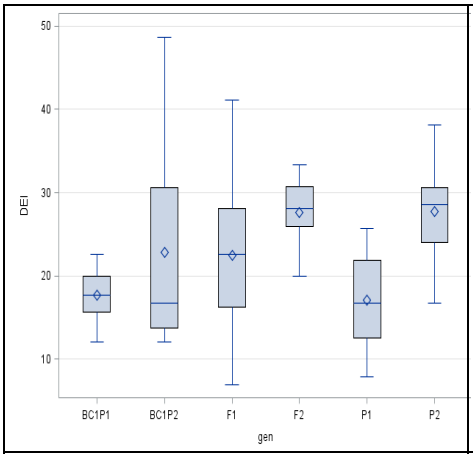
b. Days to anhtesis



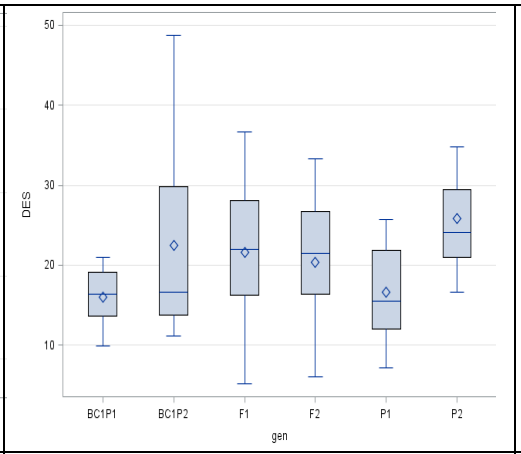
c. Ear aspect



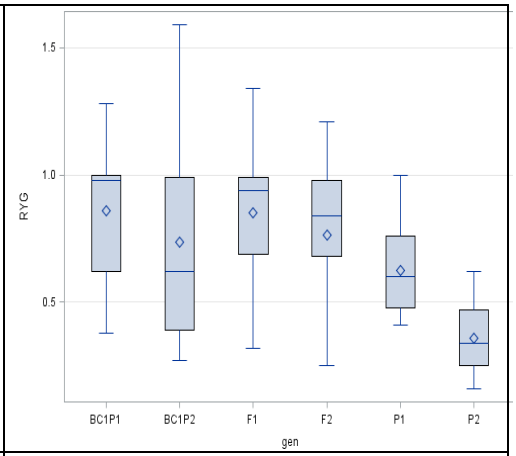
d. Plant height



a. Disease incidence

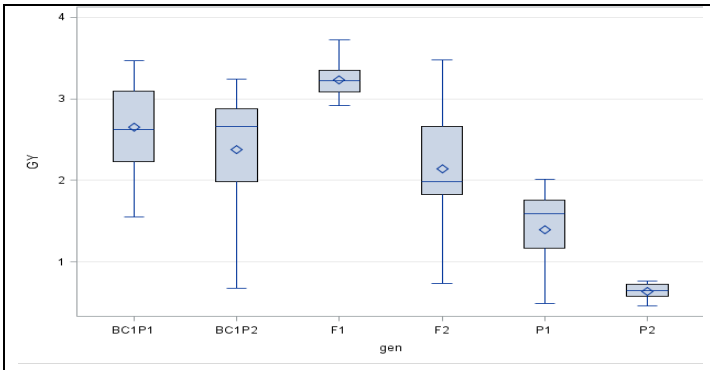


b. Disease severity

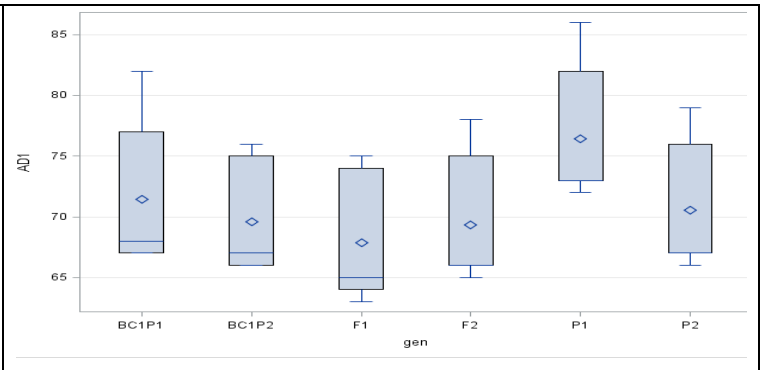


c. Weight of rotten ears

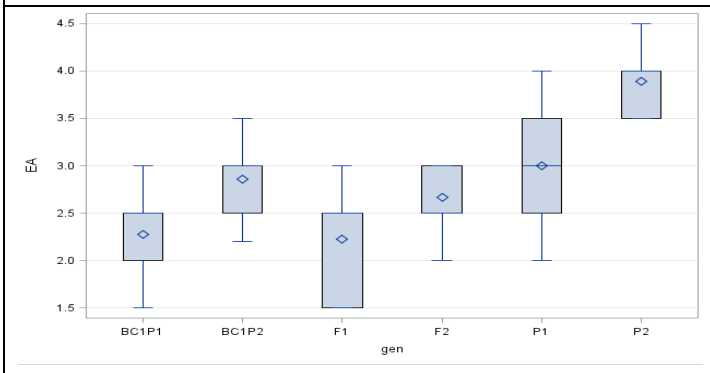
Appendix 5: Distribution of six generation means for agronomic traits and disease severity scores for the cross LPSC7 x CML442 evaluated under artificial *Diplodia* ear rot inoculation at Kibos, Kakamega and Alupe in 2014.



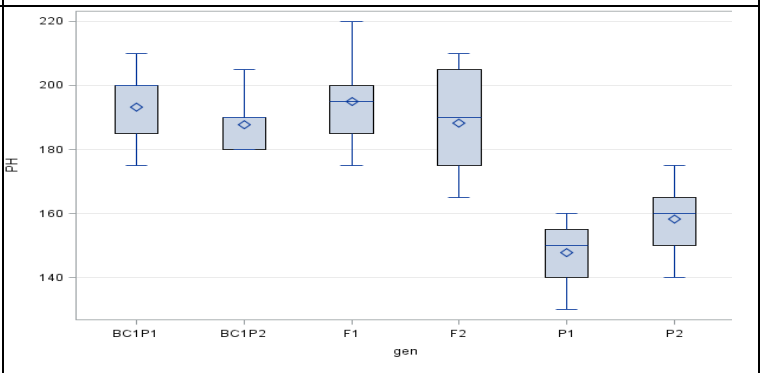
a .Grain yield



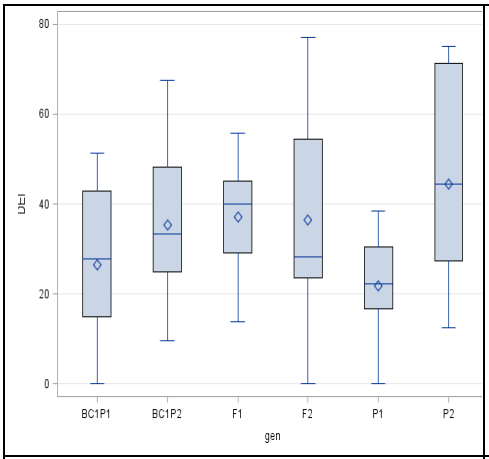
b. Days to anhtesis



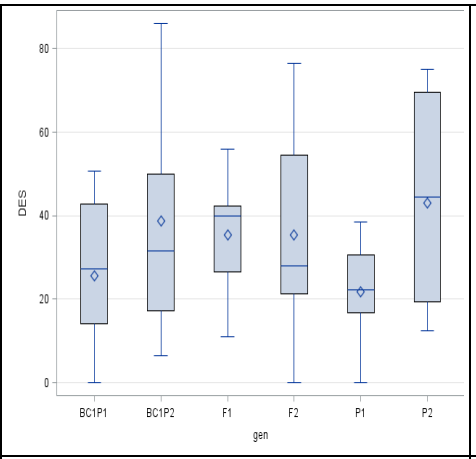
c. Ear aspect



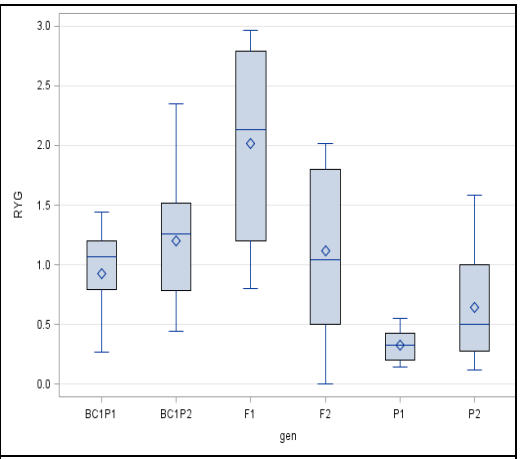
d. Plant height



a. Disease incidence

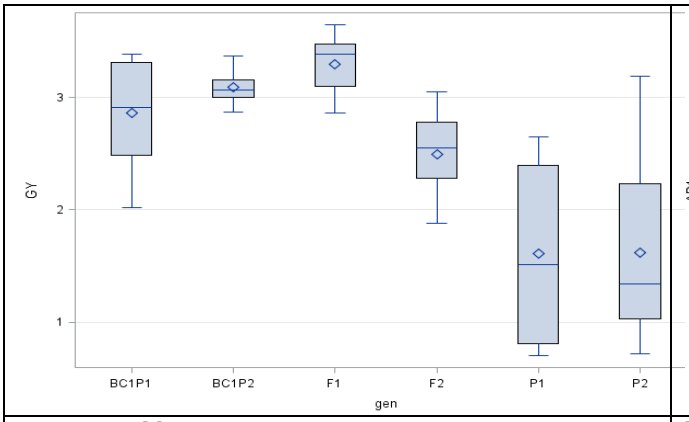


b. Disease severity

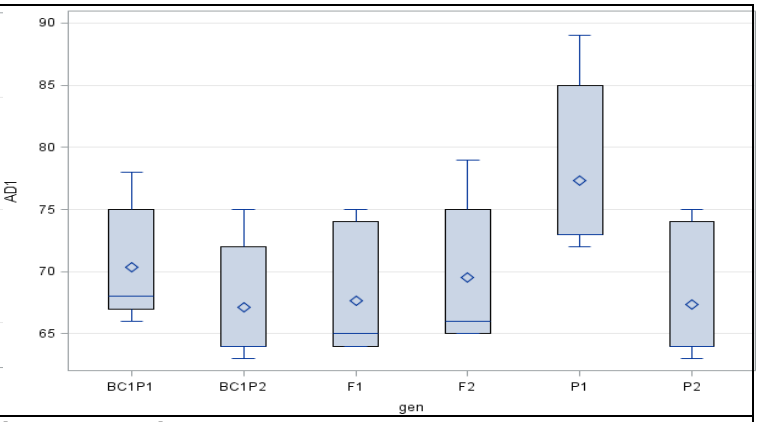


c. Weight of rotten ears

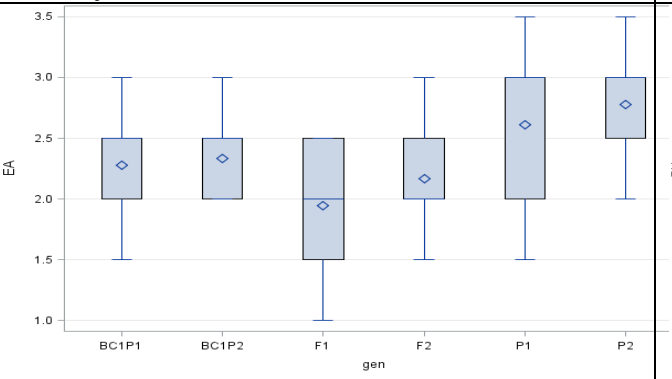
Appendix 6: Distribution of six generation means for agronomic traits and disease severity scores for the cross LPSC7 x VL06688 evaluated under artificial *Diplodia* ear rot inoculation at Kibos, Kakamega and Alupe in 2014.



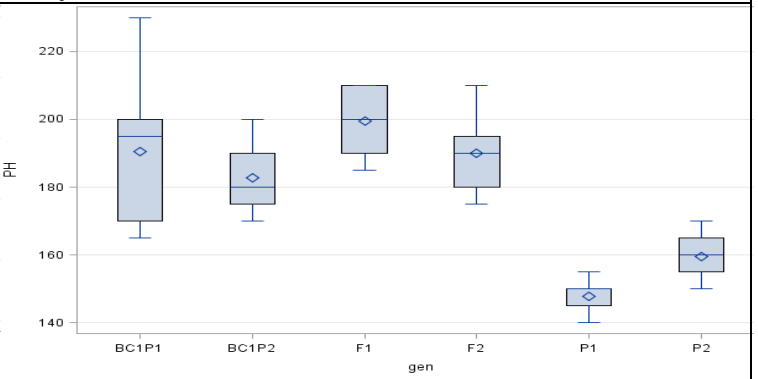
a. Grain yield



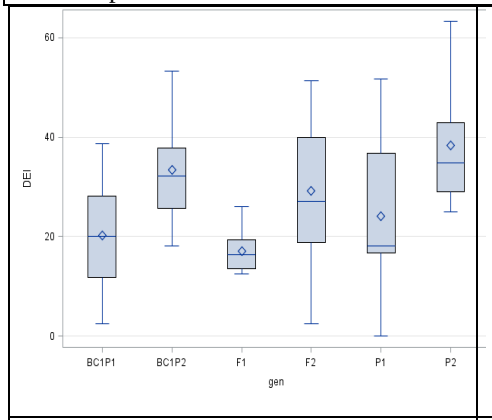
b. Days to anthesis



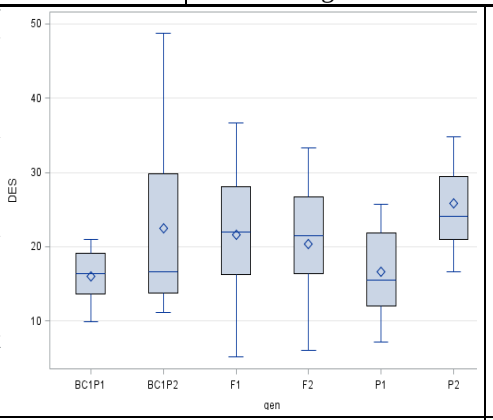
c. Ear aspect



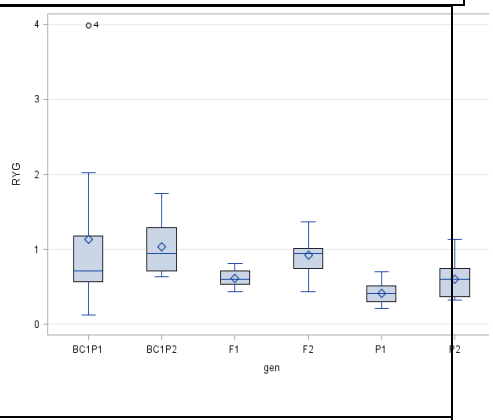
d. Plant height



a. Disease incidence



b. Disease severity



c. Weight of rotten ears