COMBINING ABILITY, HETEROSIS AND HERITABILITY OF AGRONOMIC TRAITS AND RESISTANCE TO MAIZE STREAK VIRUS IN MAIZE INBRED LINES

BY

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B. Agric. (Plant Science)

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN PLANT BREEDING

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B. Agric. (Plant Science)

NOVEMBER, 2016

DECLARATION

I hereby declare that this submission is my own work towards the Master of Philosophy in Plant breeding and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

Maize is an important cereal crop in Sub-Saharan Africa which contributes substantial portion to the diet of millions of people. The production of maize is being affected by maize streak virus disease (MSVD); an economically important foliar disease, thereby causing significant grain yield losses in farmers‟ fields. In Ghana, re-occurrence of the disease has been reported in several regions, therefore, necessitating the development of resistant hybrids which is the most sustainable and economical management option. The objectives of the study were to identify parents and hybrids that combine MSVD resistance with high yield, and also to determine the influence of maternal effect on the inheritance of MSVD resistance. Five parental inbred lines namely; TZEI-4, TZEI-7, TZEI-22, TZEI-31 and TZEI-157 were crossed in a full diallel mating design during the major season of 2015. The resulting F1 hybrids were evaluated under natural and artificial infestations during the minor and major seasons of 2015/2016 using 9 x 3 alpha-lattice design with three replications. Diagnosis of the viral disease using Polymerase Chain Reaction confirmed the presence of maize streak virus in the 27 genotypes evaluated. ANOVA for diallel crosses across environments revealed that general combining ability (GCA) and specific combining ability (SCA) mean squares were significant for MSVD severity mean score and most of the agronomic traits. Maternal effect had no significant contribution to the inheritance of MSVD resistance. GCA by environment ($P<0.01$) and SCA by environment ($P<0.001$) interactions mean squares were significant for MSVD severity mean score indicating that the disease pressure was higher under artificial infestation. Additive gene effect was preponderant for MSVD severity mean score, total leaf count, plant aspect and ear aspect whereas, the expression of other traits was influenced by non-additive gene effect. GCA effects revealed that inbred lines TZEI-7 and TZEI-22 were resistant to MSVD and could be good combiners for grain yield in addition to TZEI-31 and TZEI-157. Hybrids TZEI4*TZEI-22 and TZEI-4*TZEI-31

showed resistance to MSVD as revealed by their SCA effects and heterotic values. TZEI-7*TZEI-157, TZEI-31*TZEI-157, TZEI-22*TZEI157 and TZEI-4*TZEI-22 had positive and significant SCA effect, mid-parent heterosis and high parent heterosis for grain yield. The narrow sense heritability estimated for MSVD severity mean score, total leaf count and plant aspect were 55.3, 40.44 and

36.37 % respectively, while broad sense heritability ranged from approximately 54 to 84 % for all the measured traits. MSVD severity mean score correlated negatively and significantly ($P<0.01$) with total leaf count, plant height and 100-grain weight. Response to selection can be achieved for MSVD resistance combined with high grain yield if selection is based on MSVD severity mean score, total leaf count and plant aspect. Total leaf count, ear leaf area, plant height and 100-grain weight correlated significantly ($P<0.001$) and positively with grain yield. Promising hybrids TZEI4*TZEI-22, TZEI-22*TZEI-157, TZEI-7*TZEI-157 and TZEI-31*TZEI-157 identified in this study should be further tested in multi-locations across Ghana to determine their stability and adaptability.

DEDICATION

This thesis is dedicated to my wonderful parents, Mr and Mrs Oyewole Ige and my siblings, Titilope and Abimbola for their support during the course of the study.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CIMMYT	International Maize and Wheat Improvement Center
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
CTAB	Cetyl Trimethyl Ammonium Bromide
CV	Co-efficient of Variation
DIVA	Diffusion of Improved Crop Varieties in Africa
DNA	Deoxyribonucleic acid
DTBIA	Dot Immunoblotting Assay
ELISA	Enzyme Linked Immunosorbent Assay
et al	and others

F1	First filial
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Statistics
GCA	General Combining Ability
GDP	Gross Domestic Product
GLS	Grey Leaf Spot
ha	Hectare
h^2_b	Broad sense heritability
h^2_n	Narrow sense heritability
IITA	International Institute of Tropical Agriculture
ISEM	Immunosorbent Electron Microscopy
LSD	Least Significant Difference
MSV	Maize Streak Virus
MSVD	Maize Streak Virus Disease
OPV	Open-pollinated Variety
PCR	Polymerase Chain Reaction
PROC GLM	Procedure for Generalized Linear Model
QTLs	Quantitative Trait Loci
r	Correlation Co-efficient
R^2	Co-efficient of Determination
rpm	revolution per minute
SAS	Statistical Analysis System
SCA	Specific Combining Ability
SE	Standard Error
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeat

TZEI	Tropical *Zea mays* Early Inbred
USA	United States of America
WAP	Week after Planting

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.) is a staple and one of the most economically principal food crops for a large population of the world (Magenya *et al*., 2009). It is distributed worldwide and also ranks the first in production with over one billion tonnes produced in 2014 followed by rice (741 million tonnes) and wheat (729 million tonnes), but wheat ranks first in terms of harvestable area (FAOSTAT, 2015). According to FAOSTAT (2010), maize is produced on nearly 100 million hectares of land in 125 developing countries and is one of the most extensively grown crops in 70 % of those countries. In smallholder system, 38 million tonnes of maize were produced from 25 million hectares of land in Sub-Saharan Africa (SSA) (Smale *et al*., 2011). The demand for maize is increasing worldwide and is expected to double by 2050 because it constitutes the bulk of raw materials for some agro-based industries and livestock (Rosegrant *et al*., 2009).

In Ghana, maize is the number one cereal crop based on area cultivated and total production (MoFA, 2011); and it contributes about 30 % of Gross Domestic Product to the agricultural economy of Ghana (ISSER, 2011). FAOSTAT (2015) reported a significant reduction in maize produced throughout the country from 1,949,897 tonnes in 2012 to 1,762,000 tonnes in 2014. This reduction has been attributed to frequent biotic and abiotic stresses including pests and diseases outbreak, reduced soil fertility and drought thereby causing significant yield and economic losses in maize production every year in other SSA countries (Morris *et al*., 1999; Cairns *et al*., 2012).

Maize streak virus disease (MSVD) is caused by Maize Streak Virus (MSV) (Storey, 1925). It is a major foliar disease that affects maize throughout the SSA region (Pingali and Pandey, 2001). Amongst the diseases that cause economic damage to maize in the world, MSVD ranks third following northern leaf blight and grey leaf spot, besides it has

remained the most severe viral disease of maize in Africa resulting in the loss of returns, which ranges from US $ 120 to US $ 480 million yearly (Martin and Shepherd, 2009). With effective MSVD control, no less than half of this loss can be avoided (Martin and Shepherd, 2009). The disease can cause up to 100 % yield loss in susceptible varieties under field conditions (Magenya *et al*., 2008). However, reduction in growth and yield are directly dependent on factors such as time and stage of infection and also varies with the level of resistance (Bua and Chelimo, 2010).

Willment *et al*. (2001) reported that the most important type member of the genus *Mastrevirus* in the family Geminiviridae is MSV. It has a DNA genome of approximately 2.7 kb which is circular and single-stranded (Pratt and Gordon, 2006). It is transmitted by viruliferous leafhoppers in the genus *Cicadulina* (Storey, 1925). Storey (1933) observed that *Cicadulina mbila* (Naudé) was very efficient in the transmission of MSV; the most dominant in Africa out of all the species in its genus and its population consists of vectors that have the capability to transmit the virus (active vectors) and those that are not capable (inactive vectors). Eleven strains of MSV have been identified and are designated MSV-A to MSV-K. Merely MSV-A strain has been identified to be the most virulent and can cause significant MSVD while others attack cereals such as barley, wheat, oats, rye and millet excluding maize (Martin *et al*., 2001; Shepherd *et al*., 2010). Oppong *et al*. (2015) reported that MSV-A_1 strain variant was predominant in the transition and forest zones of Ghana and it exhibits an increased level of pathogenicity than the other MSV strain variants which are MSV-A_2, MSV-A_3, MSVA_4 and MSV-A_6 (Martin *et al*., 2001).

The incidence and severity of maize streak virus disease can be reduced by chemical control of leafhoppers and cultural or avoidance practices such as crop rotation, irrigation, inter-cropping, application of appropriate fertilizer rate and plant density manipulation

but the most sustainable and economical management option is provided by using disease resistant varieties (Martin and Shepherd, 2009).

Maize improvement programmes in SSA are aimed at improving grain yield and foliar disease resistance because the livelihood of the larger percentage of farmers with little or no resource is dependent on the headway made in maize production. Despite the successes achieved in breeding for varieties with MSVD resistance, the prevalence of MSVD continues to occur in Africa, causing huge losses in yield due to the erratic changes in climate (Legréve and Duveiller, 2010) which to some extent, makes the epidemiology of the disease complex (Martin and Shepherd, 2009). Commercial varieties in Ghana which had some degree of resistance to MSVD have become susceptible over the years probably because new strains of MSV have evolved. Therefore, it is imperative to identify stable and novel sources of resistance that can tolerate MSVD outbreak enhanced by drought or erratic rainfall resulting from change in climate in the tropical environments.

This requires studies on the degrees of resistance to MSVD and the effect of maternal inheritance on the resistant genes in the group of inbred lines, that will serve as parents for developing hybrids that are high yielding and resistant to the disease. Combining ability analysis gives information about the gene actions controlling the expression of the traits of interest and also helps the researcher to select parents with high general combining ability (GCA) and hybrids with high specific combining ability (SCA) associated with the efficient exploitation of heterosis. Heterosis is the phenomenon that describes the superiority of highly heterozygous F1 hybrids with respect to the average (mid-parent) or high parent performance of their genetically distinct homozygous parents (Virmani *et al*., 2003; Paschold *et al*., 2010). The level of heterosis manifestation in F1 hybrid is strongly associated with the genetic diversity of the parental lines (Paschold *et al*., 2010). Crosses between inbred lines from groups with divergent genetic backgrounds

are expected to exhibit high level of heterosis than those among lines from the genetically more related groups (Barata and Carena, 2006).

Thus, the main objective of the study was to identify maize genotypes with MSVD resistance for sustainable production.

The specific objectives were to:

i. determine general and specific combining abilities to identify parents that can be used to produce high yielding and MSVD resistant hybrid(s), ii. determine the effect of maternal inheritance on MSVD resistance, iii. estimate heterosis and heritability for MSV resistance and other agronomic

traits, and

iv. determine the phenotypic correlation between MSVD severity mean score and other agronomic traits.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, classification and botany of maize

Maize is one of the main cultivated crop species that originated in the Western Hemisphere and several suggestions were made that it evolved in the highlands of Guatemala and southern Mexico nearly 7,000 to 10,000 years ago (Hallauer and Carena, 2008). Wilkes (2004) reported from the information gathered over the previous six decades that teosinte (*Zea mays* L.: spp. *mexicana*) is an undomesticated weedy species; indigenous to Guatemala and southern Mexico and was presumed the parent of presentday maize. In the 16th century, maize was brought into West Africa by some

Portuguese traders passing through West Indies, Central and South America to the Gold Coast (now Ghana) (Fajemisin and Shoyinka, 1976).

Maize is a species of the tribe Andropogoneae which belongs to the grass family Poaceae. The genus *Zea* consists of five species which include *Zea nicaraguensis* (Iltis and Bruce), *Zea perennis* (Hitchcock), *Zea luxurians* (Durieu and Ascherson), *Zea diploperennis* (Iltis, Doebley and Guzman) and *Zea mays* (Linnaeus) of which the latter is economically important. It is an annual plant, monoecious and can grow to a height of 4.5m (Acquaah, 2007). The male flowers (staminate) are the tassels usually found at the apex of the stalk while the female flower (pistillate) emerges from the axils of leaves and a mass of extended styles (silks) bulge from its top as smooth threads (Hitchcock and Chase, 1971). The staminate inflorescence produces pollen and the silks being the pollen receptor must be pollinated individually so as to produce a seed or kernel. The staminate matures before the pistillate, hence, it is protandrous. Furthermore, a stalk may bear one to three cobs. A fertilized cob also called an ear may contain eight or more rows of kernels (Acquaah, 2007). It is naturally cross-pollinated and also selfpollination is usually possible.

2.2 Importance of maize

Maize is the most preferred staple in Africa where over 300 million people depend on it as their major food source compared to the developed countries where it is largely utilized as raw material for livestock feed (70 %) and only a minute fraction (5 %) is eaten by humans (ABSF, 2010). It provides food security and alleviates poverty in a number of the world‟s poverty-stricken areas especially in Africa, Asia and Latin America making it one of the most principal crops in the world. The daily per-capita consumption of maize in African regions varies between 52 to 328 g per individual per day and was estimated as 53 g per individual per day in Ghana over a 3-year period (2007-2009) (FAOSTAT, 2012). Maize production in Ghana provides employment to a larger percentage of the

populace and contributes about 30 % to the Gross Domestic Product (GDP) of the agricultural economy (ISSER, 2011). The entire maize plant is of great economic worth (Morris, 2002) and the author reported that the grains can be eaten by humans when roasted, boiled, ground into paste or powder, used as a raw material for producing animal feeds, starch, oil, sugar, protein, cellulose and ethyl alcohol. Livestock can be fed with leaves, stalks and tassels either as silage or stover. After harvesting, the plants can also be integrated into the soil to enhance its structure or dried which can be used as mulch or burned as fuel.

2.3 Maize production and its constraints

In Ghana, maize is the second most significant staple food next to cassava and it is produced in all the geographical areas showing comparable climatic conditions that determine their ability to support rain-fed agriculture with its production in the transition zone being the highest (MoFA, 2011; Adu *et al*., 2013). It is cultivated by the majority of people living in the rural community and extensively consumed all over the country. Ghana is one of the major maize producers in Africa accounting for about 9 % of the total land among assessed countries in the Diffusion of Improved Crop Varieties in Africa (DIVA) project and 7 % of the entire land in West and Central Africa (Alene and Mwalughali, 2012). It accounts for about 45 % of agricultural production and remains the main source of livelihood for a greater percentage of Ghanaians (ISSER, 2011).

In Ghana for instance, the achievable yield of maize is 6.0 t/ha whereas the average yield obtained from the field is 1.7 t/ha (MoFA, 2011) and this is among the lowest globally especially in comparison with countries such as United States of America (10.2 t/ha), China (6.3 t/ha) and South Africa (4.8 t/ha) (FAOSTAT, 2012). As a result of this, inadequate supply of food becomes an unending problem in most Sub-Saharan African countries (Magenya *et al*., 2008). The reduction in yield is due mainly to climate change,

poor soil fertility, frequent occurrence of droughts, farmers‟ limited access to fertilizer, lack of access to improved maize seeds, high incidence of insect-pests, diseases and weeds (Shiferaw *et al*., 2011; Cairns *et al*., 2012).

2.4 Maize streak virus disease

Maize streak virus disease (MSVD) is an important and the most damaging amongst the numerous viral diseases of maize that occur throughout Africa (Thottappilly *et al*., 1993; Harkins *et al*., 2009). It is caused by maize streak virus which is obligately transmitted by leafhoppers in the genus *Cicadulina* (Storey, 1925). The symptoms were initially referred to as „mealie variegation‟ and afterwards was changed to „maize streak‟ (Storey, 1925). Serious MSVD outbreaks have been reported in some African countries including Ghana, Malawi, Mozambique, Nigeria, Benin, Zambia, Zimbabwe, Republic of Congo, Angola, Kenya, Burkina Faso and Cameroon (Wambugu and

Wafula, 2000; Magenya *et al*., 2008).

MSVD symptoms are typified by broken to nearly unbroken chlorotic bands or stripes centered initially on the tertiary leaf veins and these later develop into rectangular tancoloured lesions that run parallel with leaf veins. As the disease spreads, the lesions merge resulting in blighting of the whole leaf (Agrios, 2005). The density of striping depends primarily on the resistance of the genotypes. In highly susceptible maize plants, the entire leaf lamina shows a severe, uniform white chlorosis which may progress gradually into death of cells and tissues of the plants and afterwards die back, especially when the plants are infested at the seedling stage (Rossel and Thottapilly, 1985). Severe chlorosis in susceptible maize plants leads to stunted growth, scanty ear development, reduced seed setting and ultimately huge yield losses or occurrence of premature death (Mawere *et al*., 2006; Monjane *et al*., 2011). The symptoms of MSVD in maize are most stable just after tasseling since no newer leaves are formed (Bombom *et al*., 2012). Plant species and variety have a great influence on the systemic movement of viruses and rates

of virus movement may be faster in susceptible varieties compared to tolerant varieties (Bosque-Pérez, 2000).

2.5 Maize streak virus disease distribution

MSVD appeared after maize was brought into Africa from the Americas about 40 decades ago and it was referred to as a „new-encounter" disease in maize (Buddenhagen, 1983). It is broadly spread in Africa; from Zimbabwe to Senegal, Sudan to South Africa and also on the adjacent islands including São Tomé, Mauritius, Principe Réunion and Madagascar (Rose, 1978; Thottappilly *et al*., 1993).

2.6 Maize streak virus

MSV is a type member of the genus Mastrevirus and the family Geminiviridae. It has a single-component, circular, single-stranded DNA genome of approximately 2700 bases encapsidated in 22 x 38 nm geminate particles comprising two incomplete T = 1 icosahedra, with 22 pentameric capsomers composed of a single 32-kDa capsid protein (Shephered *et al*., 2010).

Martin *et al*. (2001) identified six sub-types within the MSV-A strain and designated them as MSV-A1, MSV-A2, MSV-A3, MSV-A4, MSV-A5 and MSV-A6 and also noted significant differences in pathogenicity amongst the sub-types. Sub-types MSVA1, MSV-A2 and MSV-A5 produce more severe symptoms, MSV-A3 and MSV-A6 produce intermediate symptoms while MSV-A4 produces mild symptoms. Martin *et al*. (2001) defined sub-type as a set of isolates sharing over 98 % nucleotide sequence identity.

Rose (1978) reported that MSV was transmitted systemically by leafhoppers in the genus *Cicadulina*. Webb (1987) recounted that 22 species of *Cicadulina* have been recognized but 18 are present in Africa. Eight species which are important vectors of

MSV are *C. storeyi* (China) (= *triangula* (Ruppel)), *C. similis* (China), *C. arachidis* (China), *C. bipunctata* (Melichar), *C. latens* (Fennah), *C. ghaurii* (Dabrowski), *C. parazeae* (Ghauri) and *C. mbila* (Naudé) (Bosque-Pérez and Alam, 1992).

Storey (1925, 1928) reported that *C. mbila* could acquire the virus from infected plants within 15 s and the virus can be inoculated in 5 min after carrying out a series of classical experiments. After the virus has been acquired by *C. mbila*, the latent period of MSV within it is 6 - 12 h depending on the temperature and the virus continues to exist in the vector throughout its life time. Kimmins and Bosque-Pérez (1996) reported that inoculation of MSV into maize plants by *C. mbila* is accompanied by the injection of saliva into the phloem tissues. The vector acquires MSV particles by feeding from leaf mesophyll tissues of diseased plants thus it takes only a short time for *C. mbila* to acquire MSV when feeding on the tissue during their first contact with infected plants (Pinner *et al*., 1993). Hence, inoculation of MSV into a maize plant happens when the insect vector salivates into the phloem tissue and thus requiring additional time than acquisition.

Cicadulina mbila is the most abundant vector across the main ecological zones and seasons in SSA and also the most efficient in transmission (Asanzi *et al*., 1994; Asanzi *et al*., 1995). Within its populations, genetically distinct active and inactive vector transmitters are present (Storey, 1933), with the active transmitters having a percentage ranging from 60 and 100 % (Markham *et al*., 1984). This infers that effective transmission of virus relies on the presence of the virus in the infected plant and enough populations of active vector transmitters. Mesfin *et al*. (1995) discovered that vectors have preferences for some grasses from the study carried out on the feeding activities of the vector species. The rate of MSV transmission is reduced with lesser probing times on hosts the

leafhoppers does not have a preference for (Bosque-Pérez, 2000) showing that the feeding manner on a maize line may have an effect on its resistance to infection from MSV.

2.7 Diagnosis of maize streak virus

There are a range of immunodiagnostic and molecular diagnostic methods such as immunosorbent electron microscopy (ISEM), polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA), dot immunoblotting assay (DTBIA) and nucleic acid hybridization appropriate for making findings on viruses affecting maize; apart from information for epidemiological purposes given by these methods, they also assist in developing maize stocks that are free of disease (Sharma and Misra, 2011).

The detection of MSV in the genomic DNA sequence using degenerate oligonucleotide primers (markers) has been achieved with the use of PCR-DNA amplification technique (Sharma and Misra, 2011). Saiki *et al*. (1988) reported that the technique was developed in the mid-1980s and since then it is being swiftly taken on to recognize pathogens via their genetic material (DNA). PCR assays are fast, specific, reliable, highly versatile and extremely sensitive. It was used to detect the new strain of MSV in Cameroon (Leke *et al*., 2009). The presence of the virus can be diagnosed visually on the field based on the characteristic symptoms on plants but this is not always reliable because viral diseases can happen in a latent condition without expressing any visual symptoms (Sastry and Zitter, 2014). PCR technique was compared to ELISA by Rybicki and Hughes (1990), they reported that the endpoint dilution used in PCR was 10^4 times lesser than that normally attained in ELISA with a purified virus. Apart from diagnosis of virus, PCR technique can also be used for mapping of lines genetically against maize streak virus resistance (Pernet *et al*., 1999) and to study the transient and transgenic expression of MSV replication-associated protein mutants (Shepherd *et al*., 2007a).

Simple sequence repeats (SSRs) also known as micro-satellites is one of the DNA-based molecular markers (Molnar *et al*., 2003). It has short sequences comprising tandemly recurring replicas of between one to six nucleotide portions (Rafalski *et al*., 1996). The SSR technique circumvents the limitations and drawbacks of other molecular profiling techniques, it is not only simple but also; highly informative, reproducible, codominant, locus-specific and has the advantage of being amenable to PCR automation (Acquaah, 2007; Molin *et al*., 2013).

2.8 Epidemiology of maize streak virus disease

A susceptible host, virulent pathogen and favourable environmental conditions such as rainfall, relative humidity and temperature must be present for a disease to occur and develop (Legrève and Duveiller, 2010). Changes in the environmental factors under the influence of climate change have a significant influence on the predominance of diseases and development of new strains of diseases. MSVD epidemiology is mostly controlled by environmental influences on its vector species bringing about irregular outbreaks every 3 - 10 years (Shepherd *et al*., 2010). Alegbejo and Banwo (2005) reported that there was a significant and positive correlation between MSVD incidence and temperature but the incidence correlated negatively with rainfall and relative humidity. Swift build-up in population of virus and fast-spreading of MSVD are mostly ascribed to the convergence of factors such as: (a) the population density of wild grasses that are alternative hosts of *Cicadulina* spp. (Autrey and Ricaud, 1983); (b) staggered cultivating periods in which population of *Cicadulina* spp. build-up in early planted maize and severely damage seedlings that grow in the following season (Fajemisin and Shoyinka, 1976; Dabrowski *et al*., 1991); (c) the presence of a high proportion of active MSV transmitters within leafhopper populations, and (d) environmental factors that powers the sustained movement of *Cicadulina* spp. (Rose, 1978). Furthermore, MSVD epidemic is as a result of the effects from several interacting factors such as agroecological zones,

variety, planting date and availability of alternative hosts (Asanzi *et al*., 1994; Bosque-Pérez *et al*., 1998). Bosque-Pérez (2000) reported that epidemics of MSVD in West Africa especially in Nigeria and Ghana is related to early or erratic rains and drought.

The varying proportions of different leafhoppers in the genus *Cicadulina* ranging from 15 to 45 % capable of transmitting the virus makes the epidemiology of MSVD complicated (Asanzi *et al*., 1995). *Cicadulina storeyi* (China) has a higher capability of transmitting MSV but only this does not bring about the efficiency of the vector (Oluwafemi *et al*., 2007). Other factors to consider include distribution of vectors (*C. mbila* is the most extensively dispersed species all over Africa) in addition to the point that a greater percentage of *C. mbila* population is capable of transmitting MSV likened to other species of *Cicadulina* (Storey, 1928, 1933; Markham *et al*., 1984). *C. mbila* females are more efficient in transmitting the virus because their proportion in a population is twice or thrice times greater than other species (Wambugu and Wafula, 2000). Studies have shown that MSVD outbreaks is mostly connected to *C. mbila* species (Dabrowski, 1987; Magenya *et al*., 2008).

2.9 Economic importance of maize streak virus disease

The vulnerability of maize plants to MSVD starts from emergence to tasseling and often infection at seedling stage results in no ear formation (Magenya *et al*., 2008) but this varies with the level of resistance (Bua *et al*., 2010). Infection at the six to eight weeks stage after planting has a little effect on the vigour of the plant but eventually results in undersized and poorly filled ears (Fajemisin, 2003). In susceptible varieties, yield reductions often exceed 70 % depending on the stage of plant maturity when infection occurs (Magenya *et al*., 2008). MSV infestation significantly reduced the plant height and leaf area index of the varieties used (Bua *et al*., 2010). The extent of yield loss due to MSVD is dependent on weather, vector population densities, percent carry over

inoculums, growth stage of the crop when infection occurred and time of infection (Bjarnason, 1986; Bosque-Pérez *et al*., 1998). Severe outbreaks are often associated with late plantings or second season cropping (Bjarnason 1986; Efron *et al*., 1989). Small-holder farmers keep on suffering from significant yield losses in spite of the substantial progresses made in control measures which may perhaps reduce these deficits (Martin and Shepherd, 2009) but are unavailable to farmers without the resources to obtain them.

2.10 Management of maize streak virus disease

The epidemiology of MSVD is complex thus it requires an Integrated Disease Management; a concept which combines several control measures, for effective control or management. Present-day management strategies depend on the use of host plant resistance, chemical and cultural measures. The practicability, availability and value of cost for each method vary with regions of production and type of agriculture (subsistence or commercial) (Pratt *et al*., 2003).

Cultural practices such as roguing and destruction of alternative host plants and volunteer crops, early planting, crop rotation, planting of resistant cultivars, etc. aims at decreasing the movement of leafhoppers, interrupting their mating activities and the succeeding disperse of MSVD between farms (Bosque-Pérez, 2000). Early planting is suggested for MSVD management because it makes the crop to grow past the susceptible stages before the population of leafhopper builds up to an adequate level to spread the virus (Magenya *et al*., 2008; Shepherd *et al*., 2010). Crop rotation can be practiced with broad-leaved crops such as groundnut, soybean, pumpkin and cowpea because MSV does not infect broad-leaved plants (Damsteegt, 1983; Shepherd *et al*., 2010). Subsistence farmers find it difficult to completely rotate their maize crop because of the challenge of small-sized land they have and therefore, they prefer to practice inter-cropping which significantly reduce the yield of the main cereal crop. The use of cultural practices to control MSVD is inexpensive and easily reached by most African farmers.

However, it is impossible to get complete MSVD control with these practices given the characteristic changeability of MSVD epidemiology (Adejumo, 2005; Martin and Shepherd, 2009).

Application of systemic insecticides such as endosulphan, dimethoate, imidacloprid and aldicarb is intended for controlling the vectors (leafhoppers) (Magenya *et al*., 2008). It protects the maize crop past the susceptible stages because it residual effect lapses after seven weeks of application. Besides the alarms such as the probable poisoning of people who spray the insecticides or handle insecticide-treated seeds (Martin and Shepherd, 2009), the high prices of insecticides and equipment for spraying often limit the use of this MSVD control option especially by the small-scale farmers (Mawere *et al*., 2006).

The use of resistant cultivar is possibly considered the most suitable method of MSVD management because it is cost-effective, environmental friendly, economically viable and a long-term method to bringing down yield losses to MSVD epidemics (Ngwira and Pixley, 1998; Lagat *et al*., 2008). Collaborative efforts by several international maize breeding programmes such as the International Maize and Wheat Improvement Center (CIMMYT) and the International Institute of Tropical Agriculture (IITA) have produced a huge collection of germplasm with improved MSVD resistance (Welz *et al*., 1998; Bosque-Perez, 2000). Resistant cultivars are developed by conventional breeding or alternatively using genetic engineering. Shepherd *et al*. (2007b) developed the first maize with transgenic MSV resistance. It was reported that the transgenic maize had delayed symptoms development and reduced severity scores compared to nontransgenic maize when both were inoculated with MSV. However, genetic engineering has numerous major shortcomings, some of which includes probable interruption of vital coding or regulatory sequences, transformation and regeneration complications, awareness responsiveness and expensive risk evaluation (Shepherd *et al*., 2010).

2.11 Breeding for resistance to maize streak virus disease

Conventional breeding involves making crosses between selected parent plants that have desirable characteristics such as high yield or disease resistance. Selection of superior plant traits involves visual assessment thus the breeder"s skills lie in selecting the best plants with desirable characteristics from the large segregating offspring populations (Ulukan, 2011). Breeding for disease resistance entails maneuvering the genetic systems of the host plant and the pathogen, not individually, but in connection with the interaction between the host plant and the pathogen (Acquaah, 2007). It involves the identification and incorporation of genetic resistance into adapted cultivars or economically important varieties (Acquaah, 2007). Resistance to MSVD as suggested from studies carried out by Storey (1938) and Kairo *et al*. (1995) can be achieved by developing mechanism that can interfere with virus transmission at initial settling or sustained feeding on maize by the vector, which could be potentially exploited in breeding programmes. Efforts in breeding for MSVD resistance have relied on the availability of leafhoppers as well as alternative host plants of MSV (Mawere *et al*.,

2006) or employing artificial infestation technique which entails mass rearing of *Cicadulina* spp. and maintenance of appropriate MSV inoculum (Bosque-Pérez and Alam, 1992). Cairns *et al*. (2012) reported that breeding for disease and insect-pest resistance is guided by the genetic variability in the insect-pest or pathogen population therefore requiring a thorough understanding of the biology and ecology of the pathogen or insect pest, life cycle of diseases and other factors inducing the development of plantpathogen interactions.

Maize research programme at IITA started a project in 1979 with the aim of developing essential germplasm needed for production of hybrids. High combining inbred lines that are vigorous, resistant to MSVD, stable and adapted to the tropics were developed from

the project (Efron *et al*., 1989). Furthermore, over the decades, different researchers have made significant progress in identifying stable genetic resistance for maize diseases of economic importance (Bosque-Pérez, 2000; Welz and Geiger, 2000; Pratt and Gordon, 2006) and it is of utmost importance to identify more stable sources of genetic resistance.

2.12 Genetic basis of maize streak virus resistance

Previous studies reported that the genetics of resistance to MSV in „Mex. 37-5" and „Yellow Bounty" were considered to be non-Mendelian (Gorter, 1951). Later the resistance in „Peruvian Yellow" x „Arkell"s Hickory" was reported to be controlled by a single gene lacking dominance (Storey and Howland, 1967). Kim *et al*. (1989) gave an account that resistance to MSV is controlled by a minimum of two or three major gene pairs with the possible contribution of minor genes in the resistant inbred line „IB32". Major genes at chromosome 1 were first mapped by Kyetere *et al*. (1999) and quite a lot of authors have subsequently verified the importance of this chromosome segment, which explains 50 to 60 % of phenotypic difference on the average (Welz *et al*., 1998; Pernet *et al*., 1999; Lu *et al*., 1999).

Redinbaugh *et al*. (2004) reported that resistance to MSV is generally related to one or two major resistant loci which makes possible selection with the use of markers.

However, these resistant genes have been found to huddle in the maize genome. Numerous studies have identified the genomic regions linked to resistance to the MSVD using diverse populations of maize in different environments, and these studies have shown that resistance to MSV is quantitatively inherited with an unstable number of genes involved (Welz *et al*., 1998; Pernet *et al*., 1999; Mawere *et al*., 2006).

2.13 Diallel mating design

This involves crossing in all possible combinations among a given number of parental genotypes (Kang, 1994). Depending on the objective of the study, parental genotypes

involved in a diallel can be inbred lines or heterozygous populations (open-pollinated populations). This has been used successfully for more than 50 years in plant breeding to estimate the relative combining ability of lines. It can provide genetic information such as variance components of general combining ability (GCA) and specific combining ability (SCA) or heritability for a population when parents are randomly chosen (Baker, 1978; Kang, 1994). Information provided by diallel can also be used to measure hybrid performance, devise breeding methods and strategies in the process of developing new genotypes (Baker, 1978; Kang, 1994; Zhang *et al*., 2005; Qi *et al*., 2010).

According to Griffing (1956), depending on whether parents and reciprocals are incorporated or excluded in a particular design, there are four diallel techniques proposed for determining the combining ability of lines and their gene action. Analysis of the components of variance and genetic estimates can be performed based on fixed model (model 1) or random model (model 2) depending on whether parents were a fixed set or randomly chosen respectively (Christie and Shattuck, 1992).

The resistance of maize varieties to MSV for specific crosses cannot be reliably envisaged all the time by evaluating parental performance or from pedigree information (Diallo, 1999) thereby making diallel analysis in hybrid combinations important.

2.14 Combining abilities, heterosis and heritability

Information on the combining abilities of the materials to be utilized in breeding programs is very important for the successful development of new high yielding hybrids combined with resistance or tolerance to diseases (Alam *et al*., 2008; Legesse *et al*., 2009). Such information can show the gene action(s) controlling the expression of various traits inherited quantitatively (Hallauer and Miranda, 1988). The analysis of combining abilities of parental germplasm provides valuable information about the parents used and helps in choosing parents with high general combining ability and hybrids with high specific

combining ability. Superior hybrids can be identified by comparing the estimated SCA effects and the trait mean for each combination (Sughroue, 1995). General combining ability (GCA) is the mean performance of a line in its hybrid combinations while specific combining ability (SCA) denotes the deviation of single crosses from the mean performance of the parents involved (Sprague and Tatum, 1942). Significant values of GCA and SCA indicates additive and non-additive (dominance and epistasis) gene actions respectively (Hallauer and Miranda, 1988).

Baker (1978) suggested a ratio that can be used to assess the relative importance of GCA and SCA in predicting the performance of progeny from inbred parents. As the ratio approaches unity, the larger the predictability of selecting superior progeny on the basis of GCA estimates alone. Predominance of additive effects has been observed in controlling MSVD resistance (Vivek *et al*., 2010; Gichuru *et al*., 2011). Menkir and Ayodele (2005) observed that GCA explained 60 % of the variation for grey leaf spot (GLS) resistance in hybrids developed from 24 maize inbred lines.

The manifestation of heterosis in maize has been reported since early 1900s based on the findings of Shull (1908) and East (1909). In the United States of America, exploitation of heterosis is a great underlying factor responsible for the remarkable increase in the yield of maize between the 1930s and the 1970s (Duvick, 2001). Acquaah (2007) reported that 0.1 % of maize production in USA was dedicated to hybrid seeds in 1933 and at present, hybrids are cultivated on virtually all maize fields.

Heterosis or hybrid vigour is an occurrence wherein the progeny of the first filial (F1) generation shows superiority over their parents (mid-parent or high parent) and this could be expressed in several characters for instance agronomic yield, increased biomass, size, tolerance to abiotic stresses, pest resistance, growth rate and other reproductive factors (Falconer and Mackay, 1996; Duvick, 1999; Virmani *et al*., 2003). Nonetheless,

superiority is lost at each subsequent generation of self-fertilization; thus highest heterosis is expressed in the first filial generation (Meyer *et al*., 2004). The expression of heterosis relies on the genetic divergence between two parental lines

(Hallauer and Miranda, 1988). The complimentary interactions of alleles at each locus (dominance) and the interactions of alleles between loci (epistasis) of the two inbred parents determine the manifestation of heterosis (Carena, 2008). Heterosis has been intensively exploited in hybrid breeding of maize because of its large expression for grain yield (100-200 %) (Reif *et al*., 2005). Although, several hypotheses have been proposed to give an explanation for heterosis; its biochemical, physiological and genetical bases still remain largely inexplicable (Reif *et al*., 2005).

To create a suitable breeding programme, it is imperative to know the fraction of phenotypic variation of a trait that is heritable (Kearsey and Pooni, 1996) since the efficiency of a selection program is primarily reliant on the degree of genetic variation and heritability of a trait (Falconer and Mackay, 1996). Heritability presumes that genotypes which are very much related have the tendency to look like one another than the distant ones (Falconer and Mackay, 1996) and its estimate helps breeders to apportion resources required to efficiently select for preferred traits and to realize the highest genetic gain within short time (Smalley *et al*., 2004). It can be estimated as narrow or broad sense subject to the genetic variance used or on an individual plant basis and on a progeny mean basis depending on the generation used (Hallauer *et al*., 2010). Njoroge and Gichuru (2013), Vivek *et al*. (2010) found out the fraction of phenotypic variation that is due to genes are high for most foliar diseases ranging from 70 to 85 %.

2.15 Hybrid maize production

Hybrid cultivars represent the F1 progeny of matings that may involve inbred lines or populations, with crossing of two or more inbred lines being the commonest (Fehr, 1987).

They exhibit superior qualities over their parental inbred lines due to the exploitation of hybrid vigour or as a result of heterozygosity and hence seeds cannot be saved for the next growing season. In the United States of America (USA), there was a significant increase in maize production by planting hybrid varieties without expanding production area (Duvick, 2001). Despite the increased hybrid seeds utilization among the USA farmers, there are still some limited circumstances where open-pollinated varieties (OPVs) are still desirable (Kutka and Smith, 2007). Therefore, hybrids have not permanently replaced OPVs even in developed countries, they are still used alongside with the OPVs. However, OPVs are predominant in African countries including Ghana where the seed industry is not well developed to ensure accessibility of seed to farmers making the country not self-sufficient in maize production. In Ghana, production of hybrid maize varieties is in its infant stages with only about 3 % of farmers planting hybrid seeds which were generally imported (Ragasa *et al*., 2013).

Ragasa *et al*. (2013) reported from a nationwide investigation of 630 maize farmers that were assessed on production practices in 2012 that only 1.6 % of the farmers planted hybrids giving an account for about 3 % of the area cultivated with maize. The yields of the hybrids reported in the study were approximately 70 % higher than those of the common OPVs, even though an additional fertilizer was applied. Despite the higher rate of fertilizer applied, calculation on the basis of the study revealed that the hybrids were more cost-effective than the OPVs (Ragasa *et al*., 2013; Ragasa *et al*., 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental materials

Five inbred lines tolerant to maize streak virus disease were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Table 3.1). Two checks (Omankwa and Aburohemaa) were also obtained from the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI), Fumesua, Ghana (Table 3.1).

Table 3.1: Characteristics of maize genotypes selected for the study

Genotypes	Pedigree	Maturity	Colour	Source
TZEI-4	TZE-W Pop x 1368 STR S7 Inb. 6	Early	White	IITA
TZEI-7	WEC STR S7 Inbred 12	Early	White	IITA
TZEI-22	WEC STR S7 Inbred 9	Early	White	IITA
TZEI-31	TZE-W Pop x LD S6 Inbred 4	Early	White	IITA
TZEI-157	TZE-Y Pop STR Co S6 Inbred 102-1-2	Early	White	IITA
Omankwa	TZE-W POP STR QPM C4	Early	White	CRI, Fumesua
Aburohemaa	EVDT-Waa STR QPM CO	Early	White	CRI, Fumesua

3.2 Experimental sites

A crossing block was established for the five inbred lines in full diallel at the Finatrade Farm, Department of Animal Science, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology ($6^{o}\,41^{1}$N; $1^{o}\,33^{1}$ W), Kumasi, Ghana from April to July, 2015. It falls within the Semi-deciduous Rain forest zone and is characterized by a bimodal rainfall pattern, from March to July and then from September to November, with an average yearly precipitation of 1500 mm. The soil type is haplic alisols (Jones *et al*., 2013).

An evaluation trial was carried out at Wenchi (7.7333N; 2.1W), Ghana from October, 2015 to January, 2016. Wenchi is known to be a hot spot for MSV-A_1 strain, the most virulent strain of MSV especially in the minor season under natural conditions as reported by (Oppong *et al*., 2015; Muiru *et al*., 2015). The evaluation site lies in the heart of the Transition zone of Ghana, characterized by two seasons of rainfall with the major season starting from March and ending in July while the minor season begins from September and stops in November or December. The soil is sandy loam (Ghanadistricts, 2006)

Rearing of leafhoppers (*Cicadulina mbila)* colonies and artificial infestation took place at the Entomology section of the CSIR-CR1, Kwadaso Station. The maize seedlings were transplanted to the field at nine days after planting after being infested with MSV.

3.3 Establishment of crossing block

In the crossing block, each inbred line was planted in seven rows of 5m length at a spacing of 75 cm by 25 cm. Two seeds were planted per hill and later thinned to one plant at three weeks after planting. Full diallel mating design was used in crossing the five inbred lines namely TZEI-4, TZEI-7, TZEI-22, TZEI-31 and TZEI-157. Artificial pollination was done by collecting and bulking pollen from male parents and then crossed with the female parents. The pollen was collected by covering tassels of male plants very early in the morning (before 6:00 am) until mid-day to allow enough pollen to be collected for the day and then bulked for each line. Sufficient pollen was poured to completely cover the emerged silks of each female plant that had previously been covered with a transparent polythene. Five to ten female plants of each line were pollinated. Cross inscriptions were made on pollinating bags for easy identification of similar cross combinations during harvesting. The pollination bags stayed on the ear until maturity to prevent any contamination that could occur after manual pollination.

Ears from crosses of the same parents were harvested dried, bulked and shelled. The seeds of 20 F1 single cross hybrids and their five parents were cleaned and stored at 18^0 C before evaluating them on the field. All cultural practices including fertilizer application and weeding were done to ensure good growth and yield.

3.4 Evaluation of F1 single cross hybrids and parents for maize streak virus disease resistance

The experimental field was sprayed with rid-out (glyphosate, 360g/l) at 5.0 l/ha before ploughing and harrowing were done. The 27 genotypes including the checks (Omankwa and Aburohemaa) were planted in 9 x 3 alpha-lattice design with three replications. A plot consisted of two-rows of 5m long each. The rows were spaced 75 cm apart while hills were spaced 40 cm apart. Three seeds were sown per hill and later thinned to two plants per hill at three weeks after planting (WAP). Hence, a planting density of approximately 66,667 plants/ha. Recommended crop management practices were applied. Fertilizer equivalent to 90:0:40 kg ha^{-1} of N-P_2O_5-K_2O (26:0:4) and sulphate of ammonia fertilizers were applied at two weeks after planting and at ear emergence respectively. Post emergence weeds were controlled with the application of caliherb (2, 4 - dichlorophenoxy acetic acid, 360g/l) at 4.5 l/ha using a knapsack sprayer and manual weeding when necessary.

3.5 Artificial infestation of the maize genotypes with maize streak virus

Non-viruliferous leafhoppers, *Cicadulina mbila* were collected from the field with the use of pooters and were reared on pearl millet (*Pennisetum americanum* L.) in insect proof cages. They had an acquisition access period of 48 h from maize plants severely infected with MSV. The 27 genotypes planted in cups filled with loamy soil were infested at two-leaf stage as described by Bosque-Pérez and Alam (1992) but modified. The modification was done by placing the maize seedlings in insect proof cages and after 48 h of feeding

period by the viruliferous leafhoppers, they were transplanted to a field which has been ploughed, harrowed and laid out using 9 x 3 alpha-lattice design with three replications.

3.6 Data collected

Ten plants were selected randomly on each plot for collection of data on morphological and agronomic traits. Border plants were excluded. Data collected were:

i. Stand count: Number of plants in a plot after thinning ii. Days to 50 % anthesis (DA): Number of days from planting to the date when 50 % of the plants in a plot have tassels shedding pollen.

iii. Days to 50 % silking (DS): Number of days from planting to the date when 50 % of the plants in a plot have emerged silks.

iv. Anthesis-silking interval (ASI): Difference between days to 50 % anthesis and days to 50 % silking.

v. Total leaf count (TLC): Mean of the number of leaves per plot after silking.

vi. Plant height (PLHT): Average height of plants (cm) from the base of the plant to where tassel branching begins.

vii. Ear height (EHT): Average height (cm) from the base of the plant to the node bearing the upper ear.

viii. Ear leaf length (ELL): Length of leaf (cm) which subtends the uppermost ear after flowering.

ix. Ear leaf width (ELW): Width of leaf (cm) which subtends the uppermost ear was measured mid-way along its length after flowering.

x. Ear leaf area (ELA): This was calculated using the formula: 0.75 x leaf length (cm) x maximum leaf width (cm) according to (Montgomery, 1911).

xi. Plant aspect (PASP): This is a general score for the appearance of the plants in the plot. Factors such as relative plant and ear heights, uniformity, reaction to MSVD

and lodging were considered. PASP was rated on a scale of 1 to 5 where: 1 = excellent overall phenotypic appeal, 2 = very good overall phenotypic appeal, 3 = good overall phenotypic appeal, 4 = fair overall phenotypic appeal and 5 = poor overall phenotypic appeal (Badu-Apraku *et al*., 2012).

xii. Ear aspect (EASP): This is a score for the general appearance of all ears in the plot. Factors considered were ear size, grain filling, disease and insect damage and uniformity of size, color and grain texture. It was rated on a scale of 1 to 5 where: 1 = best, 2 = good, 3 = average, 4 = fairest, 5 = poorest ear aspect (Badu-Apraku *et al*., 2012).

xiii. Number of ear(s) per plant (NE/PLT): Total number of ears at harvest that bear kernels including the second ear as well as the top ear.

xiv. Field weight (FWT): Weight of harvested cobs (kg) per plot after harvest. xv. Ear length (EL): Length (cm) of the cob with grains per plot.

xvi. Ear diameter (ED): Diameter (mm) of the cob with grains per plot. xvii. 100-grain weight (HGW): Weight of hundred grains per plot.

xviii. Moisture percentage (MOIST %): Moisture tester (Aqua-Boy, Germany) was used to determine the moisture content of the grains per plot at harvest.

xix. Grain yield (GY) (t/ha): The field weight at 80 % shelling percentage was adjusted to 12.5 % moisture content. Yield per hectare (ha) was estimated by multiplying the yield per plant by plant density per ha and then converted to t/ha xx. Maize streak virus disease severity (MSVDS) mean score: This was done by scoring all the plants in the plot and then calculated the average. The scoring was done at 3, 6 and 9 WAP by using a scale of 1-5 (Beyene *et al*., 2012) as follows: 1= no symptoms on leaves, 2 = light disease symptoms on 20 to 40 % leaf area, 3 = moderate symptoms on 40 to 60 % leaf area, 4 = severe symptoms on 60 % of leaf area, 5 = severe symptoms on 75 % or more of the leaf area. Therefore, a genotype having a severity mean score

<1 - 1.0 was considered highly resistant, 1.1 - 2.0 resistant, 2.1 - 3.0 moderately resistant, 3.1 - 4.0 susceptible and 4.1 - 5.0 highly susceptible.

xxi. Maize streak virus disease incidence (MSVDI): Number of plants affected in each plot was counted at 3, 6 and 9 WAP and the incidence was estimated based on the number of plants affected and expressed as a percentage of the total number of plants per plot (Bua *et al*., 2010).

3.7 Detection of maize streak virus using SSR markers

3.7.1 Sampling of leaves, DNA extraction and DNA quality assessment

Leaves from 14-day-old maize seedlings were sampled, cleaned with 70 % ethanol, transferred immediately into plastic bags and transported to the laboratory on ice cubes for storage at -80 ºC until further processing. The total DNA was extracted from the leaf tissue using CTAB method (Dellaporta *et al*., 1983) with slight modifications by Kirkhouse Trust Mobile Laboratory of the Cocoa Research Institute of Ghana, New Tafo, Ghana. About 2 g of the chilled leaf sample was placed in 2 ml eppendorf microfuge tube, freeze-dried with liquid nitrogen and ground into a fine powder. 800 µl of CTAB buffer containing 2 % CTAB, 2 % PVP, 1.4 M NaCl, 20 mM EDTA pH 8.0, 0.1 M Tris-HCl pH 8.0 and 1 % β-mercaptoethanol was added with gentle shaking and placed in water bath at 65 ºC for 30 min. The tubes were allowed to cool at room temperature and then equal volume of chloroform to iso-amyl alcohol in the ratio 24:1 was added and spun with the centrifuge (Eppendorf, Germany) for 12 min at 12000 rpm. The supernatant was pipetted into 1.5 ml eppendorf microfuge tube and the chloroform iso-amyl alcohol wash was repeated. The supernatant was again pipetted into a new 1.5 ml eppendorf microfuge tube, ice-cold isopropanol was added and kept in the freezer overnight to enhance DNA precipitation. The precipitated DNA was centrifuged at 14,000 rpm for 5 min to obtain DNA pellets and the isopropanol was carefully decanted. Pellets were washed with 10 mM ammonium acetate on a shaker for 15 min and spun at 6000 rpm for 4 min.

Ammonium acetate was decanted and 80 % ethanol was added to the pellets and spun at 6000 rpm for 4 min. Ethanol was decanted and pellets were vacuum dried in DNA mini centrifuge (Jouan Nordic Gydevang, Denmark). The precipitated DNA was re-suspended in 50 µl of 1.0 mM Tris-HCl pH 8.0 and 0.1 mM EDTA pH 8.0 1X TE buffer.

The quality of each DNA isolate was established by electrophoresis on 0.8 % agarose gel stained by adding 5 µl/ml ethidium bromide solution. Each DNA sample (10 µl) was added to 2 µl loading dye (6X Bromophenol blue) in different eppendorf tubes. The mixtures were spun for 30 s at 4680 rpm and then loaded separately in the wells on the gel submerged in 1X TBE loading buffer. After loading, they were then run at 120 volts for 45 min and observed under the UV transilluminator (Scie-plas, UK). Samples were finally stored at 4 °C until required for use.

3.7.2 Markers and PCR amplification

The maize DNA samples were amplified using a set of SSR markers identified from previous study for detecting the presence of MSV (Oluwafemi *et al.*, 2008). The markers were first tested and only those that showed amplifications were selected. The PCR master-mix for each of the marker was prepared by adding all the constituents listed in Table 3.2 except the DNA in 0.2 ml PCR tubes on ice. In a 15 µl PCR reaction volume, 13.5 µl of master-mix was mixed with 1.5 µl of DNA. The sequences of the markers used are given in Table 3.3.

Table 3.2: The constituents of the 15 µl reaction volume

Constituents	Quantity (µl)
DNA template	1.5
10 µM forward primer	0.45
10 µM reverse primer	0.45
25 Mm $MgCl_2$	0.3

100x KAPA plant PCR enhancer	0.015
2.5 U/µl KAPA3G plant Taq DNA polymerase	0.12
2x KAPA plant PCR buffer	7.5
PCR grade water	4.67

Table 3.3: Names and sequences of the two SSR markers used for the detection of MSV in the 27 genotypes evaluated under natural and artificial infestations

Marker	Forward primer (5´- 3´)	Reverse primer (5´- 3´)
MSV11	TTCATCCAATCTTCATC	GGAAAATCTACTTGGGC
MSV13	TGCAGCCAGTCTTCATC	GGAAAGACTTCTTGGGC

The reaction mixtures were centrifuged briefly before placing in a thermocycler (Flex cycler^2 Base Unit, Germany). The PCR cycles for the reaction mixtures containing each of the marker were programmed at a temperature of 94 °C for 2 min for initial denaturation; 35 cycles of denaturation at 94°C for 30 s, annealing at 50 °C for 40 s, extension at 72 °C for 1 min followed by 1 cycle of denaturation 94 °C for 30 s, 50 °C for 40 s, 72 °C of final elongation for 7 min.

3.7.3 Electrophoresis and visualization of amplified products

The PCR products were electrophoresed on 2 % agarose gel system. The gel was prepared by weighing 2 g of agarose into a beaker containing 200 ml of 1X TBE buffer. The mixture was swirled to mix, melted in a microwave oven and allowed to cool to about 45 °C. The molten gel was stained by adding 5 µl/ml ethidium bromide solution before pouring into an electrophoresis tank with combs creating wells. The gel was allowed to solidify before being used. The solid gel was then placed in a gel box containing 1X TBE buffer making sure that it was completely submerged prior to removing the combs. 2 µl of bromophenol blue loading dye was added to the PCR products and 10 µl was loaded into each 1.5 mm wide gel well. The first well was loaded with 5 µl of the 100 bp (100 ng/µl) KAPA Universal DNA ladder, followed by a negative control (purified water

instead of DNA) and then the PCR products from the DNA extracted from all the genotypes under natural and artificial infestations, and then known positive control for the presence of MSV. The electrodes of the gel box were joined (red to red and black to black) and switched on to 90 volts. The gel was removed, visualized under UV transilluminator (Scie-plas, UK) and photographed after running for about 120 min.

3.8 Statistical analysis

3.8.1 Analysis of variance for genotype and full diallel

Analyses of variance (ANOVA) to detect differences among the genotypes were performed separately on the data collected from the natural and artificial infestations and then combined ANOVA across the two environments for MSVD severity mean score and other agronomic traits using the PROC GLM in Statistical Analysis System (SAS) software version 9.1. Genotypes were considered as fixed effect while environments, replications and blocks within replications as random effects. Least significant difference (LSD) was used to determine the significant differences amongst the least square means of the genotypes at the probability level of 0.05. MSVD severity mean score was square root ($\sqrt{x}$) transformed ahead of performing the analysis, but the original value was reported after back-transformation.

The GCA effects of the parents and SCA effects of the hybrids as well as reciprocal effects for each and across environments for MSVD severity mean score and other agronomic traits were estimated without the checks by following Griffing"s Method 1, Model l (fixed effects) Griffing (1956) using DIALLEL-SAS program developed by Zhang *et al*. (2005) adapted to SAS software version 9.1. Effects of GCA, SCA and reciprocal for the traits were computed from the mean values adjusted for the block effects for each environment and across environments. T-test was used to detect the significance of GCA, SCA and reciprocal effects. Standard errors were estimated as square root of the GCA, SCA and

reciprocal variances (Griffing, 1956). The relative importance of GCA and SCA were investigated by using the formula: $2\sigma^2_{gca} / (2\sigma^2_{gca} + \sigma^2_{sca})$ by Baker (1978). The format of combined ANOVA for diallel (Griffing"s Method 1, Model 1) across environments (Table 3.4). The mathematical model for diallel across environments was as follows:

$Y_{ijk} = \mu + env_k + g_i + g_j + s_{ij} + rij + g_i env_k + g_j env_k + s_{ij} env_k + r_{ij} env_k + e_{ijk}$ where *Yijk* is the mean over replications of the single crosses in the k[th] environment, μ is the overall mean, env_k is the k[th] environment effect, (*gi*, *gj*), *sij* and *rij* are the GCA, SCA and reciprocal effects, respectively as described by Griffing (1956), *eijk* is the error term and the remaining parameters correspond to interactions of the main effects with environment.

Table 3.4: Format of combined ANOVA for GCA, SCA and reciprocal effects across environments according to Griffing's Method 1, Model 1 (Griffing, 1956)

Source of variation	D. F.	Mean Squares	Expected Mean Squares
GCA	*p-1*	MS_g	$r^2 + 2p\left(\frac{1}{p-1}\right)\sum g_i{}^2$
SCA	*[p(p-1)]/2*	MS_s	$\sigma^2 + \frac{2}{p(p-1)}\sum_i\sum_j s_{ij}{}^2$
			$\sigma^2 + 2\left(\frac{2}{p(p-1)}\right)\sum_{i<j}\sum R_{ij}{}^2$

Reciprocal effect (R)	*[p(p-1])/2*	MS_R		
GCA*E	*(p-1)(e-1)*	MS_{ge}	$\sigma^2_{error} + r\sigma^2$	*ge*
SCA* E	*[p(p-1)(e-1])/2*	MS_{se}	$\sigma^2_{error} + r\sigma^2$	*se*
R*E	*[p(p-1)(e-1)]/2*	MS_{Re}	$\tau^2_{error} + r\sigma^2$	*Re*
Error	*m*	MS_{error}	τ^2_{error}	

where*:* MS_g = general combining ability mean square, MS_S= specific combining ability mean square, MS_R = reciprocal effect mean square, MS_{ge} = general combining ability by environment interaction mean square, MS_{se} = specific combining ability by environment interaction mean square, MS_{Re} = reciprocal effect by environment interaction mean square, MS_{error} = error mean square, σ^2_{ge} = variance due to general combining ability effect by environment interaction, σ^2_{se} = variance due to specific combining ability effect by environment interaction, σ^2_{re} = variance due to reciprocal effect by environment interaction, σ^2_e = variance due to error, p= number of parents, r = number of replications and e = number of environment.

The format of ANOVA for diallel (Griffing's Method 1, Model 1) for a single environment (Table 3.5). The mathematical model for a single environment described by Griffing (1956) was as follows:

$$x_{ij} = \mu + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{bc}\sum_k\sum_j e_{ijkl}$$

where μ is the population mean, g_i (g_j) is the general combining ability effects for the i^{th} (j^{th}) parents, s_{ij} is the specific combining ability effect for the cross between the i^{th} and j^{th} parents, r_{ij} is the reciprocal effect involving reciprocal crosses between i^{th} and j^{th} parents and e_{ijkl} is the environmental effect associated with the $ijkl^{th}$ individual observation.

Table 3.5: Format of ANOVA for GCA, SCA and reciprocal effects according to Griffing's Method 1, Model 1 (Griffing, 1956)

Source	D. F.	Mean Squares	Expected Mean Squares

GCA	$p - l$	MS_g	$\sigma^2 + 2\,p\left(\frac{1}{p-1}\right)\sum {g_i}^2$
SCA	$[p(p-1)]/2$	MS_s	$\sigma^2 + \frac{2}{p\,(p-1)}\sum_i\sum_j {s_{ij}}^2$
Reciprocal effect (R)	$[p(p-1)]/2$	MS_R	$\sigma^2 + 2\left(\frac{2}{p(p-1)}\right)\sum_{i<j}\sum {R_{ij}}^2$
Error	m	MS_e	$\sigma^2 e$

where: MS_g = general combining ability mean square, MS_S = specific combining ability mean square, MS_R = reciprocal effect mean square, MSe = error mean square, σ^2_e = variance due to error and p = number of parents

3.8.2 Estimation of heritability

Broad sense heritability (h^2_b) and narrow sense heritability (h^2_n) were estimated across environments using the formula by Teklewold and Becker (2005) below:

$$\sigma_{2scaenv} = \frac{MSscaenv}{MSe}$$

$$\sigma_{2gcaenv} = \frac{MSgcaenv}{MSscaenv}$$

$$\sigma^2_{sca} = \frac{MSsca}{MSscaenv}$$

$$\sigma^2_{gca} = \frac{MSgca+MSscaenv}{MSsca+MSgcaenv}$$

$$h_{2b} = \frac{(2\sigma^2gca+\sigma^2sca)}{2\sigma^2gca+\sigma^2sca+\left(\frac{2\sigma^2gca}{env}\right)+\left(\frac{\sigma^2sca}{env}\right)+\left(\frac{\sigma^2e}{renv}\right)}$$

$$h_{2n} = \frac{2\sigma^2gca}{2\sigma^2gca+\sigma^2sca+\left(\frac{2\sigma^2gca}{env}\right)+\left(\frac{\sigma^2sca}{env}\right)+\left(\frac{\sigma^2e}{renv}\right)}$$

where: σ^2_{gca} is the genetic variance due to general combining ability effect, σ^2_{sca} is the genetic variance due to specific combining ability effect, σ^2_{gcaenv} = genetic variance due to general combining ability effect by environment interaction, σ^2_{scaenv} = genetic variance due to specific combining ability effect by environment interaction, MS_{gca} = general

combining ability mean square, MS_{sca} = specific combining ability mean square, MS_{gcaenv} = general combining ability by environment interaction mean square, MS_{scaenv} = specific combining ability by environment interaction mean square, MS_e = mean square error, env = number of environments and r = number of replications.

3.8.3 Estimation of heterosis

The least square means for some trait was used to estimate heterosis in F1 over midparent and high parent according to Rai (1979).

Mid-parent heterosis (MPH) = $\frac{F1-MP}{MP} \times 100$, MP = $\frac{P1+P2}{2}$

High Parent Heterosis (HPH) = $\frac{F1-HP}{HP} \times 100$

"T" test was then performed to know whether the F1 hybrid means were significantly different from the mid-parent and high parent means as described by Wynne *et al.* (1970).

"t" for MPH = $\frac{F1-MP}{\sqrt{3}/2r(EMS)}$

"t" for HPH = $\frac{F1-HP}{\sqrt{2}/r(EMS)}$

where: F1 = mean of the hybrid, MP (mid-parent) = average of the two inbred parents, P1 and P2 = mean of the inbred parents, HP = mean of the high inbred parent, r = number of replications and EMS = error mean square.

3.8.4 Estimation of correlation co-efficients

Correlation analysis across environments was performed between MSVD severity mean score and some of the agronomic traits using Statistix version 9.1.

CHAPTER FOUR

4.0 RESULTS

Twenty-seven genotypes including two commercial varieties were evaluated for resistance to maize streak virus disease under natural and artificial infestations in Wenchi and Kwadaso respectively. The objectives were to determine the combining ability, heterosis, heritability of maize streak virus resistance and other agronomic traits and also the phenotypic correlation between maize streak virus disease severity mean score and some agronomic traits, different statistical analyses were conducted and the results are presented herein.

4.1 Analysis of variance for diallel crosses under natural infestation, artificial infestation and across the two environments.

The combined ANOVA showed significant effects ($P<0.001$) for environment and genotype for all the traits measured (Table 4.1). Also, significant effects were observed for genotype by environment interaction for maize streak virus disease severity mean score, anthesis-silking interval, ear leaf area, plant aspect and ear aspect. General combining ability (GCA) was significant ($P<0.001$) for all the traits but not significant for anthesis-silking interval and grain yield while specific combining ability (SCA) was significant ($P<0.001$) for all the traits. Significant GCA by environment interaction was detected for maize streak virus disease severity mean score, ear leaf area, plant aspect and 100-grain weight but not for others while significant SCA by environment interaction was observed for maize streak virus disease severity mean score, anthesissilking interval, plant height, plant aspect and ear aspect (Table 4.1). Out of all the measured traits, only plant aspect and anthesis-silking interval showed significant

($P<0.05$) effect for reciprocal and reciprocal by environment interaction respectively.

Baker‟s ratio was more than 0.5 for maize streak virus disease severity mean score, plant aspect and ear aspect; equal to 0.5 for total leaf count but less than 0.5 for others (Table 4.1).

Under natural infestation, highly significant effects were observed for the genotype for all the traits measured (Table 4.2). GCA and SCA were significant for almost all the traits except for anthesis-silking interval, ear leaf area and grain yield which did not show significant effects for GCA and also, only maize streak virus disease severity mean score was not significant for SCA (Table 4.2). Significant ($P<0.05$) reciprocal effects were observed for anthesis-silking interval and plant aspect which was similar to what was observed across the environments (Table 4.2).

Similar significant effects were observed for genotype for all the traits measured as in under natural infestation and across environments (Table 4.3). GCA was significant ($P<0.001$) for all the traits but not for anthesis-silking interval and grain yield while the SCA was highly significant for all the traits except for plant aspect (Table 4.3).

Table 4.1: Mean squares from the combined ANOVA of 5*5 diallel crosses and Baker's ratios for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield across natural and artificial infestations

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield
Env	1	3.53***	73.24***	22.44***	15277.62***	130498.19***	13.00***	21.99***	898.09***	84.83***
Genotype	24	0.69***	5.05***	1.83***	1470.69***	33167.21***	1.52***	1.62***	53.13***	12.58***
GCA	4	2.49***	1.62	7.49***	2084.33***	26486.22***	2.85***	4.14***	56.19***	1.67
SCA	10	0.60***	11.08***	2.33***	3139.90***	71571.62***	1.79***	2.60***	100.82***	29.83***
Reciprocal	10	0.12	1.16	0.17	75.77	2806	0.70*	0.56	6.68	1.07
Genotype*Env	24	0.30***	1.83**	0.42	85.51	3850.61**	0.93***	0.59*	6.68	2.11
GCA*Env	4	0.38**	0.11	0.79	44.14	15639.62***	1.32**	0.04	22.72**	1.79
SCA*Env	10	0.42***	2.53**	0.46	150.40*	3292.74	1.39***	1.04**	6.01	3.53
Reciprocal*Env	10	0.13	1.56*	0.31	51.94	1962.25	0.27	0.56	2.81	2.20
Error	84	0.086	0.724	0.324	60.358	1674.337	0.280	0.318	4.376	2.120
Baker"s ratio		0.80	0.15	0.50	0.06	0.03	0.68	0.61	0.06	0.04

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$

GCA: General combining ability, SCA: Specific combining ability, Env: Environment

Table 4.2: Mean squares from the ANOVA of 5*5 diallel crosses for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under natural infestation

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield

Genotype	24	0.20***	1.99**	1.13***	864.46***	12143.76***	1.39***	0.97***	32.96***	10.38***
GCA	4	0.70***	0.97	3.67***	1364.60***	835.36	0.59*	2.06***	36.51***	0.53
SCA	10	0.09	3.09**	1.40***	1836.03***	30823.93***	2.58***	1.43***	59.99***	25.61***
Reciprocal	10	0.04	2.12*	0.26	53.79	1825.31	0.42*	0.45	2.40	1.44
Error	42	0.042	0.834	0.192	47.067	1112.169	0.185	0.317	4.594	2.156

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$

GCA: General combining ability, SCA: Specific combining ability

Table 4.3: Mean squares from the ANOVA of 5*5 diallel crosses for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under artificial infestation

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield
Genotype	24	0.80***	4.89***	1.12**	691.74***	24874.07***	1.06**	1.24***	26.84***	4.31*
GCA	4	2.17***	0.76	4.61***	763.88***	41290.48***	3.57***	2.12***	42.39***	2.93
SCA	10	0.93***	10.52***	1.39**	1454.27***	44040.43***	0.60	2.22***	46.83***	7.75**
Reciprocal	10	0.21	0.60	0.22	73.92	2942.94	0.55	0.67*	7.08	1.83
Error	42	0.131	0.615	0.456	73.648	2236.504	0.376	0.319	4.158	2.085

*Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$

GCA: General combining ability, SCA: Specific combining ability

4.2 General combining ability (GCA) effects of parental inbred lines, specific combining ability (SCA) and reciprocal effects of hybrids and the means of hybrids, parental inbred lines and checks for maize streak virus disease severity mean score and other agronomic traits across environments

4.2.1 Maize streak virus disease severity (MSVDS) mean score

Generally, negative GCA effects were associated with resistance and positive effects to susceptibility. Parents TZEI-7 (-0.23) and TZEI-22 (-0.21) had significant and negative GCA effects while others had positive GCA effects (Table 4.4). The single cross hybrids which expressed MSVD resistance in terms of their SCA effects includes TZEI4*TZEI-31 (-0.37), TZEI-4*TZEI-22 (-0.20), TZEI-22*TZEI-4 (-0.14), TZEI-31*TZEI-4 (-0.08), TZEI-22*TZEI-157 (-0.06), TZEI-31*TZEI-22 (-0.06), TZEI31*TZEI-7 (-0.01) and TZEI-157*TZEI-4 (0.01) (Table 4.5). The SCA effects of the first two hybrids were significant. In most of these hybrids, one of both parents had corresponding negative GCA effect except for TZEI-4*TZEI-31, TZEI-31*TZEI-4 and TZEI-157*TZEI-4.

Inbred line TZEI-7 had the lowest mean value for maize streak virus disease severity mean score and it was significantly different ($P<0.05$) from the other parents. The highest mean value was observed for inbred TZEI-4 (Table 4.6). This result supported the highest negative and highest positive GCA effects for TZEI-7 and TZEI-4 respectively. Hybrids TZEI-22*TZEI-7 (2.32), TZEI-7*TZEI-22 (2.48), TZEI-4*TZEI22 (2.48), TZEI-157*TZEI-22 (2.54), TZEI-157*TZEI-7 (2.64), TZEI-22*TZEI-157 (2.67), TZEI-22*TZEI-4 (2.70), TZEI-4*TZEI-31 (2.71), TZEI-7*TZEI-157 (2.72), TZEI-7*TZEI-31 (2.72), TZEI-22*TZEI-31 (2.75), TZEI-31*TZEI-7 (2.79), TZEI-7*TZEI-4 (2.81), TZEI-31*TZEI-4 (2.86) and TZEI-31*TZEI-22 (2.94) can be

Table 4.4: General combining ability (GCA) effects of parental inbred lines for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100grain weight (HGW) and grain yield across natural and artificial infestations

Parents	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain yield (t/ha)
TZEI-4	0.19***	0.03	-0.47***	-8.82***	-34.63***	0.08	0.20*	-0.16	-0.29
TZEI-7	-0.23***	-0.01	0.42***	0.58	16.62*	-0.16	0.16	1.26**	0.10
TZEI-22	-0.21***	0.26	0.09	3.32	11.43	-0.29**	0.03	0.70	0.06
TZEI-31	0.19***	-0.12	0.20*	-1.88	-5.02	0.24	0.06	-0.86*	0.01
TZEI-157	0.06	-0.16	-0.23*	6.81***	11.59	0.13	-0.45***	-0.95*	0.12
SE (gi)	0.071	0.038	0.103	0.767	14.441	0.133	0.022	0.550	0.15
SE (gi-gj)	0.112	0.060	0.162	1.213	22.832	0.210	0.035	0.870	0.24

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error

Table 4.5: Specific combining ability (SCA) effects of F1 hybrids for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield across natural and artificial infestations

F1 hybrids	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4*TZEI-7	0.24*	0.72*	-0.02	11.52**	40.35**	-0.03	-0.15	0.99	0.45
TZEI-4*TZEI-22	-0.20*	0.04	0.19	12.32***	43.13**	-0.31	-0.35	1.59	1.09*

TZEI-4*TZEI-31	-0.37***	0.42	-0.23	2.45	33.82*	-0.18	-0.05	0.69	0.60
TZEI-4*TZEI-157	0.10	2.60***	-0.32	30.64***	176.84***	-0.30	-0.43	3.51*	2.03*
TZEI-7*TZEI-22	0.08	0.07	0.37*	-2.12	5.86	-0.16	-0.06	0.16	0.26
TZEI-7*TZEI-31	0.02	0.21	0.25	8.31*	46.61**	-0.36*	-0.10	1.86*	0.63
TZEI-7*TZEI-157	0.44*	1.32*	1.37***	40.80***	184.59***	-1.20***	-1.13**	9.13***	4.78***
TZEI-22*TZEI-31	0.10	1.19***	0.19	5.00	29.78*	-0.06	0.04	1.83*	0.49
TZEI-22*TZEI-157	-0.06	2.42***	1.23***	26.57***	120.05***	-0.75*	-0.60	5.78**	2.60**
TZEI-31*TZEI-157	0.09	1.37*	0.66	39.36***	195.81***	-0.88*	-1.40***	6.06***	3.88***
TZEI-7*TZEI-4	0.21	0.08	0.06	-0.94	15.88	0.33	0.17	0.04	-0.11
TZEI-22*TZEI-4	-0.14	-0.17	0.12	0.35	25.00	-0.42	-0.17	0.59	0.12
TZEI-22*TZEI-7	0.04	-0.50	0.32	2.28	9.45	0.00	-0.25	-0.83	0.01
TZEI-31*TZEI-4	-0.08	0.33	-0.05	-0.25	-21.34	-0.08	0.17	0.30	-0.46
TZEI-31*TZEI-7	-0.01	-0.08	-0.09	-4.59	-17.19	0.00	-0.08	-1.97	-0.56
TZEI-31*TZEI-22	-0.06	0.50	-0.03	-1.68	15.85	0.00	0.08	0.05	-0.21
TZEI-157*TZEI-4	-0.01	0.42	-0.04	0.67	-8.20	-0.08	0.25	0.36	-0.18
TZEI-157*TZEI-7	0.07	-0.33	-0.11	4.34	9.79	0.42	-0.25	0.26	0.14
TZEI-157*TZEI-22	0.07	0.00	-0.02	3.71	9.96	0.00	-0.42	-0.52	0.42
TZEI-157*TZEI-31	0.12	0.17	-0.01	0.09	10.36	0.33	-0.08	-0.32	-0.25
SE (sij)	0.153	0.379	0.162	2.919	13.660	0.281	0.243	0.583	0.448
SE (sij-sik)	0.235	0.581	0.249	4.478	20.953	0.430	0.373	0.895	0.686
SE (rij-rkl)	0.147	0.510	0.227	2.942	18.084	0.211	0.305	0.685	0.606

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error

Table 4.6: Means of F1 hybrids, parental inbred lines and checks for maize streak virus disease severity (MSVDS) mean score, anthesissilking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield across natural and artificial infestations

	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield

										F1 hybrids
TZEI-4*TZEI-7	3.21	3.46	11.85	100.72	384.99	3.49	2.75		4.77	(1-5)
										(days)
TZEI-4*TZEI-22	2.48	2.81	11.93	104.10	390.23	2.44	2.19	20.81	5.16	(cm)
TZEI-4*TZEI-31	2.71	2.99	11.31	89.75	318.46	3.47	2.72	17.71	4.05	(cm^2)
TZEI-4*TZEI-157	3.21	3.14	11.04	98.30	345.12	3.58	2.36	17.28	3.94	(1-5)
TZEI-7*TZEI-22	2.48	1.97	12.95	100.26	379.27	2.69	2.09	19.33	5.16	(1-5)
TZEI-7*TZEI-31	2.72	2.31	12.49	102.11	388.11	3.02	2.28	18.17	4.79	(g)
TZEI-7*TZEI-157	2.72	2.11	12.37	120.86	431.88	3.27	1.39	22.01	6.85	20.35
(t/ha) TZEI-22*TZEI-31	2.75	4.06	12.21	105.90	407.05	2.97	2.31	20.38	5.40	
TZEI-22*TZEI-157	2.67	2.80	11.95	115.19	387.74	3.08	1.32	18.42	5.63	
TZEI-31*TZEI-157	3.34	2.13	12.24	112.97	406.24	3.89	1.33	16.97	5.30	
TZEI-7*TZEI-4	2.81	3.23	11.93	102.62	359.67	2.73	2.40	20.94	5.00	
TZEI-22*TZEI-4	2.70	3.05	11.59	106.30	349.61	2.94	2.29	20.55	5.79	
TZEI-22*TZEI-7	2.32	3.05	12.44	100.12	377.67	2.53	2.55	21.67	5.37	
TZEI-31*TZEI-4	2.86	2.62	11.53	89.86	360.49	3.55	2.48	17.65	5.31	
TZEI-31*TZEI-7	2.79	2.48	12.78	108.06	408.32	3.14	2.52	22.16	5.79	
TZEI-31*TZEI-22	2.94	3.07	12.26	106.55	362.95	3.04	2.16	20.29	5.78	
TZEI-157*TZEI-4	3.23	2.41	11.13	99.21	368.22	3.59	1.76	17.19	4.58	
TZEI-157*TZEI-7	2.64	2.59	12.60	111.59	405.45	2.49	1.77	21.66	6.39	
TZEI-157*TZEI-22	2.54	2.90	12.00	110.01	374.52	2.93	2.06	20.10	5.06	
TZEI-157*TZEI-31	3.04	1.77	12.22	113.71	385.21	3.16	1.45	17.79	6.15	
Parents										
TZEI-4	3.25	0.70	11.27	53.21	136.81	3.67	3.19	15.36	2.30	
TZEI-7	1.88	0.95	11.61	70.82	242.86	3.80	3.25	14.49	1.91	
TZEI-22	2.33	0.78	11.06	84.13	269.97	3.35	2.81	14.80	2.59	
TZEI-31	3.18	0.46	11.80	67.85	182.26	4.57	3.18	10.99	1.74	
TZEI-157	2.55	0.84	10.68	77.93	217.57	4.20	2.56	10.72	1.26	
Checks										
Grand mean	2.767	2.377	11.886	98.642	349.42	3.265	2.284		4.665	
LSD (0.05)	0.344	0.941	0.643	8.930	46.855	0.594	0.648	2.466	1.646	
CV (%)	5.61	16.15	4.72	7.89	11.69	15.86	11.87	11.69	15.35	

R^2	0.79	0.82	0.78	0.92	0.89	0.78	0.74	0.87	0.80		
Aburohemaa	2.76	2.66	11.87	105.02	373.72	3.31	2.20	18.37	4.28		
Omankwa	2.62	2.82	11.80	106.18	420.06	3.25	2.29	5.59		20.63	18.399

LSD: Least Significant Difference, CV: Co-efficient of Variation, R^2: Co-efficient of Determination

categorized as moderately resistant while TZEI-157*TZEI-31 (3.04), TZEI-4*TZEI-7 (3.21), TZEI-4*TZEI-157 (3.21), TZEI-157*TZEI-4 (3.23) and TZEI-31*TZEI-157 (3.34) can be categorized as susceptible. Only the mean score of TZEI-22*TZEI-7 (2.32) was significantly different from that of Aburohemaa (2.76) but no significant difference was observed when the hybrid was compared to the mean score of Omankwa (2.62) at $P<0.05$.

4.2.2 Total leaf count

Significant and positive GCA effects were observed for inbred lines TZEI-7 (0.42) and TZEI-31 (0.20). TZEI-22 had a positive GCA effect of 0.09 but not significant (Table 4.4). TZEI-7*TZEI-157 (1.37), TZEI-22*TZEI-157 (1.23) and TZEI-7*TZEI-22 (0.37) had significant and positive SCA effects.

The mean of the total leaf count for inbred TZEI-31 (11.80) was the highest, this was not significantly different from that of inbred lines TZEI-7 (11.61) and TZEI-4 (11.27) (Table 4.6). TZEI-157 (10.68) had a mean total leaf count which is 1.12 less than the mean of TZEI-31. For the hybrids, the mean total leaf count ranged from TZEI157*TZEI-4 (11.13) to TZEI-7*TZEI-22 (12.95). The mean of the hybrid producing the highest number of total leaf count was significantly higher ($P<0.05$) than that of the two checks.

4.2.3 Plant height

TZEI-157 (6.81) had a significant ($P<0.001$) and positive GCA effect but negative and significant effects were recorded for TZEI-4 (-8.82) and TZEI-31 (-1.88) (Table 4.4). Table 4.5 revealed that the following hybrids TZEI-7*TZEI-157 (40.80), TZEI31*TZEI-157 (39.36), TZEI-4*TZEI-157 (30.64), TZEI-22*TZEI-157 (26.57), TZEI4*TZEI-22 (12.32), TZEI-4*TZEI-7 (11.52) and TZEI-7*TZEI-31 (8.31) had significant and positive SCA effects. TZEI-157 appeared to be a good combiner for taller plant heights (as

observed in its GCA effect) because its combination with other inbred lines in straight crosses gave the highest SCA effects.

TZEI-22 (84.13 cm) had the highest plant height which was not significantly different from that of TZEI-157 (77.93 cm) but it differed significantly ($P<0.05$) from the mean values of TZEI-7 (70.82 cm), TZEI-31 (67.85 cm) and TZEI-4 (53.21 cm) (Table 4.6). Hybrid TZEI-7*TZEI-157 (120.86 cm) had the highest plant height across environments but its mean value was not significantly different ($P<0.05$) from the plant heights of TZEI-22*TZEI-157 (115.19 cm) and TZEI-157*TZEI-31 (113.71 cm) (Table 4.6). The plant height of TZEI-4*TZEI-31 (89.75 cm) was 31.11 less than the height observed for TZEI-7*TZEI-157.

4.2.4 Ear leaf area

In terms of significant and positive GCA effects, TZEI-7 (16.62) had the highest value, distantly followed by TZEI-157 (11.59) and TZEI-22 (11.43) but were not significant. TZEI-4 (-34.63) and TZEI-31 (-5.02) had negative GCA effects, however, these parents gave a positive SCA effect in its straight cross (Table 4.4). All the straight crosses had significant and positive SCA effects except for TZEI-7*TZEI-22 which was not significant.

The mean value of inbred TZEI-22 (269.97 cm^2) was not significantly ($P<0.05$) different from inbred TZEI-7 (242.86 cm^2) (Table 4.6). TZEI-7*TZEI-157 (431.88 cm^2) had the highest ear leaf area followed by TZEI-31*TZEI-7 (408.32 cm^2), TZEI22*TZEI-31 (407.05 cm^2), TZEI-31*TZEI-157 (406.24 cm^2), TZEI-157*TZEI-7 (405.45 cm^2), TZEI-4*TZEI-22 (390.23 cm^2), TZEI-7*TZEI-31 (388.11 cm^2) and TZEI-22*TZEI-157 (387.74 cm^2); their means were not significantly different from one another at $P<0.05$ (Table 4.6). It was found that either one or both of the parents involved in the crosses had positive GCA effects (Table 4.4).

4.2.5 Plant aspect

Significant ($p<0.01$) and negative GCA effect were detected for inbred line TZEI-22 (0.29), but TZEI-7 had negative GCA effect but not signifcant. Positive GCA effects were detected for TZEI-4 (0.08), TZEI-157 (0.13) and TZEI-31 (0.24) (Table 4.4). All the straight crosses and few of the reciprocal crosses which include TZEI-22*TZEI-4 (0.42), TZEI-157*TZEI-4 (-0.08) and TZEI-31*TZEI-4 (-0.08) had negative SCA effects, but the hybrids with significant effects were TZEI-7*TZEI-157 (-1.20), TZEI31*TZEI-157 (-0.88), TZEI-22*TZEI-157 (-0.75) and TZEI-7*TZEI-31 (-0.36) (Table 4.5). It is interesting to know that some of the reciprocal crosses had zero SCA effects.

The worst parent (TZEI-31) and the best parent (TZEI-22) in terms of their mean values also had the highest and smallest GCA effects, respectively (Table 4.6). Majority of the hybrids had a good overall phenotypic appeal based on the scale used for the scoring (Table 4.6).

4.2.6 Ear aspect

Parental inbred line TZEI-157 (-0.45) had a negative and significant ($P<0.001$) GCA effect while others had positive effects (Table 4.4). Significant and negative SCA effects were observed for TZEI-31*TZEI-157 (-1.40) and TZEI-7*TZEI-157 (-1.13) (Table 4.5). Although, the SCA effects of most of the hybrids in which TZEI-157 was involved had non-significant negative effects except for TZEI-157*TZEI-4 (0.25) which had positive effect.

The lowest mean score was observed for TZEI-157 (2.56) and the highest for TZEI-7 (3.25) (Table 4.6). The mean score for hybrids ranged from 1.32 to 2.75.

4.2.7 Grain yield

Table 4.4 shows that the GCA effects of inbred lines TZEI-157 (0.12), TZEI-7 (0.10),

TZEI-22 (0.06) and TZEI-31 (0.01) were positive but not significant. Hybrids TZEI7*TZEI-157 (4.78), TZEI-31*TZEI-157 (3.88), TZEI-22*TZEI-157 (2.60), TZEI 4*TZEI-157 (2.03) and TZEI-4*TZEI-22 (1.09) arranged in the decreasing order of their positive SCA effects were significant (Table 4.5). Some of the reciprocal crosses had negative SCA effects.

TZEI-22 (2.59 t/ha) had the highest grain yield, followed by TZEI-4 (2.30 t/ha), TZEI-7 (1.91 t/ha), TZEI-31 (1.74 t/ha) and TZEI-157 (1.26 t/ha) (Table 4.6). Grain yields of TZEI-7*TZEI-157 (6.85 t/ha), TZEI-157*TZEI-7 (6.39 t/ha) and TZEI-157*TZEI-31 (6.15 t/ha) were significantly ($P<0.05$) different from that of Aburohemaa (4.28 t/ha) but not from Omankwa (5.59).

4.3 Heterosis estimates for some measured traits under natural and artificial infestations

The mid-parent heterosis (MPH) and high parent heterosis (HPH) for maize streak virus disease severity mean score were significant and negative for hybrids TZEI-4*TZEI-31 (MPH = -15.83, HPH = -14.92) and TZEI-31*TZEI-4 (MPH = -11.17, HPH = 10.21) but the HPH for TZEI-31*TZEI-4 was not significant ($P<0.05$) (Table 4.7). TZEI4*TZEI-22 (-11.31), TZEI-22*TZEI-4 (-3.26) and TZEI-22*TZEI-31 (-0.41) had negative MPH but were not significant ($P<0.05$). All the hybrids had significant and positive MPH and HPH for plant height, ear leaf area, 100-grain weight and grain yield except for TZEI-4*TZEI-31 and TZEI-4*TZEI-157 which did not show significant effect for HPH of grain yield and also TZE-4*TZEI-22, TZEI-4*TZEI-31, TZEI22*TZEI-7, TZEI-31*TZEI-22 and TZEI-157*TZEI-31 for 100-grain weight.

4.4 Heritability estimates for measured traits under natural and artificial infestations

Narrow sense heritability for the considered traits ranged from 3.14 to 55.3 % (Table

4.8). The highest and the lowest narrow sense heritabilities were observed for maize streak virus disease severity mean score and grain yield, respectively. Broad sense heritability for maize streak virus disease severity mean score (68.84 %), anthesissilking interval (72.80 %), total leaf count (80.36 %), plant height (65.76 %), ear aspect (78.77 %), 100-grain weight (60.06 %) and grain yield (83.81 %) were high while that of plant aspect (53.59 %) was moderate (Table 4.8).

4.5 Pearson correlation co-efficients among measured traits across natural and artificial infestations

Maize streak virus disease severity mean score correlated significantly ($P<0.01$) and negatively with total leaf count ($r = -0.22, -0.18$), plant height ($r = -0.23, -0.19$) and 100-grain weight ($r = -0.24, -0.18$) (Table 4.9). However, negative but not significant correlation was observed between maize streak virus disease severity mean score and ear leaf area (-0.03), grain yield (-0.10). Grain yield correlated significantly ($P<0.001$) and positively with total leaf count ($r = 0.43$), plant height ($r = 0.74$), ear leaf area ($r = 0.47$) and 100-grain weight ($r = 0.74$) (Table 4.9).

Table 4.7: Heterosis estimates of F1 hybrids for maize streak virus disease severity (MSVDS) mean score, plant height (PLHT), ear leaf area (ELA), 100-grain weight (HGW) and grain yield across natural and artificial infestations

	MSVDS		PLHT		ELA		HGW		Grain Yield	
F1 hybrids	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
TZEI-4*TZEI-7	25.22**	71.06***	62.41***	42.22***	102.80***	58.52***	36.36***	32.49***	126.58**	107.25*
TZEI-4*TZEI-22	-11.31	6.17***	51.59***	23.74**	91.86***	44.55**	38.01***	35.52	111.35**	99.67*
TZEI-4*TZEI-31	-15.83**	-14.92*	48.27***	32.27**	99.62***	74.73***	34.43**	15.31	100.41*	76.11
TZEI-4*TZEI-157	10.73	25.91**	49.91***	26.13**	94.77***	58.63***	32.51**	12.49**	121.21*	71.15
TZEI-7*TZEI-22	17.66*	31.91**	29.41***	19.17*	47.91****	40.49**	31.97**	30.56*	129.93**	99.78*
TZEI-7*TZEI-31	7.47	44.84***	47.27***	44.19***	82.59***	59.81***	42.62***	25.40***	162.56**	151.18*
TZEI-7*TZEI-157	23.02**	45.12***	62.50***	55.08***	87.60***	77.83***	74.67***	51.94***	332.53***	259.00***
TZEI-22*TZEI-31	-0.41	17.75**	39.35***	25.87**	80.02***	50.78***	57.99***	37.64*	149.55**	108.84*
TZEI-22*TZEI-157	9.39	14.54	42.16***	36.92***	59.06***	43.63**	44.34***	24.42***	192.88**	117.74*
TZEI-31*TZEI-157	16.45*	30.84**	54.99***	44.96***	103.20***	86.72***	56.33***	54.39***	253.54***	204.52**
TZEI-7*TZEI-4	9.48	49.55***	65.47***	44.90***	89.47***	48.10**	40.29***	36.31**	137.92**	117.62*
TZEI-22*TZEI-4	-3.26	15.82	54.79***	26.34**	71.89***	29.50*	36.24***	33.78***	136.86**	123.77**
TZEI-22*TZEI-7	10.16	23.51*	29.23***	19.00*	47.29**	39.90**	47.97***	46.39	139.15**	107.79*
TZEI-31*TZEI-4	-11.17*	-10.21	48.44***	32.43**	125.96***	97.78***	34.00**	14.94***	162.76**	130.90*
TZEI-31*TZEI-7	10.29	48.64***	55.84***	52.58***	92.09***	63.13***	73.96***	52.96***	217.51***	203.75**
TZEI-31*TZEI-22	6.51	25.94**	40.21***	26.64**	60.51***	34.44**	57.30***	37.04	167.17**	123.59**
TZEI-157*TZEI-4	11.39	26.66**	51.29***	27.29**	107.81***	69.24***	31.82**	11.90***	157.57**	99.28*
TZEI-157*TZEI-7	19.15*	40.56**	50.03***	43.18***	76.12***	66.95***	71.89***	49.52**	303.69***	235.07***
TZEI-157*TZEI-22	3.99	8.89	35.76***	30.76***	53.64***	38.73**	57.48***	35.75***	163.51**	95.90*
TZEI-157*TZEI-31	6.14	19.26*	55.99***	45.90***	92.68***	77.05***	63.94***	61.91	309.72***	252.91***

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$

MPH: Mid-Parent Heterosis, HPH: High Parent Heterosis

Table 4.8: Narrow sense and Broad sense heritabilities of maize streak disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield across natural and artificial infestations

Traits	MSVDS	ASI	TLC	PLHT	PASP	EASP	HGW	Grain Yield
Narrow sense heritability (%)	55.30	10.57	40.44	4.14	36.37	48.16	3.40	3.14
Broad sense heritability (%)	68.84	72.80	80.36	65.76	53.59	78.77	60.06	83.81

Table 4.9: Correlation co-efficients among maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield for the twenty-seven genotypes evaluated across natural and artificial infestations

Traits	MSVDS	TLC	PLHT	ELA	EASP	HGW
TLC	-0.2216**					
PLHT	-0.2270**	0.5532***				
ELA	-0.0345	0.2536**	0.5061***			
EASP	0.0463	-0.2868***	-0.6615***	-0.2637***		
HGW	-0.2371**	0.5232***	0.7052***	0.3491***	-0.4069***	
Grain yield	-0.1039	0.4344***	0.7428***	0.4677***	-0.5852***	0.7404***

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$

4.6 Detection of maize streak virus (MSV)

MSV detection by polymerase chain reaction (PCR) using two SSR markers (MSV11 and MSV13) was carried out on the 27 maize genotypes infested with MSV naturally and artificially. They produced good quality amplification products which were seen as the clear and sharp bands for MSV11 (Plates 4.1a and b) but not for MSV13. The amplicons were observed at a range of 220-254 bp across the environments.

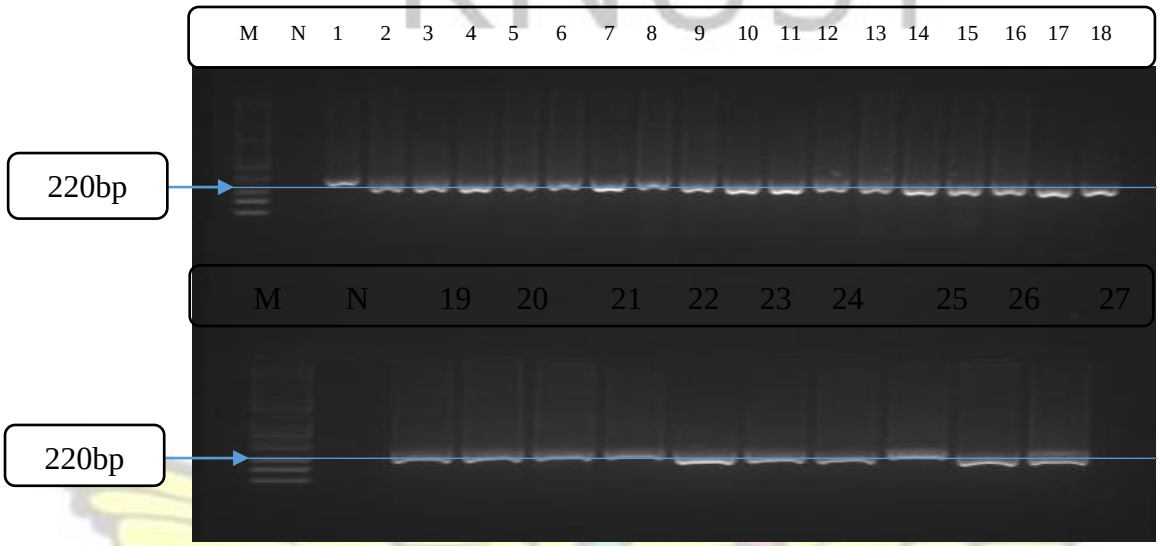

Plate 4.1a: MSV naturally infested maize plants

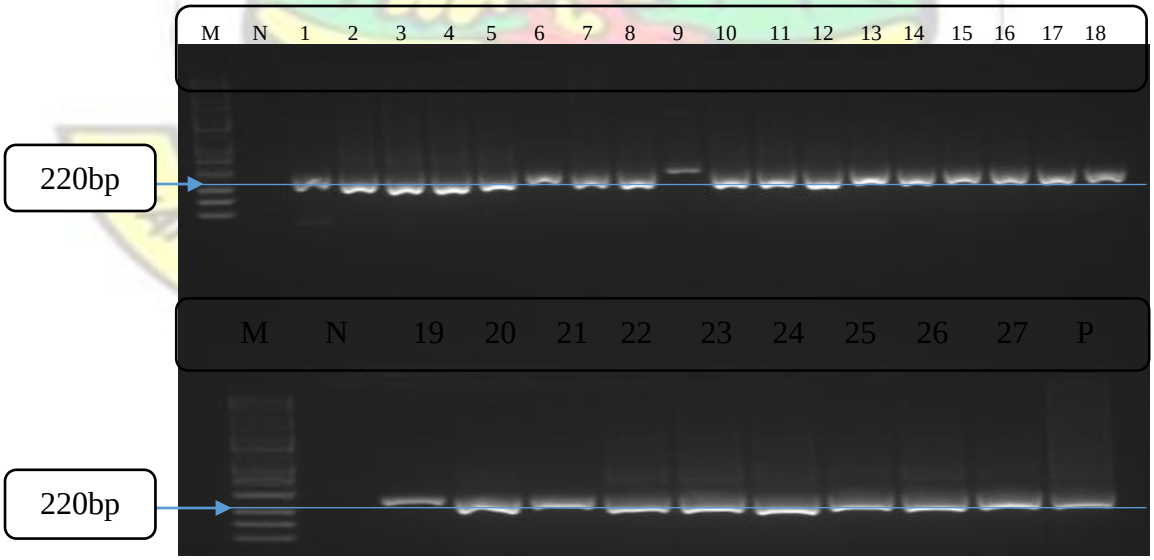

Plate 4.1b: MSV artificially infested maize plants

Plates 4.1a and 4.1b: PCR amplification profiles of 27 genotypes with SSR marker (MSV11).

bp = base pair, M = molecular marker (100 ng/µl), N = Negative control, P = Positive control (LA-3), 1 = TZEI-22*TZEI-22, 2 = TZEI-7*TZEI-7, 3 = TZEI-31*TZEI-31, 4 = TZEI-157*TZEI-157, 5 = TZEI-4*TZEI-4, 6 = TZEI-157*TZEI-31,7 = TZEI-7*TZEI22, 8 = TZEI-7*TZEI-157, 9 = TZEI-22*TZEI-157, 10 = TZEI-31*TZEI-157, 11 = TZEI-4*TZEI-157, 12 = Aburohemaa,13 = TZEI-31*TZEI-4, 14 = TZEI-4*TZEI-22, 15 = TZEI-4*TZEI-31, 16 = Omankwa, 17 = TZEI-31*TZEI-7, 18 = TZEI-7*TZEI-31, 19 = TZEI-4*TZEI-7, 20 = TZEI-22*TZEI-4, 21 = TZEI-157*TZEI-7, 22 = TZEI31*TZEI-22, 23 = TZEI-22*TZEI-31, 24 =TZEI-7*TZEI-4, 25 = TZEI-157*TZEI-22, 26 = TZEI-157*TZEI-4, 27 = TZEI-22*TZEI-7.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Performances of parental inbred lines and hybrids

Significant effects observed for genotype and environment for all the traits measured showed that enough genetic differences were present amongst the genotypes and that the environments were distinct. This would then permit effective progress to be made from selection for MSVD resistance and yield. The observed significant genotype by environment interaction mean square for maize streak virus disease severity mean score explained that the response of genotypes to MSV differed across environments, suggesting that there was an uneven transmission of MSV by the vectors to all the genotypes, resulting from escapes under natural infestation. This led to higher disease pressure under artificial infestation as compared to natural infestation which was revealed by the maize streak virus disease severity mean score for each environment. Bosque-Pérez *et al*. (1998) reported that infestation of plant with MSV at early stages leads to greater disease severity. This would then make selection of resistance lines difficult only under natural infestation therefore, stressing the necessity to evaluate inbreds and hybrids under artificial infestation thus, enhancing stable performance and productivity of hybrids. Significant genotype by environment observed for ear leaf area could be as a result of the

differences in quantity of soil macronutrients responsible for large leaf area. Plant aspect scored on the basis of the overall phenotypic appeal of each genotype also differed significantly across environments, this confirms the differences in reaction of all the genotypes to MSVD in which some were showing more unbroken chlorotic stripes, non-uniform plant and ear heights. These differences were influenced by the disease severity (Bosque-Pérez *et al*., 1998).

Maize streak virus disease severity mean score across environments observed for inbreds TZEI-7 and TZEI-22 were low, and were therefore considered as resistant and moderately resistant, respectively according to Beyene *et al*. (2012). The mean of hybrid TZEI-22*TZEI-7 was significantly lower compared to the check "Aburohemaa", even though some other hybrids had lower mean scores which were not significantly different. This suggests that these inbred lines and hybrid have the potential to be used in hybrid maize breeding programmes targeting resistance to MSVD. Bello *et al*. (2012) reported that grain yield is influenced by plant height as a result of dry matter produced by the leaves, therefore, hybrids TZEI-7*TZEI-157 and TZEI-22*TZEI-157 could also be selected for taller plant heights because of their excellent performance across environments. The mean of grain yield across environment for the single cross hybrids was 5.31 t/ha and this was 0.37 t/ha higher than the means of the checks, indicating that some of the hybrids performed significantly better than the checks used in this study.

5.2 General combining ability, specific combining ability and reciprocal effects across environments

Studies on GCA, SCA and reciprocal effects are essential because they reveal the worth of genotypes in hybrid combinations (Mutengwa *et al*., 2012). Significant GCA and SCA mean squares observed for maize streak virus disease severity mean score, total leaf count, plant height, ear leaf area and plant aspect reveal the relative contributions of additive and non-additive gene effects to the expression of these traits. However, grain

yield was solely controlled by non-additive gene effect as revealed by its significant SCA. Substantial breeding progress can be made for these traits using recurrent selection, backcrossing and hybridization methods for population improvement as well as in the development of hybrids and synthetic varieties. Baker‟s ratio for maize streak virus disease severity mean score indicated that additive gene effect was preponderance in the control of MSVD resistance in the genotypes evaluated. This result agrees with those of Vivek *et al*. (2010), Gichuru *et al*. (2011) and Mutengwa *et al*. (2012) who found out that additive gene effects were predominant in the inheritance of resistance to MSVD. Moreover, the preponderance of GCA over SCA variance implied that early generation testing may be efficient for selecting resistant genotypes. High GCA mean square implied that the per se performance of the inbred lines used in this study should be a suitable pointer of the performance of their hybrids, that is, the inbreds transmitted

MSVD resistant genes to the hybrids (Gethi and Smith, 2004; Badu-Apraku *et al*., 2011). There was a noteworthy difference in the combining abilities of the parental inbreds and hybrids under the two environments as shown by the significant GCA by environment and SCA by environment interactions, suggesting that selection for resistance to MVDS should be done in specific target environment. Negative GCA and SCA effects observed for maize streak virus disease severity mean score indicates resistance while a positive effect suggests susceptibility (Owolade *et al*., 2006; Bokmeyer *et al*., 2009). Good general combiners for MSVD resistance were TZEI-7 and TZEI-22, implying that they contributed towards resistance in the single crosses they were involved in. The large negative GCA effects of these inbred lines make them qualify to be used as testers in selection of MSVD resistant genotypes (Pswarayi and Vivek, 2008). Significant SCA effects reveals that the level of resistance of certain hybrids were higher or lower than expected on the basis of the GCA of the two parents involved in the cross (Falconer and Mackay, 1996) and these effects are pinpointing to dominant gene action. One-tenth of

hybrids evaluated which include TZEI-4*TZEI-31 and TZEI-4*TZEI-22 were resistant as revealed by their SCA effects. Despite the positive GCA effects observed for parents TZEI-4 and TZEI-31, the SCA effect observed for the resultant straight cross was negative. This could be as a result of the presence of quantitative trait loci (QTLs) that were too small in effect to be expressed in each of the parents but sufficient to be detected when they combine together.

TLC was controlled by additive and non-additive gene effects as revealed by its Baker"s ratio. Non-significant GCA by environment and SCA by environment interactions revealed that the phenotypic expression of this trait was consistent under natural and artificial infestations for the inbred lines and single cross hybrids, thus selection of genotypes for yield based on total leaf count can be done in any of the environments. TZEI-7 and TZEI-31 contributed favourable genes to a higher number of leaves in the plants as revealed by their GCA effects. These inbreds can be selected for vigour and increased yields because of the large amount of photosynthate produced. TZEI-7*TZEI157, TZEI-22*TZEI-157 and TZEI-7*TZEI-22 expressed a higher number of leaves in terms of their SCA effects.

Non-additive gene effect played more vital role in determining the plant height. This is in agreement with Zare *et al*. (2011) who found a σ^2gca/σ^2sca ratio of 0.15 but in contrast, other researchers Legesse *et al*. (2009) and Gichuru (2013) indicated that variance due to general combining ability was large implying that additive gene effects were preponderant. Significant SCA by environment interaction detected suggests that the heights of the hybrids were fluctuating under different environments possibly due to factors such as soil and climate. The significance is expected because single cross hybrids are responsive to environmental factors (Hallauer *et al*., 2010). TZEI-157 contributed favourable genes towards taller plant height based on its GCA effect. TZEI7*TZEI-157,

TZEI-31*TZEI-157, TZEI-4*TZEI-157, TZEI-22*TZEI-157, TZEI4*TZEI-22, TZEI-4*TZEI-7 and TZEI-7*TZEI-31 could be recommended for taller plant heights because of their SCA effects.

Ear leaf area was more influenced by non-additive gene effect as revealed by Baker‟s ratio. Findings from Zare *et al*. (2011) revealed that additive gene effect was more important than non-additive gene effects, thereby contrasting the result from this study. Aliu *et al*. (2008) observed that the GCA/SCA ratio of leaf area was 0.40. The GCA effects of the parents were not the same in the environments in which they were evaluated, therefore selection of parents should be done specifically for target environments. This difference was also observed by Zare *et al*. (2011) for the set of inbred lines evaluated. Only inbred TZEI-7 contributed favourable genes towards larger ear leaf area. A large ear leaf area is desirable because the energy absorbed by leaves increases with increasing leaf area and this facilitates photosynthetic activities in the plants. The significant and positive SCA effects observed for all the straight cross hybrids but one suggests that exploitation of heterosis for larger ear leaf area by plant breeders is possible by crossing the set of parental inbred lines used in this study.

Plant aspect was largely controlled by additive gene effect as it was clearly shown by Baker‟s ratio, implying that GCA was the major component accounting for the differences among the hybrids evaluated in this study. This concurs with the findings from Menkir and Ayodele (2005). In separate trials, the authors evaluated 96 hybrids and 24 inbreds in five environments with the hybrids produced using the Design II mating design. They found out that GCA accounted for 60 % of the variation observed for plant aspect. The observed significant reciprocal effects for PASP indicated that the trait was under the control of cytoplasmic (maternal) effect. Significant SCA by environment and GCA by environment interactions inferred varying performance of hybrids and inbreds across

environments, which corroborates higher disease pressure recorded under artificial infestation. TZEI-22 had negative GCA effect, thus a good combiner for plant aspect because it could improve the trait by 0.29 unit. On the basis of SCA effects, genotypes with overall good phenotypic appeal that could be incorporated into maize breeding programs targeting resistance to MSVD include TZEI-7*TZEI-157, TZEI-22*TZEI-157 and TZEI-7*TZEI-31.

Grain yield was exclusively controlled by non-additive gene effect, indicating that heterosis could be exploited from crossing the set of parental lines used in the study in order to develop hybrids that are high yielding. It is therefore expedient to assess the parental inbred lines with different testers to be able to identify superior hybrids since the performance of the hybrids cannot be based on GCA alone (Hallauer and Miranda, 1988). This result agrees with Bhatnagar *et al*. (2004). In contrast, Sibiya *et al*. (2013) found out that additive gene effect was more predominant in controlling grain yield. Varying gene action controlling grain yield is dependent on the parents and environment under consideration (Gichuru, 2013). Parental inbred lines TZEI-157, TZEI-7, TZEI-22 and TZEI-31 contributed 0.12, 0.10, 0.06 and 0.01 t/ha to the expression of yields observed in the hybrids. One or both of the parents involved in the following crosses TZEI-7*TZEI-157, TZEI-31*TZEI-157, TZEI-22*TZEI-157, TZEI 4*TZEI-157 and TZEI-4*TZEI-22 had positive GCA effect suggesting that favourable genes were transmitted to the progenies (Badu-Apraku and Oyekunle, 2012). This implies that these hybrids can be used as testers in subsequent breeding programmes.

5.3 Combined heterosis estimates

Plant breeders exploit heterosis by crossing distantly related genotypes in order to achieve an increase in desirable traits as compared to the mid-parent or high parent values. Negative MPH observed in TZEI-4*TZEI-22, TZEI-22*TZEI-31 and TZEI4*TZEI-22

for maize streak virus disease severity mean score indicated resistance. TZEI-4*TZEI-31 and its reciprocal had negative MPH and HPH even though their parents had the worst maize streak virus disease severity mean scores. This could be as a result of the sufficient quantitative trait loci (QTLs) expressed in the hybrid compared to the QTLs present in the parents which are too small in effect. Negative heterosis observed could be due to dominance (Clements *et al*., 2004), an oblique effect of hybrid vigour (Hung and Holland, 2012) and their negative SCA effects (Sprague and Tatum, 1942). For plant height, ear leaf area, 100-grain weight and grain yield; all the hybrids showed significant and positive superiority over the mid-parent and high parent except for the non-significant HPH for TZE-4*TZEI-22, TZEI-4*TZEI-31, TZEI-22*TZEI-7, TZEI-31*TZEI-22 and TZEI-157*TZEI-31 for 100-grain weight and also for the grain yield of TZEI-4*TZEI-31 and TZEI-4 and TZEI-157. This implies the likelihood of using these crosses for hybrid maize production. The MPH and HPH of all the hybrids for grain yield exceeded 100 % but hybrids with exceptional heterosis were TZEI7*TZEI-157, TZEI-157*TZEI-31, TZEI-157*TZEI-7, TZEI-31*TZEI-157 and TZEI31*TZEI-7. Heterosis in maize for yield has been reported by several authors (Kara,

2001; Betran *et al*., 2003; Gissa *et al*., 2007; Flint-Garcia *et al*., 2009). The average MPH and HPH estimates for set of hybrids evaluated by Betran *et al*. (2003) across environments were 171 and 132 %, respectively compared closely to approximate estimates of 179 and 139 % observed in this study. The significant, positive and high heterosis expressed in F1 hybrids for grain yield revealed the preponderance of dominant gene action. This is buttressed by the significant SCA observed for grain yield. Hull (1945) was of the view that non-additive effects (dominance and/or epistasis) were of greater importance for the expression of heterosis and that selection should be emphasized for specific combining ability (Sprague and Tatum, 1942). According to Sprague (1983) and Hill *et al*. (1998), the accumulation of good dominant alleles and masking of

deleterious effects of recessive alleles by their dominant alleles in the F1 as well as the superiority of F1 heterozygote at a number of its loci to both homozygote parents have brought about the heterosis. Therefore, the exploitation of heterosis for higher grain yields from these set of single cross hybrids may possibly be a breeding advantage.

5.4 Broad sense and narrow sense heritabilities across environments

Narrow sense heritability (h^2_n) is so essential to plant breeders since the efficiency of selection for a trait relies on the proportion of additive variance in the genetic variance to the variation in phenotypes among the genotypes (Fehr, 1987; Falconer and Mackay, 1996). Low h^2_n observed for plant height, 100-grain weight and grain yield is an indication that they are less heritable possibly because of the high number of genes that control their expression (Suzuki *et al*., 1986). The aforementioned traits were under the influence of non-additive gene effect because of the predominance of SCA variance, hence slower rate in genetic improvement would be expected from selection. High h^2_n recorded for maize streak virus disease severity mean score apparently revealed that resistance to MSVD is highly heritable (Suzuki *et al*., 1986) thus increase in response to selection is predicted.

Broad sense heritability (h^2_b) recorded for all the measured traits revealed the existence of a large amount of genetic variation among the hybrids evaluated. The generally high broad sense heritability estimate indicated that the environments in which the genotypes were evaluated had a lower effect on the expression of the trait. Gichuru (2013) found a higher h^2_b (0.88) for maize streak virus disease severity mean score in the set of genotypes used.

5.5 Phenotypic correlation

Information on correlation among traits is important so as to determine the traits to be used as selection criteria for a more effective breeding program. The positive and significant correlation between grain yield and total leaf count, plant height, ear leaf area

and 100-grain weight across environments implies that an indirect selection for any of these traits would increase yield to some extent. High dry matter accumulation by the high number of leaves possessed by tall plants might have brought about the correlation between grain yield and plant height (Bello *et al*., 2012). This result agrees with findings from Bello *et al*. (2012) and Nazir *et al*. (2010). Negative and significant correlation observed between maize streak virus disease severity mean score and plant height elucidates the influence of MSVD on the height of maize plants. This result is in agreement with Bosque-Pérez *et al*. (1998) and Bua *et al*. (2010), who found out that MSV infestation significantly reduced the height of maize plants. The correlation coefficient between grain yield and maize streak virus disease severity mean score was negative but not significant, thus suggesting that some of the genotypes evaluated in this study were tolerant. The reduction in grain yield is still certain because some of the traits that contribute to increased yield such as total leaf count and plant height were negatively and significantly correlated with maize streak virus disease severity mean score.

5.6 Detection of maize streak virus (MSV) using SSR markers

PCR plays an essential task in identification, detection and diagnosis of plant viruses with the use of degenerate oligonucleotide markers (Sharma and Misra, 2011). The amplicons obtained from using marker "MSV11" were at an expected range of 220 - 254bp (Rybicki and Hughes, 1990) and the amplification was specific for the virus. Decisively, the marker detected MSV in all the genotypes under natural and artificial infestations.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study was undertaken under natural and artificial infestations in order to identify parents and hybrids that combine MSVD resistance with high yield and also to determine the influence of maternal effect on the inheritance of MSVD resistance. Combined ANOVA for diallel revealed significant differences amongst the genotypes and environments for all the measured traits but not all the traits were significant for genotype by environment interaction. Baker"s ratio revealed the gene effect that is preponderant for all the measured traits. Inbred lines and hybrids resistant to MSVD combined with other agronomic traits were identified based on their GCA and SCA effects. The performance of the single cross hybrids over mid-parent and high parent were determined. The contribution of genetic variance (additive and non-additive) to the phenotypic variance for all the measured traits were estimated. The phenotypic relationship between maize streak virus disease severity mean score and other agronomic traits was also established.

Important genetic materials which can be utilized for succeeding breeding programmes were identified. Across environments, estimates of GCA revealed that TZEI-7 and TZEI-22 were good combiners for MSVD resistance, TZEI-7 and TZEI-31 were the most suitable parents for total leaf count, TZEI-157 contributed favourable genes for tall plant stature, TZEI-7 was appropriate for larger ear leaf area and TZEI-22 for good overall phenotypic appeal. TZEI-7, TZEI-22, TZEI-31 and TZEI-157 can be considered for increased grain yield. Although, it was challenging to have an inbred parent with desirable GCA effects for all the traits, this implies that hybridization is unavoidable in order to integrate important traits in a particular line except for TZEI-7 that had the favourable

genes for nearly all the traits. TZEI-4*TZEI-22, TZEI-22*TZEI-157, TZEI7*TZEI-157 and TZEI-31*TZEI-157 were the best performing hybrids in terms of combining resistance or tolerance with high yield based on SCA effects and heterosis. Thus, they can be further evaluated in multi-locations for possible release for commercial production by farmers. TZEI-7*TZEI-157 and TZEI-31*TZEI-157 can be further improved for resistance by using them as females in the development of threeway cross hybrids so that their potential for high yield can be fully exploited.

Maternal effect had no significant contribution to the inheritance of MSVD resistance, therefore in future hybrid breeding for MSVD resistance the choice of a maternal parent is not very important.

In breeding for genotypes that combine resistance to MSVD with high yield, response to selection could be achieved if selection for high yield is combined with total leaf count. Correlation among traits particularly the positive correlation between grain yield and total leaf count, plant height, ear leaf area and 100-grain weight gives an indication that these traits can be used as selection indices for increased yield in subsequent maize breeding program.

6.2 Recommendations

Considering the economic importance of MSVD in Sub-Saharan Africa, a large number of parental inbred lines should be used and the number of replications or hotspot environments should also be increased during the evaluation of the parental inbred lines and the single cross hybrids.

The PCR amplified fragments should be sequenced after MSV detection in order to ascertain the MSV sub-type in each environment towards breeding for resistant varieties.

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APPENDICES

Appendix 4.1: Preparation of reagents

1. CTAB
    a. 2 % CTAB (Cetyltrimethylammonium bromide)
    b. 0.1 M TrisHCl {pH = 8}
    c. 20 mM EDTA
    d. 1.4 M NaCl
    e. 2 % (w/v) PVP (polyvinyl polypyrrolidine)
    f. 1.0 % β-mercaptoethanol (added just before use)
    g. mg/ml proteinase K (added just before use)

2. TE buffer (1000 ml)
    a. 1 M Tris pH 8.0 10 ml
    b. 0.5 M EDTA pH 8.0 2 ml
    c. 5 M NaCl 200 ml
    d. Distilled H_2O complete volume to 1000 ml

3. Chloroform:isoamyl alcohol (24:1)
    a. Measure 960 ml/l Chloroform in beaker

b. Add 40 ml/l Isoamyl alcohol into the beaker

4. 70 % ethanol (100 ml): Measure and mix 70 ml of absolute ethanol with 30 ml distilled water

5. 0.8 % Agarose: Weigh 0.8 g of agarose, add 100 ml of 1X TBE and heat in a microwave to dissolve

Appendix 4.2: Mean squares from combined ANOVA for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield across natural and artificial infestations

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield
Env	1	0.211***	7.086***	23.695***	17432.174***	158087.65***	11.414***	0.300**	963.810***	4.601***
Rep(Env)	4	0.014	0.365***	1.975***	602.510***	6325.11**	1.673***	0.218***	40.262***	1.415***
Block(Env*Rep)	12	0.016*	0.150*	0.623*	231.652***	4252.93**	0.775**	0.056	11.356**	0.218*
Genotype	26	0.061***	0.520***	1.758***	1380.796***	31499.77***	1.423***	0.162**	50.458***	0.787***
Env*Genotype	26	0.031***	0.158**	0.378	87.22	3850.61**	0.944***	0.058*	6.297	0.112
Error	92	0.0086	0.0694	0.3142	60.6431	1669.671	0.2680	0.0350	4.6248	0.1030
CV (%)		5.61	16.15	4.72	7.89	11.69	15.86	11.87	11.69	15.35
R^2		0.79	0.82	0.78	0.92	0.89	0.78	0.74	0.87	0.80

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$, Env: Environment, CV: Co-efficient of Variation, R^2: Co-efficient of Determination.

Appendix 4.3: Mean squares from ANOVA for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under natural infestation

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield
Block(Rep)	6	0.021***	0.2	0.575*	231.952***	3871.73**	0.107***	0.052	20.334**	7.672**
Genotype	26	0.019***	0.223**	1.067***	825.958***	11388.61***	0.092***	0.088**	31.237***	9.827***
Error	46	0.0038	0.0936	0.1797	45.6732	1023.641	0.0134	0.0337	4.6091	2.1653
CV (%)		3.79	21.52	3.46	6.20	10.06	6.22	12.04	10.30	27.17
R^2		0.76	0.67	0.82	0.93	0.89	0.84	0.69	0.83	0.79

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$, CV: Co-efficient of Variation, R^2: Co-efficient of Determination

Appendix: 4.4: Mean squares from for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under artificial infestation

Sources of variation	df	MSVDS	ASI	TLC	PLHT	ELA	PASP	EASP	HGW	Grain Yield
Block(Rep)	6	0.012	0.1	0.671	231.352*	4634.127	0.019	0.059	2.379	1.708
Genotype	26	0.073***	0.455***	1.069**	642.058***	23875.910***	0.075***	0.131***	25.519***	4.216*
Error	46	0.0135	0.0452	0.4487	75.6131	2315.7018	0.0258	0.0356	4.6406	1.9573
CV(%)		6.86	11.55	5.82	9.85	12.64	8.61	11.71	13.50	35.74
R^2		0.77	0.86	0.68	0.85	0.87	0.65	0.76	0.79	0.66

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$, CV: Co-efficient of Variation, R^2: Co-efficient of Determination

Appendix 4.5: General combining ability (GCA) effects of parental inbred lines for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under natural infestation

Parents	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4	0.13**	-0.04	-0.33***	-10.17***	-7.84	-0.02	0.23*	0.84	-0.04
TZEI-7	-0.10*	0.03	0.41***	0.63	6.75	-0.09	0.16	1.31**	0.11
TZEI-22	-0.19***	0.29	0.02	3.71*	1.07	-0.09	-0.01	0.05	0.09
TZEI-31	0.17**	-0.11	0.27**	-1.95	-1.28	0.25*	0.06	-1.09*	-0.22
TZEI-157	-0.02	-0.17	-0.38***	7.78***	1.30	-0.05	-0.44***	-1.11*	0.06
SE (gi)	0.033	0.149	0.072	1.120	5.446	0.070	0.092	0.350	0.240
SE (gi-gj)	0.053	0.236	0.113	1.771	8.611	0.111	0.145	0.553	0.379

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error.

Appendix 4.6: General combining ability (GCA) effects of parental inbred lines for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under artificial infestation

Parents	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4	0.24***	0.09	-0.62***	-7.47***	-61.42***	0.17	0.17	-1.16**	-0.54
TZEI-7	-0.35***	-0.04	0.43**	0.53	26.50**	-0.23*	0.17	1.22**	0.10
TZEI-22	-0.22***	0.23	0.15	2.92	21.79**	-0.49***	0.07	1.35***	0.03
TZEI-31	0.20**	-0.14	0.13	-1.82	-8.76	0.24*	0.07	-0.62	0.24
TZEI-157	0.13*	-0.14	-0.08	5.83***	21.88**	0.31**	-0.47***	-0.78*	0.17

SE (gi)	0.059	0.128	0.110	1.401	7.723	0.100	0.092	0.333	0.236
SE (gi-gj)	0.093	0.202	0.174	2.216	12.211	0.158	0.146	0.527	0.373

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error.

Appendix: 4.7: Specific combining ability (SCA) effects of F1 hybrids for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under natural infestation

F1 hybrids	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4*TZEI-7	0.12	0.71*	0.11	10.15**	38.59**	-0.05	-0.46*	1.24	0.71
TZEI-4*TZEI-22	0.08	-0.06	0.12	8.71*	35.10*	-0.21	-0.46*	1.98*	1.642**
TZEI-4*TZEI-31	-0.17	0.34	-0.22	2.94	33.81*	-0.05	-0.03	0.96	0.87
TZEI-4*TZEI-157	0.27	1.47*	0.35	34.38***	198.32***	-0.47	-1.00*	4.83*	3.11*
TZEI-7*TZEI-22	-0.11	-0.13	0.16	-0.46	11.82	-0.15	-0.23	-0.34	0.29
TZEI-7*TZEI-31	-0.08	-0.06	0.31	8.04*	37.70*	-0.48*	0.04	0.91	0.88
TZEI-7*TZEI-157	-0.002	0.87	1.34***	44.29***	153.96***	-1.70***	-1.57***	9.34***	6.52***
TZEI-22*TZEI-31	0.09	1.34***	0.26	11.29**	43.29**	-0.31	-0.13	1.53	0.73
TZEI-22*TZEI-157	-0.14	1.30*	1.10**	34.33***	140.11***	-1.70***	-0.73	6.30**	3.68**
TZEI-31*TZEI-157	0.09	0.57	1.03**	39.20***	137.53***	-1.53***	-0.50	5.95**	4.18**
TZEI-7*TZEI-4	0.11	0.33	0.13	-0.25	17.79	0.17	0.17	0.11	-0.18
TZEI-22*TZEI-4	0.01	-0.50	-0.05	-1.31	19.86	-0.33	0.00	0.80	-0.20
TZEI-22*TZEI-7	0.02	-0.83*	0.49*	4.19	28.35	0.00	-0.17	-0.33	0.55
TZEI-31*TZEI-4	-0.09	0.83	-0.15	-0.73	-7.29	-0.17	0.17	1.07	-0.26
TZEI-31*TZEI-7	-0.10	0.17	0.08	-1.00	1.74	0.00	-0.50	-1.12	0.19
TZEI-31*TZEI-22	-0.12	1.17**	0.24	-0.61	25.83	0.17	-0.17	-0.37	-0.19
TZEI-157*TZEI-4	-0.02	0.33	-0.20	-2.52	-22.18	-0.17	0.33	0.56	-0.19
TZEI-157*TZEI-7	-0.11	0.00	-0.03	5.06	-6.44	0.33	0.17	-0.44	-0.07
TZEI-157*TZEI-22	-0.09	-0.17	0.22	5.15	0.13	-0.33	-0.50	-0.38	1.30
TZEI-157*TZEI-31	0.05	0.50	0.00	3.16	16.44	0.50	0.00	-0.27	-0.38

SE (sij)	0.069	0.307	0.148	2.310	11.227	0.145	0.190	0.722	0.494
SE (sij-sik)	0.106	0.471	0.226	3.543	17.222	0.222	0.291	1.107	0.758
SE (rij-rkl)	0.118	0.527	0.253	3.961	19.254	0.248	0.325	1.237	0.848

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error.

Appendix: 4.8: Specific combining ability (SCA) effects of F1 hybrids for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under artificial infestation

F1 hybrids	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4*TZEI-7	0.36**	0.74*	-0.14	12.89***	42.10*	-0.01	0.17	0.74	0.20
TZEI-4*TZEI-22	-0.47***	0.14	0.26	15.92***	51.16**	-0.41	-0.23	1.20	0.53
TZEI-4*TZEI-31	-0.57***	0.51	-0.23	1.95	33.83**	-0.31	-0.07	0.42	0.34
TZEI-4*TZEI-157	-0.07	3.73***	-0.98	26.91***	154.37***	-0.13	0.13	2.19	0.95
TZEI-7*TZEI-22	0.27*	0.27	0.57*	-3.78	-0.11	-0.17	0.10	0.67	0.23
TZEI-7*TZEI-31	0.12	0.47	0.20	8.58**	55.51**	-0.24	-0.23	2.80***	0.38
TZEI-7*TZEI-157	0.88***	1.77**	1.42**	37.30***	215.22***	-0.70	-0.70	8.92***	3.05*
TZEI-22*TZEI-31	0.11	1.04**	0.11	-1.29	16.27	0.19	0.20	2.12**	0.24
TZEI-22*TZEI-157	0.03	3.53***	1.38**	18.82**	99.99**	0.20	-0.47	5.25**	1.51
TZEI-31*TZEI-157	0.08	2.17**	0.28	39.53***	254.08***	-0.23	-2.30***	6.18***	3.58**
TZEI-7*TZEI-4	0.31*	-0.17	0.00	-1.64	13.96	0.50	0.17	-0.02	-0.04
TZEI-22*TZEI-4	-0.29	0.17	0.28	2.01	30.14	-0.50	-0.33	0.39	0.44
TZEI-22*TZEI-7	0.06	-0.17	0.15	0.38	-9.44	0.00	-0.33	-1.33	-0.53
TZEI-31*TZEI-4	-0.07	-0.17	0.04	0.23	-35.38	0.00	0.17	-0.48	-0.66
TZEI-31*TZEI-7	0.08	-0.33	-0.26	-8.18*	-36.13	0.00	0.33	-2.81**	-1.31
TZEI-31*TZEI-22	-0.01	-0.17	-0.29	-2.75	5.87	-0.17	0.33	0.46	-0.22
TZEI-157*TZEI-4	0.004	0.50	0.12	3.85	5.78	0.00	0.17	0.16	-0.17
TZEI-157*TZEI-7	0.25	-0.67	-0.19	3.61	26.02	0.50	-0.67*	0.96	0.36

TZEI-157*TZEI-22	0.23	0.17	-0.26	2.27	19.79	0.33	-0.33	-0.66	-0.46
TZEI-157*TZEI-31	0.19	-0.17	-0.02	-2.98	4.27	0.17	-0.17	-0.37	-0.13
SE (sij)	0.122	0.264	0.227	2.889	15.921	0.206	0.190	0.686	0.486
SE (sij-sik)	0.187	0.405	0.349	4.432	24.421	0.316	0.292	1.053	0.746
SE (rij-rkl)	0.209	0.453	0.390	4.955	27.304	0.354	0.326	1.177	0.834

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$, *** Highly significant at $p < 0.001$. SE: Standard error.

Appendix 4.9: Means of F1 hybrids, parental inbred lines and checks for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under natural infestation

F1 hybrids	MSDVS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4*TZEI-7	3.05	3.17	12.45	104.49	350.23	3.07	2.24	24.04	5.56
TZEI-4*TZEI-22	2.79	1.63	12.00	105.42	349.32	2.65	1.89	23.49	5.91
TZEI-4*TZEI-31	2.61	2.69	11.71	96.90	325.05	3.24	2.28	21.00	4.83
TZEI-4*TZEI-157	2.72	1.69	11.44	109.77	338.83	2.70	1.91	20.22	4.65
TZEI-7*TZEI-22	2.26	0.85	13.15	112.76	352.54	2.67	1.70	21.06	6.36
TZEI-7*TZEI-31	2.49	1.89	13.11	112.18	346.37	2.67	1.63	19.94	5.96
TZEI-7*TZEI-157	2.53	1.83	12.52	132.47	343.96	2.54	1.54	24.45	7.76
TZEI-22*TZEI-31	2.50	4.19	13.06	122.18	389.06	2.54	1.56	21.67	6.29
TZEI-22*TZEI-157	2.17	1.52	12.43	133.54	350.25	2.15	0.89	20.84	7.73
TZEI-31*TZEI-157	3.03	1.30	12.49	123.52	343.44	3.44	1.52	19.00	5.14
TZEI-7*TZEI-4	2.76	1.93	12.53	108.61	339.50	2.52	1.81	24.73	6.25
TZEI-22*TZEI-4	2.75	2.46	12.22	110.96	322.09	2.61	1.83	24.25	7.72
TZEI-22*TZEI-7	2.05	2.52	12.61	114.25	329.69	2.35	1.74	23.25	6.24
TZEI-31*TZEI-4	2.93	1.50	12.23	100.51	345.18	3.56	2.09	20.53	6.26
TZEI-31*TZEI-7	2.73	1.52	13.17	115.64	349.74	3.02	2.59	22.57	5.95
TZEI-31*TZEI-22	2.84	1.81	12.58	120.04	325.98	2.41	2.00	22.45	6.59
TZEI-157*TZEI-4	2.77	0.98	12.04	116.51	387.38	2.78	1.20	20.34	5.51
TZEI-157*TZEI-7	2.65	1.28	12.81	123.37	362.32	1.98	0.98	25.29	7.84
TZEI-157*TZEI-22	2.37	1.81	12.20	124.94	354.18	2.56	1.85	22.84	5.60
TZEI-157*TZEI-31	2.88	0.50	12.62	121.67	322.64	2.35	1.39	20.61	6.86
Parents									
TZEI-4	2.78	0.46	11.76	63.80	163.30	3.09	3.35	19.70	3.02
TZEI-7	2.36	0.89	11.93	78.30	208.42	3.80	3.26	17.36	1.42
TZEI-22	2.12	0.89	11.53	93.35	221.20	3.94	2.44	16.03	2.84
TZEI-31	2.90	0.56	12.12	76.65	191.00	4.81	2.15	13.42	1.68
TZEI-157	2.45	1.31	10.23	83.58	184.20	4.78	1.72	10.09	0.90
Checks									
Aburohemaa	2.81	1.98	12.16	117.32	344.01	3.24	1.69	21.26	5.45
Omankwa	2.83	2.19	12.14	120.67	351.09	3.54	1.74	22.20	5.88
Mean	2.634	1.679	12.269	109.015	318.185	3.000	1.889	20.838	5.415

LSD (0.05)	0.326	1.444	0.697	11.107	52.583	0.709	0.930	3.529	2.419
CV (%)	3.79	21.52	3.46	6.20	10.06	6.22	12.04	10.30	27.17
R^2	0.76	0.67	0.82	0.93	0.89	0.84	0.69	0.83	0.79

LSD: Least Significant Difference, CV: Co-efficient of Variation, R^2: Co-efficient of Determination

Appendix 4.10: Means of F1 hybrids, parental inbred lines and checks for maize streak virus disease severity (MSVDS) mean score, anthesis-silking interval (ASI), total leaf count (TLC), plant height (PLHT), ear leaf area (ELA), plant aspect (PASP), ear aspect (EASP), 100-grain weight (HGW) and grain yield under artificial infestation

F1 hybrids	MSVDS (1-5)	ASI (days)	TLC	PLHT (cm)	ELA (cm^2)	PASP (1-5)	EASP (1-5)	HGW (g)	Grain Yield (t/ha)
TZEI-4*TZEI-7	3.37	3.76	11.25	96.96	419.74	3.91	3.26	16.66	3.97
TZEI-4*TZEI-22	2.16	4.00	11.86	102.79	431.15	2.24	2.50	18.13	4.41
TZEI-4*TZEI-31	2.81	3.30	10.91	82.60	311.88	3.70	3.17	14.42	3.27
TZEI-4*TZEI-157	3.71	4.59	10.64	86.8253	351.41	4.46	2.81	14.33	3.23
TZEI-7*TZEI-22	2.69	3.09	12.74	87.76	406.00	2.72	2.48	17.60	3.97
TZEI-7*TZEI-31	2.95	2.72	11.88	92.05	429.85	3.37	2.93	16.39	3.62
TZEI-7*TZEI-157	2.92	2.39	12.21	109.25	519.79	4.00	1.24	19.58	5.93
TZEI-22*TZEI-31	2.99	3.93	11.37	89.62	425.04	3.41	3.07	19.08	4.51
TZEI-22*TZEI-157	3.17	4.07	11.46	96.85	425.23	4.02	1.76	16.00	3.53
TZEI-31*TZEI-157	3.65	2.96	11.99	102.43	469.04	4.33	1.15	14.93	5.47
TZEI-7*TZEI-4	2.85	4.54	11.34	96.62	379.84	2.94	2.98	17.14	3.75
TZEI-22*TZEI-4	2.65	3.63	10.97	101.63	377.13	3.28	2.74	16.85	3.85
TZEI-22*TZEI-7	2.59	3.57	12.27	85.99	425.65	2.70	3.35	20.10	4.50
TZEI-31*TZEI-4	2.78	3.74	10.83	79.21	375.80	3.54	2.87	14.78	4.36
TZEI-31*TZEI-7	2.85	3.44	12.39	100.47	466.89	3.26	2.44	21.75	5.63
TZEI-31*TZEI-22	3.03	4.33	11.93	93.06	399.91	3.67	2.31	18.12	4.97
TZEI-157*TZEI-4	3.69	3.83	10.22	81.90	349.06	4.41	2.31	14.04	3.65
TZEI-157*TZEI-7	2.62	3.91	12.39	99.81	448.59	3.00	2.56	18.03	4.94
TZEI-157*TZEI-22	2.71	3.98	11.81	95.09	394.86	3.30	2.26	17.35	4.53
TZEI-157*TZEI-31	3.20	3.04	11.83	105.75	447.77	3.96	1.52	14.98	5.43
Parents									
TZEI-4	3.72	0.94	10.78	42.63	110.32	4.24	3.02	11.02	1.58
TZEI-7	1.39	1.02	11.28	63.34	277.30	3.80	3.24	11.61	2.39
TZEI-22	2.55	0.67	10.60	74.92	318.74	2.76	3.19	13.57	2.33
TZEI-31	3.46	0.37	11.47	59.05	173.53	4.33	4.20	8.56	1.81
TZEI-157	2.65	0.37	11.13	72.29	250.94	3.63	3.41	11.34	1.62

Checks									
Aburohemaa	2.70	3.33	11.59	92.71	403.42	3.39	2.72	15.48	3.12
Omankwa	2.41	3.46	11.45	91.69	489.02	2.96	2.83	19.06	5.30
Mean	2.899	3.074	11.504	88.269	380.663	3.531	2.679	15.960	3.914
LSD (0.05)	0.615	1.244	1.101	14.291	79.089	0.973	0.928	3.541	2.299
CV (%)	6.86	11.55	5.82	9.85	12.64	8.61	11.71	13.50	35.74
R^2	0.77	0.86	0.68	0.85	0.87	0.65	0.76	0.79	0.66

LSD: Least Significant Difference, CV: Co-efficient of Variation, R^2: Co-efficient of Determination