ASSESSMENT AND GENETIC CHARACTERISATION OF MAIZE (*Zea mays* L.) GERMPLASM FOR LEAF BLIGHT (*Helminthosporium turcicum* Pass.) RESISTANCE

BY

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DECLARATION

I, **Victor B. Simelane**, declare that all the work presented in this dissertation is my own work and has not been submitted for a degree at this or any other University.

Signature.....

Date.....06/07/2007.....

APPROVAL

This dissertation of **Victor B. Simelane** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Plant Breeding) by the University of Zambia

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DEDICATION

Like my previous academic works, this one again is for the loving memory of my parents who inspired and encouraged me in many ways. I know they would have loved to see this.

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ABSTRACT

Maize leaf blight caused by the fungus *Helminthosporium turcicum (Exerohilum turcicum)* is one of the constraints affecting maize production and thereby eroding income and food security in Zambia and the Southern African region. Maize leaf blight severity on susceptible varieties can range between 40-70% plant coverage and can reduce maize yields by up to 60% when infection is high. Six F_1 maize hybrids and their F_2 populations were evaluated for leaf blight resistance in 2006 and 2007 at the Golden Valley Agricultural Research Trust, Chisamba, Zambia. They were artificially inoculation with the pathogen. Disease resistance parameters used to assess the disease included, disease severity scores, percent affected ear leaf area, number of lesions, lesion sizes, incubation periods, area under disease progress curve and apparent infection rates. From the F_1's evaluation, disease resistance parameters indicated that hybrids J34-2 and J34-4 were the most susceptible and J34-1 and J34-3 were most resistant hybrids respectively. All F_2 populations showed continuous variation for leaf blight resistance. Variability parameters namely genetic coefficient of variation (GCV), broad sense heritability (h^2b) and genetic advance (GA) as measured by the resistance parameters were used to determine the gene action for leaf blight resistance on individual populations. The variability parameters revealed that in populations J34-1, J34-2, J34-3 and J34-5 leaf blight resistance was under additive gene action, while in populations J34-4 and J34-6 they revealed that leaf blight resistance was under non additive gene action. In all cases genotypic correlations in general, were higher than the corresponding phenotypic correlations, indicating little influence of the environment on any inherent association among the traits studied. Male flowering dates were found to be significantly but negatively correlated with most disease resistance parameters, indicating that such a trait could be used indirectly for the improvement of leaf blight resistance in maize. The inferences of the results of the present study and possible implications in maize breeding for leaf blight resistance have been discussed.

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Chapter 1

1.0 Introduction

Maize (*Zea mays* L.) is native to Mexico and Central America (Galaninat, 1976; Pursglove, 1972 and Dowell *et al.*, 1996) and is an important food crop. Among the world's major cereal crops it ranks third after wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) in production. In the year 2000 about 140 million hectares were put into maize globally and of this approximately 96 million hectares were in developing countries (Pingali and Pandey, 2000). Current annual maize production is estimated at 500 million tonnes and by 2020 the projected demand for maize in developing countries will surpass the demand for both wheat and rice. Table 1 shows the consumption of maize for direct human consumption in the Southern African Development Community (SADC) region.

Table 1. Average % of maize used as direct human consumption in the SADC region

Country	Average % of maize used as direct human consumption
Lesotho	92
Madagascar	85
Malawi	81
Mozambique	90
South Africa	53
Swaziland	30
Zambia	84
Zimbabwe	77

Source: Heisey and Edmeades (2001).

In Zambia maize is the most important staple food crop followed by cassava (*Manihot esculenta* Cranz.), sorghum (*Sorghum bicolour* (L.) Moech) and millet (*Pennisetum americanum* (R) Leeke). Maize accounts for more than 70% of dietary carbohydrate intake for most Zambians. Almost 90% of the maize produced is used directly for human consumption, with livestock and industry (beer making) taking up the rest (Mungoma and Mwambula, 1996; Ministry of Agriculture Food and Fisheries, 1995). Maize is also an important cash crop grown by the majority of smallholder farmers in the country.

Successful maize production may result in improved food security in the country since maize is the main source of food and income for the majority of the people of Zambia. Cultivation of maize is done in Zambia throughout the three agro-ecological zones namely regions I, II, IIb and III (Figure 1) and by all categories of farmers (Bunyolo *et al.*, 1995). The average productivity stands at 1.5 t/ha and this hardly results in food self-sufficiency in the country (Ministry of Agriculture Food and Fisheries, 1995).

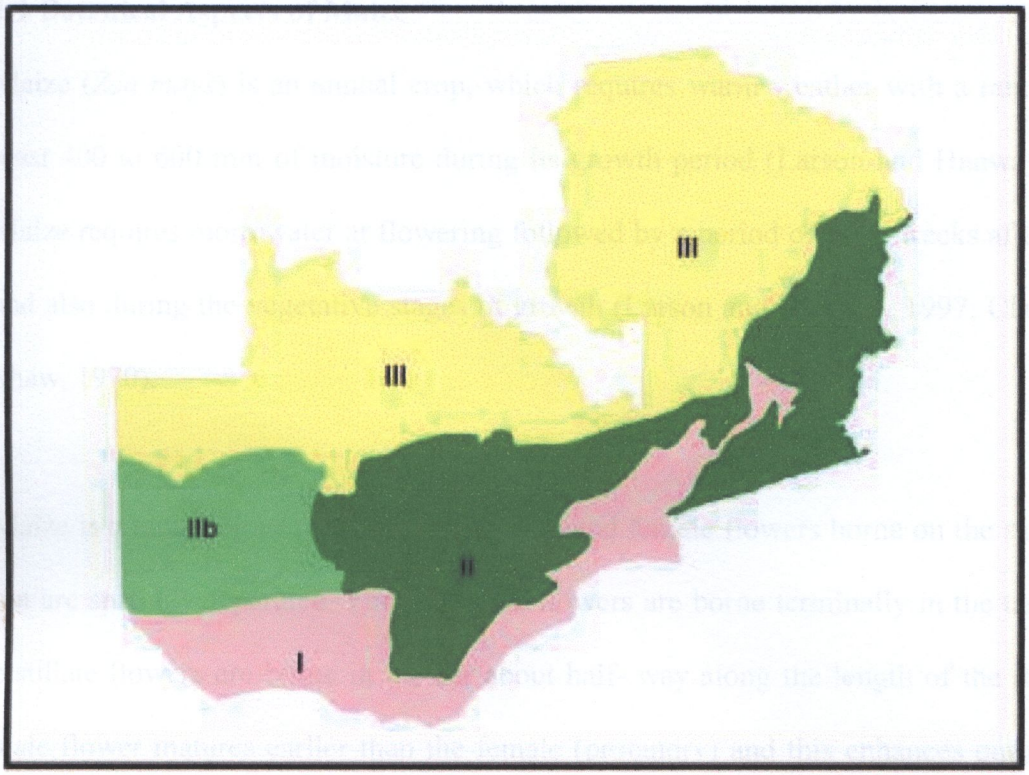

Figure 1: Zambia agro-ecological regions I, IIa, IIb and III

Source: http://www.fao.org/ag/AGP/AGPC/doc/Counprof/zambia/zambia.htm

1.1 Botanical Aspects of Maize

Maize (*Zea mays*) is an annual crop, which requires warm weather with a rainfall of at least 400 to 600 mm of moisture during its growth period (Larson and Hanway, 1997). Maize requires more water at flowering followed by a period of three weeks after silking and also during the vegetative stages of growth (Larson and Hanway, 1997; Classen and Shaw, 1970).

Maize is a monoecious plant with both male and female flowers borne on the same plant but are spatially separated. The staminate flowers are borne terminally in the tassel. The pistillate flowers are borne in the ear about half- way along the length of the stalk. The male flower matures earlier than the female (protandry) and this enhances out crossing. Consequently, the plant is normally 98% cross pollinated (Poehlman, 1987). The tassel normally sheds mature pollen before the silk is receptive. Repeated selfing of maize results in inbreeding depression which is characterised by small plant stature, reduced pollen shed, seed set as well as vigour and increased tenderness. Crossing of the repeated artificial selfed plants has been effectively used by breeders to produce more vigorous hybrid plants which perform better than the parents through heterosis (Poehlman, 1987).

The seed develops from the ovule following a double fertilisation process in which the diploid (2n) embryo is formed from paternal and maternal gamete fusion. The endosperm is formed by fusion of the two maternal nuclei and the paternal nucleus forming triploid (3n) tissue. After fertilisation seed development and grain filling continue for a period of

about 60 days of physiological maturity after which moisture loss occurs up to the time harvesting is done (Larson and Hanway, 1997).

The plant is divided into several growth stages. The staging system employed divides the plant development into vegetative (V) and reproductive (R) stages. Sub divisions of the V stages are designated numerically as V1, V2, and V3 through V (*n)*, where *n* represents the last leaf stage before the tasselling stage (VT). The first and the last V stages are designated as VE (emergence) and VT (tasseling). The (*n*) will fluctuate with the variety and environmental differences. The reproductive stages are divided into 6 stages, from silking to physiological maturity (Larson and Hanway, 1977) and designated as R1 to R6.

According to Duveiller *et al.* (1994), this system accurately identifies the stages of the maize plant. However, all plants in a given field will not be in the same stage at the same time. They stressed that each specific R and V stages are defined only when 50% or more of the plants in the field are in or beyond that stage.

Chapter 2

Literature Review

2.1 Maize Production Constraints

Among the constraints affecting maize production and thereby eroding income and food security in Zambia and the southern African region are drought, low soil fertility, soil acidity (low pH), and pest and diseases (Zambezi and Mwambula, 1996; Banziger *et al.*, 2001; Mungoma and Mwambula, 1996; Reddy *et al.*, 1989; Betram *et al.*, 2003).

Three of the most serious leaf diseases of maize in Zambia since 1996 have been Grey leaf spot (GLS) caused by *Cercospora zeae- maydis,* Maize streak virus (MSV) and leaf blight of maize caused by *Helminthosporium turcicum (= Exerohilum turcicum*). Research on maize diseases in Zambia has focused on GLS and MSV and less has been done on maize leaf blight, which is now a big problem in most maize growing areas of Zambia (Verma, 2001; Ngwira and Pixley, 2000; Tembo and Pixley, 1998*)*. Leaf blight severity on susceptible varieties can range between 40-70% plant coverage and leaf damage by the time the crop reaches physiological maturity. The severe blighting results in weakened stems and leads to lodging. According to Ngwira and Pixley (2000) the disease can reduce maize yield by up to 80%.

The Zambia National Maize Research Programme has developed commercial maize varieties since 1964. Most varieties have been found to display different levels of susceptibility to maize leaf blight and none can be considered to be resistant. Under favourable conditions of maize leaf blight development these varieties can develop

significant levels of disease. This is expected because the varieties have not been selected for resistance to maize leaf blight.

2.2 Epidermiology and the Distribution of Maize Leaf Blight in Zambia

Maize leaf blight is considered a major disease in many parts of the world (Ward and Nowell, 1997). The disease was first reported in Italy in 1876, then later in New Jersey in the USA in 1878. A serious outbreak occurred in Connecticut in 1899 (Drechsler, 1925). It has now become one of the most prevalent and severe diseases of maize in Eastern and Southern African regions (Ngwira and Pixley, 2000; Tembo and Pixley, 1998). The mid-altitude regions of the tropics, about 900–1600 m above sea level, have a particularly favourable climate for the disease as dew periods are long and temperatures moderate. Such conditions prevail on millions of hectares of crop land in Eastern and Southern Africa, Latin America, China, and India (Renfro and Ullstrup, 1988).

In Zambia, it was first observed in 1982 almost throughout the country but did not cause economic importance by then (Verma, 2001). Most maize varieties have developed susceptibility to the disease since 1998, but this was quickly overcome by breeding resistant varieties by seed companies and the International Centre for Maize and Wheat improvement (CIMMYT) in Zimbabwe. In Mpongwe (Zambia) most resistant inbred lines and hybrids showed susceptibility to maize leaf blight. The disease is now considered to be the second most important disease of maize in Zambia after Grey Leaf Spot (Rao *et al.*, 1987). As a result, research in the maize programme is now focusing on

selecting germplasm material that has enhanced resistance to grey leaf spot and maize leaf blight (Mwansa, personal communication, 2006).

The occurrence and severity of maize leaf blight has increased over the years in Zambia. This is probably due to the increased use of reduced-tillage management systems and the introduction of maize hybrids with some degree of susceptibility to the disease. Although maize leaf blight can be found in conventionally tilled fields, it has only been a significant problem in reduced tillage fields where the new crop is planted directly into the heavy cover of old maize residues (Turner, 1975)

Levy (1997) reported that pathogenic fitness and environmental conditions are important factors in determining disease development and the occurrence of disease epidemics depend on the ability of the pathogen to infect, grow and sporulate on maize plants. The disease can be particularly severe in mid altitude humid regions where environmental conditions are favourable for infection and where heavy dew fall, moderate temperatures and long nights are common (Welz *et al.*, 1997). The fungus overwinters in infected maize leaves. The cells of the conidia can be transformed into thick walled resistant spores called chlamydospores that are capable of surviving adverse winter weather (Smith and Kinsley, 1993). Primary infection occurs on young plants that grow up through the residues of the previous maize crop. Infection and spread of the disease is favoured by moderate temperatures (18-26^{o}C) and heavy dews during the growing season (Elliot and Jenkins, 1964). The conidia germinate and penetrate leaf tissue directly or through stomata and infection occurs when free moisture is present on the leaf surface

(Agrios, 1988). Dry weather was found to severely retard disease spread in Uganda (Adipala *et al.*, 1993) but under prolonged dry weather conidia are produced abundantly on diseased leaves on which they can survive for at least one year but loose germinability thereafter (Thakur *et al.*, 1989). Chlamydospores, borne singly or in chains on dominant hyphae, germinate to form conidia which incite infection (Asare-Nyoko, 1964).

Although maize leaf blight is highly influenced by the microclimatic conditions, the disease prevalence varies depending on the location and climatic conditions (Donahue *et al.*, 1991). This makes the assessment of the disease very difficult for both inheritance studies and breeding for resistance (Saghai Maroof *et al.*, 1996). According to Ngwira and Pixley (2000) the disease spreads more rapidly in high rainfall than in dry areas.

In addition to warm and humid rainy weather, there are also other factors that cause rapid spread of maize leaf blight. In Southern Africa, for instance presence of inoculum and extensive cultivation of susceptible host plants are some of the factors (Tembo and Pixley 1998; Ngwira and Pixley, 2000). Other factors that assure disease spread include the use of minimum tillage techniques, which leave the fields covered with infected stover (Thompson *et al.*, 1987). Payne and Waldron (1983) reported that such conditions contribute to over wintering of fungus on corn debris thereby serving as a source of disease inoculum in the rainy season. This could lead to early infections of the crop in the following season with symptoms appearing about the mid season. While increased severity has been associated with no-tillage maize, Perkins *et al.* (1995) observed that the disease is just as prevalent under conventional tillage practices.

The minimum tillage is now commonly practiced as a conservation method of farming with the objective of restoring soil fertility. In Zambia, Golden Valley Agricultural Research Trust (GART) and some Non Governmental Organization (NGO) are spearheading and promoting this technology. In areas where this technology is introduced, more than 50% of the farmers have already adopted the technique (Moono personal communication, 2004).

In addition, continuous and or extensive cropping of maize is also another contributing factor. In Zambia, like in most maize growing areas, the practice has been to grow maize traditionally under a monoculture system with few farmers practicing any form of crop rotation.

2.3 The Causal Pathogen of Maize Leaf Blight

Maize leaf blight is caused by the fungus *Helminthosporium turcicum* Pass. = *Exerohelium turcicum* (Pass) Leonard & Suggs); and its perfect stage is *Trichometasphaeria turcica* Lutrell (or *Setosphaeria turcica* [Lutrell] Leonard and Suggs). It has five distinct races; of these race 0 is nearly avirulent to maize, race 1 is highly pathogenic on a few inbred lines, race 2 is much less pathogenic with no distinct specialisation to the host, and race 3 occurs on commercial hybrids, populations and some susceptible inbred lines, and is of major concern. A fifth race has been reported. (Perkins *et al.*, 1995).

The conidia are spindle shaped, often slightly curved on one side and are brown, olive grey to black in colour with an average of nine septa. The average size of the conidia is 105 x 20 mμ. A conspicuous and identifying feature of the spores of *Helminthosporium turcicum* is protruding hilum (Asare-Nyoko, 1964). The hilum of most of other species of this genus that attack maize is internal or contained within the wall of the cell attached to the conidiophore. Perithecia are black, ellipsoidal and measure about 360 to 720mμ in length by 350 to 500 mμ in diameter. The asci are long, cylindrical and measure approximately 27 x 200 mμ and produce 1 to 8 ascospores. Ascospores are fusoid, hyaline, and contain three septa, and measure 15 x 62 mμ in size. Walls of ascospores are constricted at the septa (Barnett *et al.*, 1965).

2.4 Symptomatology of Maize Leaf Blight

Maize leaf blight is recognised by characteristic narrow linear spots ranging in size from 15 - 18 cm x 2 - 4 cm. Multiple spots may develop along the leaf veins making them appear longer. Spots are greyish-tan and are usually surrounded by a pigmented border where conidia are arranged in a concentric zonate pattern (Smith and Kinsley, 1993). Within 48-72 hours of infection of leaves by the pathogen, small chlorotic flecks appear at points of penetration. These are barely visible except where a large number of penetrations occur over a small area of leaf surface. Lesions become evident 8 to 10 days after infection is established and appear as small wilted areas, which continue to enlarge. The time required for lesions to develop depends on temperature. Rapid development occurs at temperatures of 25 to 30 ^{o}C while at lower temperatures a longer time is required. Observations made under artificial and natural epidemics indicate that kernels

are not attacked by the pathogen. The spots usually elongate linearly between the veins of the leaf. The shape, size and colour of the spots may vary depending on the hybrid or variety and the type of gene action operating (Perkins and Hooker, 1981). The spots develop from the lower leaves and eventually spread to the whole plant (Elliot and Jenkins, 1964).

Jordan *et al.* (2001) studied the reaction of maize inbred lines to *Helminthosporium turcicum* at the V1 stage of plant growth and found that all plants developed small pinhead size spots 48 hours after inoculation whereas resistant plants exhibited chlorotic spots. On the susceptible cultivars the small spots developed into typical lesions. Leath (1984) was able to distinguish lesions 5 days after inoculating maize cultivars. The resistant reactions were yellowish chlorotic lesions and susceptible reactions were elliptical grey and sporulating. Miles *et al.* (1980) discovered that inbred lines that showed many small chlorotic lesions with two or three necrotic ones were considered as moderately resistant. Those with few chlorotic lesions and more than three necrotic lesions were considered moderately susceptible. The susceptible lines showed many water soaked necrotic lesions with dark margins full of profuse sporulation.

2.5 ECONOMIC IMPORTANCE OF MAIZE LEAF BLIGHT

Under ideal conditions for disease development, disease severity has been observed to reach 40-75% plant coverage (Raymundo and Hooker, 1981). This causes extensive leaf damage by the time the maize crop reaches physiological maturity and results in yield

losses. If maize crop is grown for silage, the extensive leaf damage caused by maize leaf blight can reduce the quality of silage (Ward and Nowell, 1997).

Yield losses can exceed 50% if maize leaf blight hits before flowering (Raymondo and Hooker, 1981) or moderate if it develops later. In temperate environments losses may average 4.2% grain yield per 10% disease severity (Levy, 1997). In the Corn Belt of the USA, Aryers *et al.* (1984) reported 20% yield loss in Pennsylvania and 35% in Tennessee. Others have reported losses of as much as 50% (Strongberg and Donahue, 1986; Strongberg and Flinchum, 1994), whilst losses of 10-50% have been reported in Kenya (Njunguna *et al.*, 1992) and 30% where disease is endemic (Ward *et al.*, 1993). Latterell and Rossi (1983) mentioned that losses occur even where severe disease results in lodging of stalk.

Yield losses due to maize leaf blight have been attributed to loss of photosynthetic area, increase in lodging, and premature death (Strongberg and Donahue, 1986; Strongberg and Flinchum, 1994). Due to loss of photosynthetic area, carbohydrates are diverted from the stalk and roots to the grain at greater than normal levels. This causes stems to weaken and often lodge, leading to death of the plant and, therefore, yield losses occur (Dodd, 1980).

2.6 CONTROL OF MAIZE LEAF BLIGHT

Maize leaf blight can be controlled partially through the combined use of crop rotation (Sprague and Dudley, 1988), crop sanitation, fungicide application and use of genetic

resistance (Leath and Pedersen, 1986). De Leon and Pandey (1989) suggested crop rotation of one to two years or deep burying of infested maize residues before maize hybrids can be planted. Such methods reduce over wintering of the fungus in the field. Patrick *et al.* (2005) showed that the severity of maize leaf blight is less in fields when adequate potassium in the form of potassium chloride has been applied.

The relationship of potassium to maize blight is most pronounced in impoverished soils. Several fungicides are labeled for control of maize leaf blight. Mancozeb and Propiconazole are labeled for field corn, popcorn and sweet corn. Maneb and Maneb plus Zinc are also labeled for control on popcorn and sweet corn (Nyvall, 1989). Fungicide application may be cost prohibitive in some situations and may be limited to seed production fields. Applications may also be prohibited past a certain growth stage and restrictions may apply to feeding crop residue to livestock (Shurtleff, 1973). The use of fungicides increases production costs and is usually not economical to many farmers (Tooley and Grau,1984). In addition the chemical fungicides are also viewed as environmental hazards with possible adverse effect on the farmer's health (Shurtleff, 1973).

Genetic resistance to maize leaf blight can be a sole solution or as a contribution to a better integrated approach to disease problem (Turner, 1982). Therefore, the main solution lies in the use of genetic resistance in maize cultivars, which is highly effective and a cost efficient solution (Ceballos *et al.*, 1991).

2.8 GENETICS OF MAIZE LEAF BLIGHT RESISTANCE

The genetics of resistance and susceptibility in plants was first reported by Flor (1956) working with rust disease of flax *Linum usitatissimum* caused by *Melampsora lini.* He showed that for each gene of resistance in the host there was a corresponding gene for avirulence in the pathogen and for each gene for avirulence in the pathogen, there was a gene for susceptibility in the host plant and proposed that this phenomenon can be explained by gene for gene theory.

Genetic resistance in maize to leaf blight is determined either by polygenic race non specific resistance or by monogenic race specific resistance. The former is quantitative and the latter is qualitative. Monogenic resistance appears to control the lesion size and is conferred by either *Ht1, Ht2, Ht3, HtN1 and HtM1* genes (Turner and Johnson, 1980). Polygenic resistance controls the number of lesions but not lesion size (Hughes and Hooker, 1971). Diverse sources of qualitative and quantitative resistance are available but qualitative resistances are often unstable. In the tropics especially, they are either overcome by new virulent races or they suffer from climatically sensitive expression. Quantitative resistance is expressed independently of the physical environment and has not been reported to succumb to *Helminthosporium turcicum* pathotypes in the field (Welz *et al.*, 2000).

The reaction of plants with monogenic resistance is characterised by relatively small and narrow necrotic areas which are surrounded by a chlorotic halo. The size of the necrotic lesion and the width of the chlorotic halo vary with genetic background into which the *Ht*

gene was introduced (Windes and Perderson, 1990). Thus the incorporation of both mono and polygenic resistance into inbred lines of maize is very important (Thakur *et al.*, 1989). The chlorosis associated with *Ht1* and *Ht2* can be detrimental to plants under severe epiphytotics of maize leaf blight (Ullstrup, 1970). Prior to 1961, the only known resistance to maize leaf blight was polygenic (Elliot and Jenkins, 1964; Jenkins and Roberts, 1952; Ullstrup and Miles, 1957). Later, diverse sources of monogenic resistance were reported (Hooker, 1961; Gevers, 1975 and Turner, 1982). Following the discovery of these sources of monogenic resistance, different races of the pathogen carrying new pathogenic capability were also reported (Bergquist and Masias, 1974; Smith and Kinsley, 1993 and Thakur *et al.*, 1989). Leath and Perderson (1986) gave the following virulence formulas for the different races of the pathogen: race 0: *Ht1, Ht2, Ht3, HtN,* race 1: *Ht2, Ht3, HtN/Ht1*, race2: *Ht1, HtN/Ht2, Ht3* and race 3: *Ht1/Ht2, Ht3/HtN.* Genes *Ht2 and Ht3* provide protection against races 1 and 2 of the pathogen, but Smith and Kinsley (1993) reported that they were not effective against race 3 of the pathogen.

The *Ht1, Ht2* and *Ht3* resistance genes occur as chlorotic lesions with minimum sporulation; while the *HtN* induced resistance is expressed as a delay in disease development until after pollination (Leonard *et al.*, 1989). This gene, according to Gevers (1975), was derived from the Mexican maize cultivar 'Pepitila' which is reasonably stable. In some parts of the world, however, the effects may fail to be expressed and the genetic segregation may not behave like the expected dominant gene ratios but it does, however, remain in the tolerable limits of deviation of stability and segregation. Turner and Johnson (1980) reported the presence of race 1 in Indiana that was avirulent on *Ht1*

but not *Ht2*. Lipps and Hite (1982) reported the presence of race 1 in Ohio which was virulent in *Ht1* but avirulent on *Ht2*. Welz *et al.* (1997) indicated the presence of race 0 and 1 in China, 0 in Zambia and 2 in Uganda. A new chlorotic halo gene different from the *Ht* gene has been reported by Carson (1965) which may be of limited commercial value when alone, but useful in combination with the *Ht* genes.

Pratt *et al.* (1993) reported a polygenic based resistance in 0h10 progenies expressed as rate reducing resistance or low number of lesions. The homozygotes obtained by combining *Ht1* and *Ht3* genes did not result in significantly less disease than each parent (Leath and Perderson, 1986). Dunn and Namn (1970) reported gene dosage effects for the *Ht2* gene. Smith and Kinsley (1993) suggested that a combination of *Ht1* and *Ht2* or *Ht3* genes would provide resistance against races 1, 2 and 3. Pataky (1992) showed that high levels of partial resistance with or without *Ht* genes presented a spectacular approach in reducing damage from maize leaf blight. It also eliminates the severe yield- depressing chlorosis associated with the *Ht* gene of resistance in very susceptible backgrounds. Studies by Carson (1994) indicated that the latent period is related to partial resistance, which suggested that selection for increased latent period length would be more beneficial than selection for reduced disease severity.

With the rapid variation and mutation of pathogen races, maize varieties loose their resistance to the pathogen. Most published reports have found additive, with less important, but significant non-additive gene action affecting resistance (Gevers, 1975; Ullstrup 1970 and Manwiller, 1983). However, dominance, recessive and epistatic

(Pataky, 1994) gene actions have also been implicated in maize blight resistance. It has also been reported that resistance to the pathogen by a maize plant is a quantitative trait, and the additive gene action has less important expression (Carson, 1994). Molecular marker analyses done by Bubeck *et al.* (1993) showed significant association between maize leaf blight resistance and particular chromosome segments. The polygenic resistance has been shown to be associated with 10 chromosomes in a maize plant.

2.9 BREEDING FOR LEAF BLIGHT RESISTANCE

The success of plant breeding operations relies heavily on an extent of genetic variability present in a crop species for a particular trait. In fact plant breeding uses selection for improving the architecture of a crop by management of available genetic variability (Mohammadia *et al.*, 2003). Success of breeders in changing the characteristics of a population depends on the degree of correspondence between phenotypic and genotypic values (Dabholker, 1992; Singh and Ceccarelli, 1995).

A quantitative measure, which provides information about the correspondence between genotypic variance and phenotypic variance, is heritability (Dabholker, 1992). The term heritability has been further divided into broad sense and narrow sense, depending whether it refers to the genotypic value or breeding value, respectively (Falconer, 1989). The ratio of genetic variance to phenotypic variance (VG/VP) is called heritability in the broad sense or genetic determination. It expresses the extent to which individual phenotypes are determined by the genotypes. A large percentage for a character is

regarded as highly heritable whereas if it is smaller, some environmental agency is considered responsible for phenotypic manifestation of the character (Dabholker, 1992).

A broad sense heritability estimate based on various components of variance provides information on the relative magnitudes of genetic and environmental variation in the germplasm (Dudley and Moll, 1969). However, the type of gene action involved in the expression of a character has a significant role in determining heritability values. Characters that are controlled largely by genes acting in an additive fashion have higher values of heritability than characters governed by genes with large non additive effects (Hanson, 1963; Falconer, 1989; Dabholker, 1992). According to Dabholker (1992), it is important to note that heritability is a property not only of the character being studied, but also the population being sampled and the environmental circumstances to which individuals have been subjected. More variable environmental conditions also reduce the magnitude of heritability while more uniform conditions increase it (Blum, 1988; Rosielle and Hamblin, 1981).

Different selection methods have been proposed by various authors in breeding for leaf blight resistance. Ceballos *et al.* (1991), Hughes and Hooker (1971) and Hooker and Perkins (1980) used the full sib S_1 recurrent selection to increase the levels of resistance to northern leaf blight in eight subtropical populations. There are few reports on the progress achieved through recurrent selection for quantitative resistance to diseases in maize (Miles *et al.*, 1981). Mean ratings for *Helminthosporium maydis* were decreased in three cycles of S_1 recurrent selection (Jinahyon and Russel, 1996). Jenkins *et al.* (1954)

reported average gains for resistance to northern leaf blight using mass selection with complete control of the parents. Predicted responses to selection to different diseases (including leaf blight) using S_1 half sib and mass selection have been reported (Miles *et al.*, 1980). Miles *et al.* (1981) reported average gains per cycle for resistance to leaf blight in two different populations between 7 and 9% through recurrent modified ear to row index selection.

2.9.1 Correlation Between Traits

Relationships between two metric characters can be positive or negative, and the cause of correlation in crop plants can be genetic or environmental (Hallauer & Miranda, 1988; Falconer, 1989). Two types of correlations, phenotypic and genetic, are commonly discussed in plant breeding. Phenotypic correlation (r_p) involves both genetic and environmental effects. It can be directly observed from measurements of the two characters in a number of individuals in a population (Hallauer & Miranda, 1988). Genetic correlation (r_g) is the association of breeding values (i.e., additive genetic variance) of the two characters (Falconer, 1989). Both measure the extent to which degree the same genes or closely linked genes cause co-variation in two different characters (Hallauer & Miranda, 1988).

Estimation of genetic and phenotypic correlations is based on components of variances and covariances that are estimated from analyses of variance and covariance, respectively. However genetic correlations inherently have large errors because of difficulties to avoid the directional effects of confounding factors on additive correlation estimates. A line's performance is correlated with its performance in crosses, to some

extent depending on how much of the variance is due to additive genes. Genetic correlation in particular determines the degree of association between traits and how they may enhance selection. It is useful if indirect selection gives greater response to selection for traits than direct selection for the same trait.

Chapter 3

Materials and Methods

3.1 Pathogen Identification and Isolation

Standard methods of pathogen identification and isolation were used according to Agrios, (1988). Maize leaves showing typical symptoms of maize blight caused by *Helminthosporium turcicum* were collected from Golden Valley Agricultural Research Trust (GART) in Chisamba, Zambia and stored under pressed newspapers to encourage sporulation. Affected and sporulating areas on leaves were cut into 1 cm^2 pieces and placed in a 250 ml beaker half full with sterile distilled water. They were picked up with sterilised forceps and placed in a plate with 0.5% sodium hypochlorite (jik) for 1 to 2 minutes and then pressed between two sterile filter papers to remove excess bleach. The surface disinfected diseased leaf pieces were placed in a petri dish with sterile pre-moistened filter paper and incubated for 36 hours at 25°C in a NAPCO 303 series incubator.

Culture plates were examined for the presence of conidia by transferring a small portion of infected material into a drop of sterile distilled water on a glass slide. The mycelium was stirred to dislodge conidia and the slides were then observed under a light microscope. Conidia of *Helminthosporium turcicum* were observed, compared and confirmed with diagrams of Turner (1982) and Drechsler (1923).

While observing the conidia through the dissection microscope, a bit of potato dextrose agar (PDA) medium adhering to the tip of a sterile transfer needle was brought into

contact with the identified conidia and was streaked on to PDA plates prepared earlier. These were incubated for 14 days at 30°C and thereafter stored in a refrigerator at 4°C for further observations.

3.2 Inoculum Preparation

Isolates were removed from storage in the fridge and each petri dish was moistened with a few drops of sterile distilled water using a sterile pipette. The conidia were dislodged from the surface of the colonies on a microscope slide and then drained into a beaker with 10ml of sterile distilled water. The conidial suspension was blended for 10minutes using a D4M- Laboratory Blender then filtered through double folded cheese cloth into a 1000ml beaker. A final concentration of about $1x10^3$ conidia ml^{-1} was maintained by using the drop technique using a Pipeman microliter pipette (P-20D, West Coast Scientific, Inc., Oakland, CA).

3.3 Host Plants, Field Plots and Cultural Practices

Six (6) maize genotypes of F_1 generation and their F_2 populations were planted at the Golden Valley Agricultural Research Trust, Chisamba, Zambia (1170masl, 14.17°S, 28.37°E) on the 5th of January 2006 and 8th January 2007 respectively. The F_1 materials and their F_2 populations were developed from CIMMYT Zimbabwe (Table 2). These were planted on 3 row plots of 5m long with an inter and intra row spacing of 90cm x 50cm making a planting density of 33 333 plants per hectare and they were replicated 3 times. Two F_1 trials were planted side by side for whilst one F_2 was planted in a

randomised complete block design (RCBD). Fertiliser was applied at the rate of 180-80-40 kg per hectare in terms nitrogen, phosphorous and potassium respectively to sowing and 140 kg N per hectare was applied as urea at about the V5 growth stage. All trials received standard cultural practices to control insects and weeds. Termites and stalkborers were controlled by spraying with Antkil (EC) at 5 ML per 20L of water and Sevin (WP) at 10 grams per 20 L of water, respectively. Weeding was done manually.

3.4 Inoculation and Spraying of Host Plants

At 4 weeks after planting at the V6 growth stage, one of the F_1 trials was inoculated with the isolated pathogen while the other one was sprayed as a control on a weekly basis (from V3 growth stage to V9) using Benomyl (Methyl 1-(butyl-carbamoyl)-2-benzimidazolecarbamate) (WP) at a concentration of 3grams per 10 litre of water. A backsprayer with a flat fan nozzle sprayer delivering 200L per hectare was used. In the inoculated trial, each plant in each plot received a dose of 5ml of inoculum which was applied in the whorls using a wash bottle each week. Caborandum powder (D and D Mineral Derivatives, Bronkorspruit, South Africa) was gently rubbed into the whorls of the maize plants before inoculation to encourage pathogen infection. Inoculations were done from the V6 to the R2 growth stage. A maize leaf blight susceptible inbred line L917, was planted as spreader rows around the inoculated trial to maximise infection of the host plants. There was only artificial inoculation as above on the F_2 trial.

Table 2: Experimental materials used in the study

F_1 Hybrids			F_2 Populations		
Name	**Pedigree**	**Origin**	**Name**	**Pedigree**	**Origin**
J34-1	ZM621A-10-1-1-1-2-BBBBBB/MAS[206/312]-23-2-1-1-BBBBB	MZ06B-120-5	J34-1	ZM621A-10-1-1-1-2-BBBBBB/MAS[206/312]-23-2-1-1-BBBBB]-B	MZ06B-120-5
J34-2	[SYN-USAB2/SYN-ELIB2]-12-1-1-1-BBB/[SYN USB1/SYN-ELIB1]-56-3-2-1-BBB	HA06A-N1110-2	J34-2	[SYN-USAB2/SYN-ELIB2]-12-1-1-1-BBB/[SYN USB1/SYN-ELIB1]-56-3-2-1-BBB]-B	HA06A-N1110-2
J34-3	USB1/SYN-ELIB1]-56-3-2-1-BBB1-1-2-2-]-B/CML488	HA06A-N1110-3	J34-3	USB1/SYN-ELIB1]-56-3-2-1-BBB1-1-2-2-]-B/CML488]-B	HA06A-N1110-3
J34-4	[(CML395/CML4440-B-4-1-3-1-B/CML395//DTPWC8 F31-1-1-2-2]-5-1-2-2-B/[CML444/CML395//SC/ZM605 #b-19-2-X-1-1-BBBBBB]-2-2-2-1-BBB	HA06A-N1110-4	J34-4	[(CML395/CML4440-B-4-1-3-1-B/CML395//DTPWC8 F31-1-1-2-2]-5-1-2-2-B/[CML444/CML395//SC/ZM605 #b-19-2-X-1-1-BBBBBB]-2-2-2-1-BBB]-B	HA06A-N1110-4
J34-5	TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1-1-2-1-1-2-1-1-BBB/ZM621A-10-1-1-1-2-BBBBBB	HA06A-N1110-5	J34-5	TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1-1-2-1-1-2-1-1-BBB/ZM621A-10-1-1-1-2-BBBBBB]-B	HA06A-N1110-5
J34-6	[TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-BBB/MAS[206/312]-23-2-1-1-BBBBB	HA06A-N1110-6	J34-6	[TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-BBB/MAS[206/312]-23-2-1-1-BBBBB]-B	HA06A-N1110-6

3.5 Disease Assessment

Disease severity scores

In the F_1 generation trial, eight (8) plants were chosen at random per plot and tagged for disease assessment on the inoculated trial plots. Ten (10) plants were chosen on the F_2 generation trial, as recommended by Falconer (1989). Disease damage (severity scores) on each tagged plant was assessed by using a score of (1-5), where 1 = no or very few lesions, and 5 = many lesions (heavily blighted). This was done from the V8 to the R6 growth stage and a total of six assessments were made in the F_1's and four were made in the F_2's .

Percent affected ear leaf area

Percent affected ear leaf area (PAELA) was scored on each tagged plant using the Horsfall – Barrat (1945) rating system as follows;

0 % = no disease-no lesions identifiable.

0.5 – 5 % of leaf area diseased = a few restricted small lesions.

6 – 15 % of leaf area diseased = several big lesions.

16 - 35 % of leaf area diseased = several large and coalesced lesions and

36 - >75 % of affected leaf area representing multitudes of coalesced lesions resulting in leaf wilting and tearing and blotching. If the ear leaf was not affected by the disease any leaf above the main ear was used for the assessment.

Incubation periods

Incubation periods (date from inoculation to appearance of typical symptoms) were assessed on individually tagged plants.

Lesion numbers and sizes

Lesion sizes (length and width) and number of lesions per tagged plant were recorded from the V8 to the R2 growth stage and could not be continued further because of coalescing of some lesions. The lesions were randomly selected on tagged plants in relation to lesion presence.

Area Under Disease Progress Curve (AUDPC)

The areas under disease progress curves were calculated for each genotype by using percent affected ear leaf area data at different assessment dates. A formula by Shanner and Finney (1980) was used. It was estimated by:

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where "t" is time in days of each reading, "y" is the percentage of affected foliage at each reading and "n" is the number of assessments.

Apparent Infection Rates (AIR)

The apparent infection rates (AIR) for each genotype were calculated based on disease severity scores which were regressed on assessment dates. Slopes of regression lines for

each genotype were computed representing an increase in disease severity over time using the exponential model.

3.6 Agronomic Data Collection

Agronomic Traits

Agronomic data collected on tagged plants were days to anthesis, plant and ear heights, and yield parameters. Days to anthesis were recorded as days from planting to pollen shedding, plant and ear heights as the height measured from ground level to point of ear attachment and end of tassel. In the F_1 trial anthesis date was recorded at 50% flowering per plot. Yield parameters were taken on per plot basis which included field weight (total weight of cobs), grain weight and grain moisture percentages. Field weights were taken using a PIXO-5 (20kg) spring balance and a seed moisture meter was used to get grain moisture percentages.

Grain yield and yield loss

Grain yield (t/ha) was calculated on both sprayed and inoculated trial on the F_1's as follows:

$$GY = FW \times \frac{GW}{FW} \times \frac{100\% - \text{Moisure \%}}{87.5\ \%} \times \frac{10\ 000}{\text{Plot area} \times 1000}$$

Where FW and GW are field and grain weights respectively.

Yield Loss (%) was calculated according to Campbell and Madden (1990) as follows:

$$\text{Loss (\%)} = \frac{\text{Grain yield from sprayed trial} - \text{Grain yield from inoculated trial}}{\text{Grain yield from sprayed trial}} \times 100$$

3.7 Data Analysis

Analysis of Variance

Before analysis of variance (ANOVA), data for all measured disease and agronomic traits were first tested for normality, and percent affected leaf area were normalised using the arcsine ($\sqrt{x+0.05}$) transformation (Snedecor and Cochran, 1980) while disease severity scores, number and sizes of lesions were normalized using the square root ($\sqrt{x+0.5}$) transformation (Gomez and Gomez, 1984). ANOVA and mean separation tests were computed using the transformed data. The Least Significant Differences (LSD) test was used to assess differences in means. GenStat 9th edition (Numerical Algorithms Group, LTD., Oxford, UK) statistical software was used for all analyses.

On the analyses of the F_1 hybrids, plot means were used to perform the ANOVA and to check if there were any significant differences between genotypes in all measured traits (Table 3).

Table 3: Analysis of variance for the F_1 hybrids

Source of variation	**Df**	**MS**
Hybrids (Between)	h - 1	M3
Replications	r - 1	M2
Error (Within)	(r -1) (h -1)	M1

Analysis of F_2 Populations: Variances, Heritability and Genetic advance

In the F_2 populations, an individual plant analyses (10 tagged plants) was used in the ANOVA to obtain significant differences between genotypes (plants) within a population and estimates of genetic variances in all measured traits. Variances were estimated according to Singh and Chaudhary (1985) by using the ANOVA expected mean squares for the various sources of variation (Table 4). Genetic variance ($\sigma^2 g$) between plants within a population was estimated as: $\sigma^2 g = M3 - M1/r$ and environmental (error) variance was estimated as $\sigma^2 e = M1$ where *M1* is the mean squares for error, *M3* is the mean squares for genotypes and *r* is the number of replications (Table 4). Phenotypic or total ($\sigma^2 p$) variance was calculated as the sum of genotypic and error variance ($\sigma^2 p$) = $\sigma^2 g$ + $\sigma^2 e$. Heritability (broad – sense) was calculated according to Falconer (1989) as:

$h^2 b = \sigma^2 g / (\sigma^2 g + \sigma^2 e)$.

Table 4: Analyses of variance and expected sum of squares for each F_2 population

Source of variation	Df	MS	EMS
Genotypes (Between)	g - 1	M3	$\sigma^2 e + r\sigma^2 g$
Replications	r - 1	M2	_____
Error (Within)	(r – 1)(g – 1)	M1	$\sigma^2 e$

Genetic and phenotypic coefficient of variation (GCV and PCV) within populations were estimated according to Burton (1952) as: GCV = $\sqrt{\sigma^2 g}/X$, and PCV = $\sqrt{\sigma^2 p}/X$, where X is the population mean.

Genetic advance (GA) was estimated as the percentage of the population mean following Jonhson *et al.* (1955) method. It was computed as: GA (%) = k (GCV) ($\sqrt{h^2}$ b), where the k is equal to 2.06, the expected value for a normally distributed population when 5% of the families are selected. Standard error (SE) of the population mean was computed according to the procedures of Cockerham (1963) as:

$$\sqrt{2(EMS)/r}$$

Where *EMS* and *r* are the error mean squares (variance) of an agronomic trait or resistance parameter and number of replications of the trial, respectively.

3.7.3 Genotypic and Phenotypic Correlation Analyses

Genotypic and phenotypic correlation between all disease resistance parameters and agronomic traits were calculated according to Falconer (1989). Phenotypic correlations (r_p) was calculated as: $r_p = \frac{Cov\ (X,Y)}{\sqrt{\sigma^2\ (X)\ \sigma^2\ (Y)}}$

Genotypic correlations (r_g) were estimated as:

$$r_g = \frac{CovG\ (X,Y)}{\sqrt{\sigma^2 G\ (X)\ \sigma^2 G\ (Y)}}$$

Where Cov (X,Y) is the covariance between traits X and Y and σ^2 (X) σ^2 (Y) are the variances for traits X and Y. CovG (X,Y) is the genetic covariance between traits X and Y and σ^2 G (X) σ^2 G (Y) are the genetic variances for traits X and Y, respectively. The

phenotypic and genotypic variances were pooled across the six populations. Both correlations were tested for significance using tables of Steel and Torrie (1980).

Chapter 4

Results

4.1 Conidia of *Helminthosporium turcicum*, the causal pathogen of maize leaf blight

Conidia isolated were spindly shaped, brown to dark brown in colour with a range of 5-12 septa. The average size of the conidia was 90 - 110 mμ in length and 15 – 30 mμ wide as measured with a calibrated light microscope. Some were slightly curved on one side while others were straight and their bases had a protruding hilum. These morphological results are similar to the ones obtained by Drechsler (1925) working with graminicolous species of *Helminthosporium* (Figure 2).

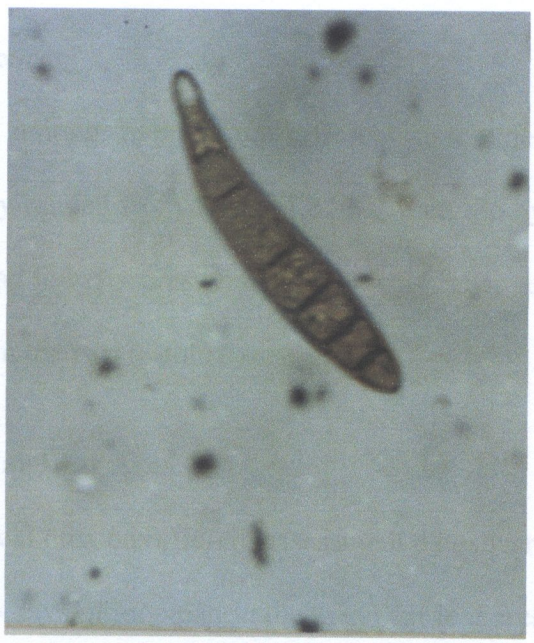

Figure 2: Conidia of *Helminthosporium turcicum*

4.2 Symptoms of Maize leaf Blight

Typical lesions appeared in all F_1 genotypes and F_2 populations after inoculation with the pathogen. They first appeared as small chlorotic white flecks at the point of penetration (whorls) which widened to linear narrow lesions ranging in size from 5 – 10 cm long and 1- 3 cm wide. The lower leaves were usually attacked first. The spots elongated linearly between the veins of the leaves and as they aged, the central area became dark brown with margins full of sporulation. As the attack ended, the lesions coalesced to form a blight and the leaves totally dried out. The chlorotic lesions on lower leaves indicated hybrid resistance (Figure 3).

4.3 Characterisation of F_1 Hybrids

4.3.1 Disease Severity Scores

Disease severity scores among hybrids on different assessment dates were highly significant ($P<0.01$). They ranged from 1.12 – 4.84 between the hybrids at 16 to 56 days after inoculation. J34-3 had lower scores while J34- 3 had higher scores. The other entries were intermediate between the two (Table 5).

4.3.2 Percent Affected Ear Leaf Area

The percent affected ear leaf area on different assessment dates were significant ($P<0.05$) between hybrids. After 56 days of inoculation the mean ear leaf area affected by the disease was 60.49% on J34-2, followed by J43-4 with 41.56%. J34-3 had the least final affected area leaf area of 4.04%. (Table 6).

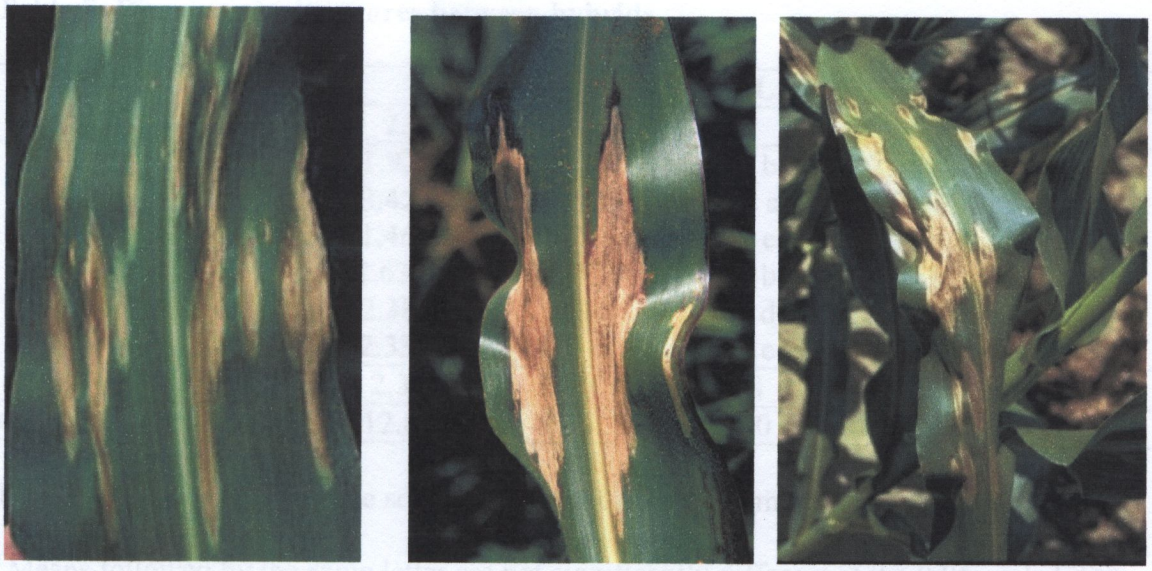

Figure 3: Symptoms of maize leaf blight caused by *Helminthosporium turcicum*

Table 5: Disease severity scores between hybrids

Hybrids	DAYS AFTER INOCULATION					
	16	**23**	**31**	**39**	**48**	**56**
J34-1	1.50 d y*	1.92 e	2.25 c	2.48 b	2.53 e	2.55 c
J34-2	2.44 a	3.42 a	4.15 a	4.50 a	4.71 a	4.84 a
J34-3	1.12 e	1.46 b	1.67 d	1.83 e	1.96 e	1.96 d
J34-4	2.18 b	2.63 b	3.29 b	3.50 b	4.03 b	4.09 b
J34-5	1.84 c	2.38 c	2.47 c	2.96 d	3.13 d	3.27 b
J34-6	1.84 c	2.33 d	2.75 c	3.22 c	3.55 c	3.77 b
MEAN	1.82	2.35	2.76	3.08	3.33	3.40
C. V. (%)	6.29	12.85	9.76	11.20	10.39	12.67

Data were transformed by the square root ($\sqrt{x+0.5}$) transformation.

[y] Means followed by the same letter are not significantly different at the 0.05 probability level according to DMR Test

Table 6: Percent affected ear leaf area between hybrids

Hybrids	DAYS AFTER INOCULATION					
	16	23	31	39	48	56
J34-1	1.07 c y *	3.92 c	5.15 b	6.38 c	7.48 d	10.26 d
J34-2	8.71 a	20.09 a	31.29 a	39.17 a	49.38 a	60.49 a
J34-3	0.31 c	0.94 d	2.33 b	3.04 c	3.79 e	4.04 e
J34-4	6.19 b	11.17 b	15.34 b	25.38 b	33.96 b	41.56 b
J34-5	1.59 c	3.88 c	5.63 b	13.14 b	20.54 c	18.51 c
J34-6	2.57 c	4.42 c	8.29 b	9.67 c	17.42 c	19.75 c
MEAN	10.86	15.38	18.06	19.57	25.69	28.54
C. V. (%)	2.24	11.76	5.18	12.4	11.48	4.18

Data were transformed by the arcsine ($\sqrt{x+0.05}$) transformation.

[y] Means followed by the same letter are not significantly different at the 0.05 probability level according to DMR Test.

4.3.3 Incubation Periods, Number and Sizes of Lesions

Incubation periods between genotypes were significant ($P< 0.05$). They ranged from 9-14 days. J34-6 took 9 days and J34-3 took 14 days. Twenty three days after inoculation, the number of lesions between genotypes was 12 and 21 for J34-3 and J34-1. J34-2 and J34-6 recorded 65 and 46 lesions respectively. Lesion sizes at 23 days after inoculation was greatest on J34-2 with 12.30cm^2 and the least was on J34-3 with 1.40 cm^2 and there were significant differences ($P<0.05$) between genotypes (Table 7).

4.3.4 Area under Disease Progress Curves (AUDPC) and Apparent Infection Rates (AIR)

There were highly significant ($P<0.01$) differences between hybrids for areas under disease progress curves. They ranged from 132 to 2188 %-days with J34-2 having the largest and J34-3 with the least. The same trend was observed for apparent infection rates with J34-2 and J34-3 having rates of 1.34 and 0.11 day $^{-1}$ which were the highest and the least rates, respectively (Table 8 and figure 4).

Table 7: Incubation periods, number and sizes of lesions between hybrids

Hybrids	Incubation period	Number of lesions [y]	Lesion sizes (cm^2) [y]
J34-1	11 [b y *]	21 [e]	6.10 [a]
J34-2	11 [b]	65 [a]	12.30 [a]
J34-3	14 [a]	12 [f]	1.40 [b]
J34-4	11 [b]	44 [c]	10.04 [a]
J34-5	10 [c]	30 [d]	9.41 [a]
J34-6	9 [c]	46 [b]	7.00 [a]
MEAN	11	36.3	7.71
C. V. (%)	11.14	8.9	7.24

Data were transformed by the square root ($\sqrt{x+0.5}$) transformation. Data was collected 23 days after inoculation

[y] Means followed by the same letter are not significantly different at the 0.05 probability level according to DMR Test

Table 8: Area under disease progress curves (AUDPC) and apparent infection rates (AIR) between hybrids

Hybrids	AUDPC (%-days)	AIR (day $^{-1}$)
J34-1	366 [d**]	0.22 [d]
J34-2	2188 [a]	1.34 [a]
J34-3	132 [e]	0.11 [e]
J34-4	1359 [b]	0.73 [b]
J34-5	570 [c]	0.55 [c]
J34-6	602 [c]	0.48 [c]
MEAN	5216	3.43
C. V. (%)	11.33	6.44

** Means followed by the same letter are not significantly different at the 0.05 probability level according to DMR Test

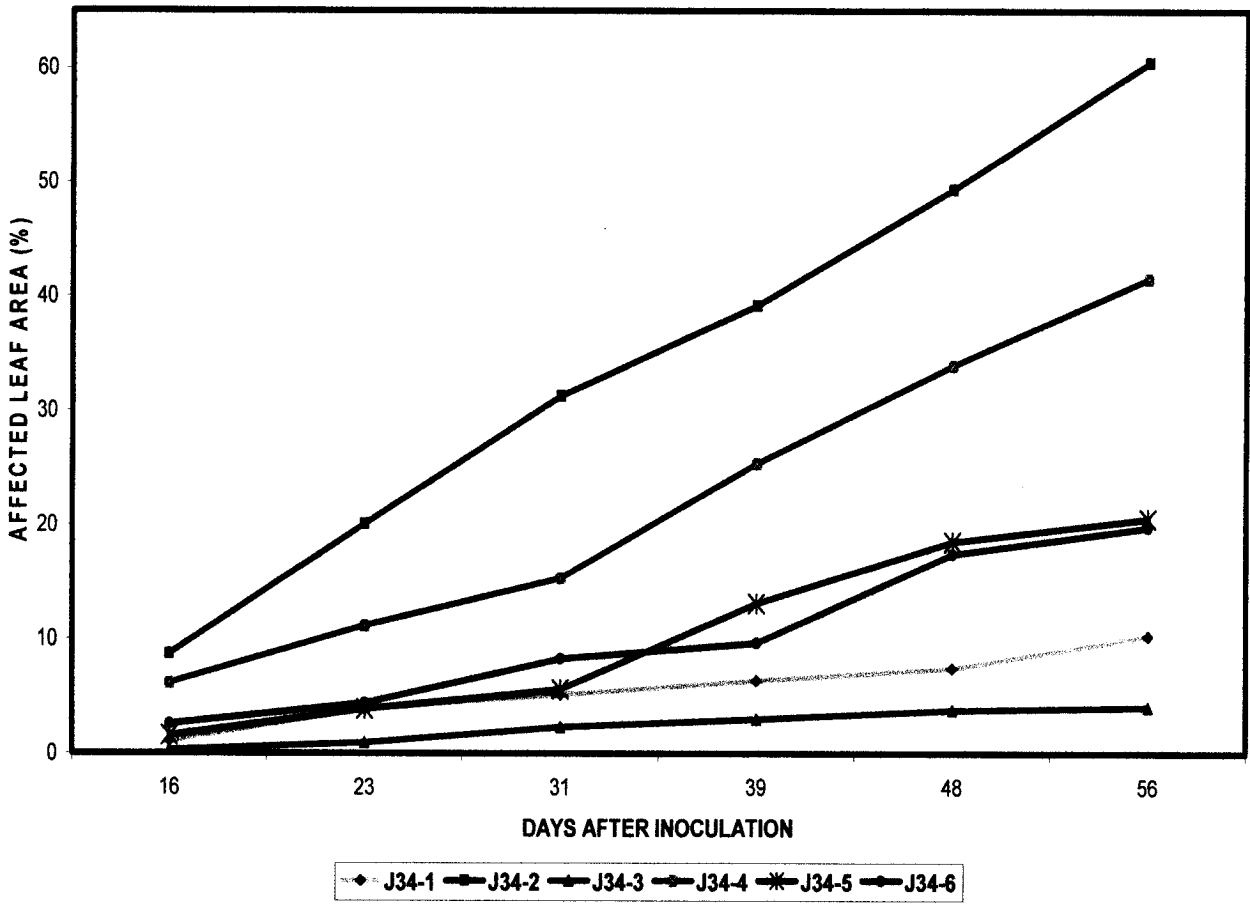

Figure 4: Disease progress curves of six F_1 maize hybrids

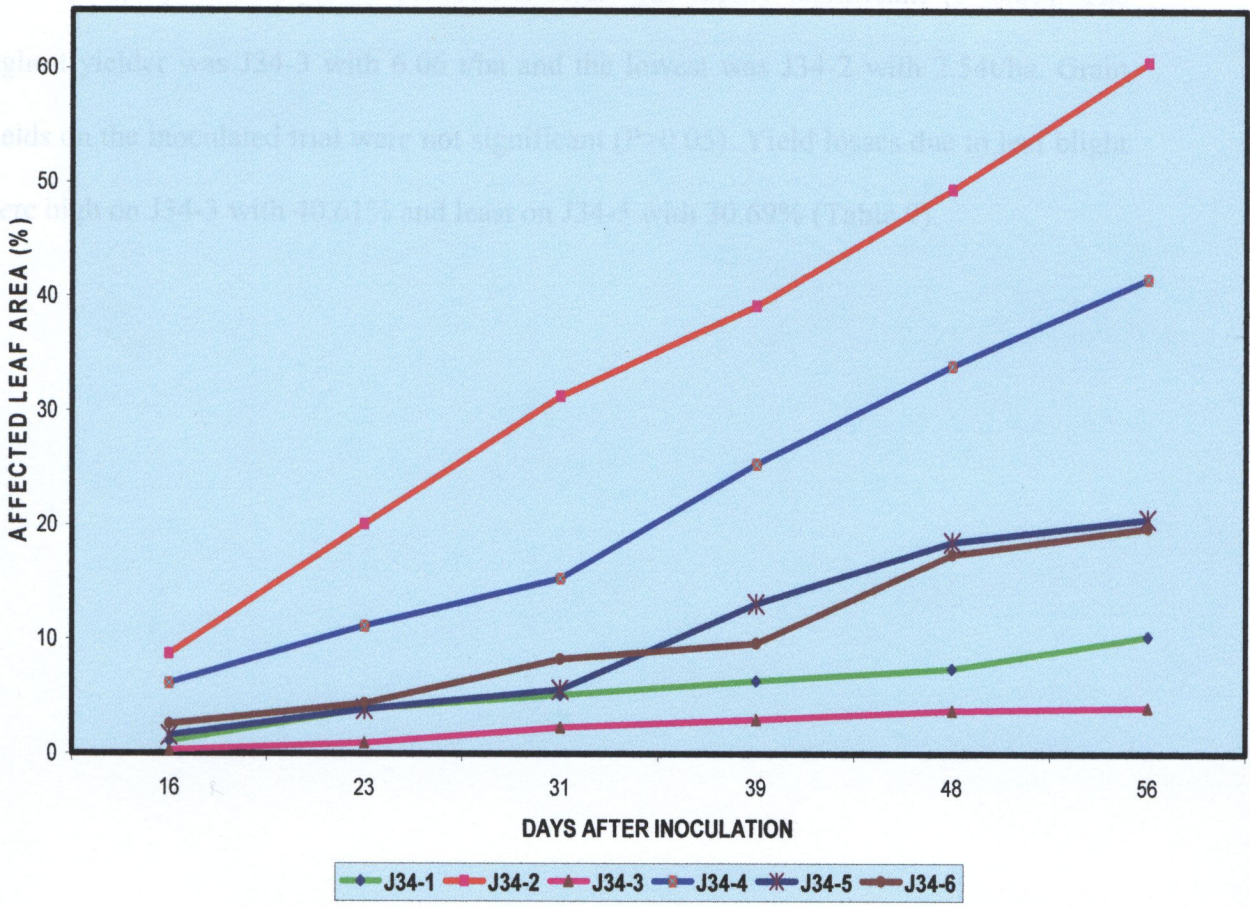

Figure 4: Disease progress curves of six F_1 maize hybrids

4.3.5 Plant heights, 50% flowering dates, Grain yields and Yield Loss

Between hybrids, plant height was significant ($P<0.05$) and it ranged from 2.2m to 2.7m high. Hybrids J34-4 was the tallest and J34-3 and J34-1 were the shortest. There were also highly significant differences ($P<0.01$) between genotypes for flowering dates. J34-6 flowered early at 56 days while J34-3 reached 50% flowering at 64 days. On the Benomyl sprayed trial, yields between hybrids was highly significant ($P<0.01$). The highest yielder was J34-3 with 6.06 t/ha and the lowest was J34-2 with 2.54t/ha. Grain yields on the inoculated trial were not significant ($P>0.05$). Yield losses due to leaf blight were high on J34-3 with 40.61% and least on J34-5 with 30.69% (Table 9).

Table 9: Plant heights, 50% flowering dates, Grain yields and Yield Loss

Genotype	Plant height (m)	50% Flowering(days)	Grain yield (t/ha)		Yield loss (%)
			Sprayed	Inoculated	
J34-1	2.2 [d]	62 [b]	4.74 [b]	3.52	38.64
J34-2	2.5 [b]	56 [d]	4.15 [d]	2.54	38.80
J34-3	2.2 [c]	64 [a]	6.06 [a]	3.60	40.61
J34-4	2.7 [a]	58 [c]	4.17 [d]	2.81	32.57
J34-5	2.5 [b]	61 [c]	4.78 [c]	3.30	30.69
J34-6	2.5 [b]	58 [c]	4.56 [c]	3.41	31.14
MEAN	2.4	60	4.74	3.20	
C. V. (%)	10.56	8.21	6.49	NS	

** Means followed by the same letter are not significantly different at the 0.05 probability level according to DMR Test

4.4 Genetic Characterisation of F_2 Populations

4.4.1 Agronomic traits

In population J34-1, plant heights ranged from 186- 258 cm and exhibited the maximum broad sense heritability of 91.81% with the highest genetic advance as percent of mean of 30.27. Ear heights had a genetic variance of 196.61, genetic coefficient of variation of 14.76%, broad sense heritability of 86.27% and genetic advance as percent of mean of 28.24. Population J34-2 had flowering dates that ranged from 52 to 62 days with a genetic variance of 108.87. Plant heights had the lowest broad sense heritability and genetic advance as percent of mean of 21.91% and 11.73 respectively. In population J34-3, the highest genetic variance was estimated in plant height (1785.19) followed by flowering dates (406.69). Similarly, the phenotypic variance was maximum in the case of plant height (2494.72%) and flowering dates (1314.99). Broad sense heritability was highest for ear height and low for flowering dates at 79.32 and 30.92% respectively.

In population J34-4, extremely low broad sense heritability was observed in flowering dates and ear heights. They had 23.04 and 17.95% respectively. There was low genetic coefficient of variation of (18.39%) in population J34-5 for plant heights, followed by flowering dates with 16.44%. Plant heights ranged from 175 to 236 cm with a broad sense heritability 59.94% and genetic advance as percent of mean of 23.57. Flowering dates exhibited a high broad sense heritability and genetic advance as percent of mean of 93.86% and 32.82 respectively. Population J34-6 had flowering dates ranging from 52 to 75 days, ear heights from 70 to 109 cm and plant heights from 183- 248 cm. Ear heights

and flowering dates exhibited a high broad sense heritability of 95.06 and 81.19% respectively (Table 10).

4.4.2 Leaf Blight Resistance

Population J34-1 showed significant ($P<0.01$) significances between genotypes in leaf blight resistance as measured by the different resistance parameters. Disease severity scores had a mean of 3.74 with genotypic and phenotypic variation of 0.99 and 1.42, respectively. Broad sense heritability and genetic advance (as percent of mean) was 69.7 and 45.75, respectively. Percent affected ear leaf areas had a mean of 42.10% with a genetic coefficient of variation of 8.34% and broad sense heritability of 60.56. Incubation periods had a mean of 11 days and ranged from 8 to 15 days. Genotypic, phenotypic variance and genetic advance (as percent of mean) was 5.25, 6.41 and 32.46 respectively.

Lesion sizes showed a mean of 6.8 cm^2 with a genotypic and phenotypic coefficient of variation of 38.40 and 39.10%, respectively. Broad sense heritability and genetic advance (as percent of mean) were 75.57 and 62.32 respectively. Area under disease progress curve had a mean of 844.48 %-days with genetic, phenotypic variance and broad sense heritability of 15.56, 18.0 and 86.43, respectively (Table 11).

Table10: Genetic Characterisation of six maize populations for 3 agronomic traits

Population	Agronomic Traits	Genetic parameters								
		Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
J34-1	Flowering date	60	55- 64	83.17	12.24	95.41	15.20	16.28	87.17	29.23
	Ear height	95	81-112	196.61	911.05	227.88	14.76	15.89	86.27	28.24
	Plant height	220	186 - 258	1138.93	1043.9	1240.59	15.34	16.01	91.81	30.27
J34-2	Flowering date	58	52-62	108.87	36.25	145.12	17.99	20.77	75.02	32.09
	Ear height	92	80-108	126.59	50.63	177.22	12.23	14.47	71.43	21.29
	Plant height	216	190-260	691.02	2462.9	3153.94	12.17	26.00	21.91	11.73
J34-3	Flowering date	58	50-66	406.69	908.3	1314.99	34.77	39.09	30.92	39.83
	Ear height	88	76-100	183.42	47.81	231.23	15.39	17.28	79.32	28.23
	Plant height	212	178-244	1785.19	709.53	2494.72	19.93	23.56	71.56	34.73
J34-4	Flowering date	57	51-64	56.87	189.91	246.78	13.23	27.56	23.04	13.08
	Ear height	93	76-111	89.46	408.72	498.18	10.217	24.00	17.95	8.88
	Plant height	215	172-260	613.33	199.93	2533.26	11.52	23.41	24.21	11.67
J34-5	Flowering date	62	54-68	103.89	6.8	110.69	16.44	16.97	93.86	32.82
	Ear height	90	79-99	273.94	47.35	371.29	18.39	21.41	73.78	32.54
	Plant height	219	175-236	1047.70	700.13	1747.83	14.78	19.09	59.94	23.57
J34-6	Flowering date	65	52-75	44.04	10.20	54.24	10.21	11.33	81.19	18.95
	Ear height	90	70-109	228.07	11.84	239.91	16.78	17.21	95.06	33.70
	Plant height	211	183-248	224.87	497.74	722.61	12.74	22.46	32.18	11.12

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

Table11: Genetic Characterisation of J34-1 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	3.74 (±0.31)	2.71 – 3.25	0.99	0.43	1.42	26.6	31.9	69.7	45.75
Percent affected ear leaf area (%)	42.1 (±0.31)	37.21 – 53.74	12.33	8.03	20.36	8.34	10.72	60.56	13.37
Incubation period (days)	11 (±0.51)	8 - 15	5.25	1.16	6.41	17.41	23.04	81.90	32.46
Number of lesions	13 (±0.41)	4- 9	2.50	8.92	11.42	12.17	26.0	21.89	11.73
Lesion sizes (cm^2)	6.8 (±0.36)	4.3 – 10.4	1.73	0.59	2.32	38.40	39.10	75.57	62.32
Area under disease progress curve (% -days	844.48 (±0.74)	236.74- 2049.83	15.56	2.44	18.00	14.77	15.89	86.43	28.29
Apparent infection rates (day^{-1})	0.58 (±0.02)	0.10 – 1.27	0.008	0.002	0.01	15.39	18.09	80.00	28.36

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

In population J34-2 all resistance parameters showed highly significant differences ($P<0.01$) between genotypes for leaf blight resistance. Disease severity scores had a mean of 3.05 with a genotypic and phenotypic coefficient of variation of 15.11 and 17.31%, respectively. Broad sense heritability and genetic advance (as percent of mean) was 76.35 and 27.19, respectively. Percent affected ear leaf area showed a mean of 36.72% with a genetic, phenotypic variance and broad sense heritability of 5.4, 6.9 and 78.31, respectively. The number of lesions per plant had a mean of 12 with a range of 5 to 21 lesions. Broad sense heritability and genetic advance was 72.31 and 27.99, respectively. Apparent infection rates had a mean of 0.43 day^{-1}. The genotypic and phenotypic coefficient of variation was 17.39 and 20.09%, respectively with a broad sense heritability of 74.24 and genetic advance (as percent of mean) of 30.87 (Table 12).

Population J34-3 showed significant ($P<0.01$) significances between genotypes in leaf blight resistance as measured by the different resistance parameters. Disease severity scores had a mean of 2.87 and ranged from 1.42 to 3.74 with genotypic and phenotypic variation of 0.59 and 0.89, respectively. Broad sense heritability and genetic advance (as percent of mean) was 66.31 and 40.98, respectively. Percent affected ear leaf areas had a mean of 33.47% with a genetic coefficient of variation of 17.47% and broad sense heritability of 65.15 (Table 13).

Table 12: Genetic Characterisation of J34-2 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	3.05 (±0.12)	2.88 – 3.26	0.21	0.07	0.28	15.11	17.31	76.35	27.19
Percent affected ear leaf area (%)	36.72 (±0.58)	10.44 – 41.70	5.4	1.5	6.9	19.94	22.59	78.31	36.35
Incubation period (days)	9 (±0.08)	8- 14	2.045	0.03	2.07	15.89	16.00	98.60	32.50
Number of lesions	12 (±0.56)	5 - 21	3.67	1.41	5.08	15.98	18.79	72.31	27.99
Lesion sizes (cm^2)	8.7 (±0.38)	5.05- 14.77	1.55	0.64	2.190	14.39	17.01	70.78	24.93
Area under disease progress curve (% -days	628.75 (±0.55)	321.73- 1388.41	10.37	1.36	11.73	16.20	17.23	88.41	31.30
Apparent infection rates (day^{-1})	0.43 (±0.21)	0.11- 0.96	0.56	0.192	0.75	17.39	20.09	74.24	30.87

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

Incubation periods had a mean of 10 days and ranged from 8 to 13 days. Genotypic, phenotypic variance and genetic advance (as percent of mean) was 2.16, 2.68 and 27.17, respectively. Lesion sizes showed a mean of 8.2 cm^2 with a genotypic and phenotypic coefficient of variation of 83.18 and 85.64%, respectively. Broad sense heritability and genetic advance (as percent of mean) were 94.34 and 66.43, respectively. Area under disease progress curve had a mean of 531.71 %-days with genetic, phenotypic variance and broad sense heritability of 3.57, 5.92 and 60.30, respectively. Apparent infection rates had a mean of 0.40 with a genetic and phenotypic coefficient of variation of 23.57 and 30.66%, respectively (Table 13).

In population J34-4, only the apparent infection rate parameter showed significant differences ($P<0.01$) between plants for leaf blight resistance. The other resistance parameters showed non significant differences ($P>0.05$) between plants. Disease severity scores had a mean of 3.44 with a genotypic and phenotypic coefficient of variation of 10.97 and 23.84 %, respectively. Broad sense heritability and genetic advance (as percent of mean) was 21.84 and 10.56, respectively (Table 14).

Table13: Genetic Characterisation of J34-3 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	2.87 (±0.29)	1.42 – 3.74	0.59	0.35	0.89	25.54	32.98	66.31	40.98
Percent affected ear leaf area (%)	33.47 (±0.02)	15.50 – 44.76	34.18	18.28	52.46	17.47	21.64	65.15	29.05
Incubation period (days)	10 (±0.34)	8- 13	2.16	0.52	2.68	14.69	16.36	80.59	27.17
Number of lesions	13 (±0.45)	6 - 14	12.31	9.41	12.72	26.99	27.43	76.78	54.79
Lesion sizes (cm^2)	8.2 (±0.79)	4.05- 12.65	46.52	2.8	49.32	83.18	85.64	94.34	66.43
Area under disease progress curve (% -days	531.71 (±0.72)	200 – 1201.33	3.565	2.355	5.92	11.29	14.74	60.30	17.96
Apparent infection rates (day^{-1})	0.40 (±0.49)	0.12- 0.86	0.0089	0.0111	0.02	23.57	30.66	44.50	32.38

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

Table14: Genetic Characterisation of J34-4 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	3.44 (±0.34)	2.88- 4.69	0.142	0.508	0.65	10.97	23.48	21.84	10.56
Percent affected ear leaf area (%)	25.59 (±1.65)	23.42- 27.74	5.36	12.3	17.66	5.69	10.33	30.35	6.46
Incubation period (days)	9 (±1.46)	7 -13	4.46	9.6	14.06	24.50	41.62	34.57	29.67
Lesion sizes (cm^2)	7.40 (±0.43)	5.51-11.50	0.482	0.816	1.298	9.38	15.40	37.13	11.77
Number of lesions	11 (±2.09)	9.00- 13.00	11.49	19.67	31.25	21.19	34.94	36.77	26.47
Area under disease progress curve (% -days)	607.58 (±2.04)	414.6 – 822.7	6.58	18.7	25.28	13.35	26.17	26.03	14.03
Apparent infection rates (day^{-1})	0.55 (±0.92)	0.26 – 0.72	0.002	0.038	0.04	8.59	11.75	55.80	13.22

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

In population J34-5, disease severity scores had a mean of 3.68 with genetic and phenotypic variances of 0.36 and 0.79, respectively. Broad sense heritability was 45.57 and genetic advance (as percent of mean) was 21.56. Percent affected ear leaf area showed a mean of 39.44 % with genotypic and phenotypic coefficient of variation of 20.41 and 24.04 %, respectively.

Mean incubation periods was 10 days with genetic, phenotypic variance and broad sense heritability being 2.49, 5.75 and 43.30. Lesion sizes showed a mean of 9.21 cm^2 and they ranged from 5.30 to 14.63 cm^2. Broad sense heritability and genetic advance (as percent of mean) was 71.81 and 36.62, respectively. The area under disease progress curve parameter had a mean of 621.41 %-days with genetic and phenotypic coefficient of variation of 30.98 and 35.44 %, respectively. Apparent infection rates showed a mean of 0.60 day^{-1} with genetic variance of 0.0032 and broad sense heritability of 88.90. (Table 15).

In population J34-6, most disease resistance parameters showed a non significant differences ($P>0.05$) between plants for leaf blight resistance. Disease severity scores had a mean of 3.07 with a genotypic and phenotypic coefficient of variation of 20.50 and 37.67%, respectively. Broad sense heritability and genetic advance (as percent of mean) was 29.10 and 22.78, respectively. Percent affected ear leaf area showed a mean of 38.94 % with a genetic, phenotypic variance and broad sense heritability of 35.73, 120.24 and 29.72 respectively. The number of lesions per plant had a mean of 14 with a range of 7 to 23 lesions. Broad sense heritability and genetic advance was 37.96 and 7.86, respectively (Table 16).

Table15: Genetic Characterisation of J34-5 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	3.68 (±0.31)	1.66- 4.32	0.36	0.43	0.79	15.5	22.98	45.57	21.56
Percent affected ear leaf area (%)	39.44 (±2.37)	14.72- 52.64	64.79	25.19	89.98	20.41	24.04	72.08	35.69
Incubation period (days)	10 (±0.85)	7 -14	2.49	3.26	5.75	15.80	23.98	43.30	21.42
Lesion sizes (cm^2)	9.21 (±0.54)	5.30- 14.63	3.73	1.31	2.42	20.98	16.91	71.81	36.62
Number of lesions	15 (±0.26)	6-18	2.51	0.31	2.82	10.56	11.21	89.00	20.52
Area under disease progress curve (% -days	621.41 (±1.59)	220.11- 937.83	37.06	11.44	48.500	30.98	35.44	76.41	55.78
Apparent infection rates (day^{-1})	0.60 (±0.03)	0.22-0.84	0.0032	0.004	0.0036	9.49	10.5	88.9	18.43

$\sigma^2 g$: genetic variance, $\sigma^2 e$: error variance, $\sigma^2 p$: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 b: broad sense heritability

Area under disease progress curves had a population mean of 849.97 %-days with a broad sense heritability of 45.20. Apparent infection rates had a mean of 0.65 day^{-1}. The genotypic and phenotypic coefficient of variation was 11.94 and 24.44 %, respectively with a broad sense heritability of 24.0 and genetic advance (as percent of mean) of 12.05. (Table 16).

4.4.3 Genotypic and Phenotypic Correlation Analysis

An analysis of genotypic and phenotypic correlation revealed that there were highly significant ($P<0.01$) correlations between all resistance parameters. When considering genotypic correlations, area under disease progress curves and disease severity scores were tightly correlated at 0.88 followed by lesion sizes with disease severity scores at 0.86 and apparent Infection rates and area under disease progress curves at 0.83. Incubation periods showed a negative correlation with other parameters. Flowering dates were also negatively associated with most resistance parameters. There were no significant ($P>0.05$) correlations between ear and plant heights with all disease resistance parameters, but plant and ear heights were tightly correlated at 0.57 (Table 17).

Table16: Genetic Characterisation of J34-6 Population for maize leaf blight resistance.

Disease resistance parameters	Genetic parameters								
	Population mean	Range	σ^2g	σ^2e	σ^2p	GCV (%)	PCV (%)	h^2 b	Genetic Advance as % of mean
Disease severity score (1-5)	3.07 (±0.46)	2.99- 3.48	0.39	0.95	1.34	20.50	37.67	29.10	22.78
Percent ear affected leaf area (%)	38.94 (±4.33)	10.66- 42.72	35.73	84.51	120.24	15.35	28.16	29.72	11.49
Incubation period (days)	10 (±1.38)	7-14	1.05	8.56	9.61	10.24	31.00	10.93	6.97
Lesion sizes (cm^2)	10.9 (±1.14)	5.25-16.99	5.86	1.81	7.67	12.34	25.40	23.60	12.35
Number of lesions	14 (±0.59)	7-23	0.75	1.6	2.35	6.19	10.94	37.96	7.86
Area under disease progress curve (% -days	849.97 (±1.48)	322.41- 1486.63	8.10	9.82	17.92	10.59	15.75	45.20	14.67
Apparent infection rates (day^{-1})	0.65 (±0.6)	0.10-0.97	0.0060	0.019	0.025	11.94	24.44	24.00	12.049

σ^2g: genetic variance, σ^2e: error variance, σ^2p: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variance, h^2 b: broad sense heritability

Table 17: Genotypic and Phenotypic Correlation Analysis

Traits	DSS (1-5)	PAELA (%)	I P (days)	LS (cm^2)	NL	AUDPC (% days)	AIR (day^{-1})	Flowering date (Days)	Ear height (cm)	Plant height (cm)
DSS (1-5)		0.69**	-0.50*	0.86**	0.69**	0.88**	0.64**	-0.82 **	0.26	0.11
PAELA (%)	0.64**		-0.55*	0.67**	0.68**	0.67**	0.79**	-0.61**	0.12	0.14
I P (days)	-0.47*	-0.53*		-0.65**	-0.51*	-0.73**	-0.44*	-0.85**	0.27	-0.15
LS (cm^2)	0.81**	0.62**	-0.61**		0.63**	0.69**	0.62**	-0.44*	0.29	0.12
NL	0.67**	0.63**	-0.47*	0.58*		0.59*	0.66**	-0.44*	0.17	0.17
AUDPC (% -days)	0.84**	0.56*	-0.63**	0.61**	0.53*		0.83**	-0.61**	0.12	0.09
AIR (day^{-1})	0.66**	0.77**	-0.41*	0.53*	0.61**	0.81**		-0.70**	0.11	0.21
Flowering dates (Days)	-0.77**	-0.55*	-0.79**	-0.31	-0.39*	-0.43*	-0.66**		-0.13	0.19
Ear height (cm)	0.21	0.19	0.30	0.06	0.23	0.16	0.08	-0.09		0.57*
Plant height (cm)	0.08	0.11	0.03	0.27	-0.12	-0.04	0.14	-0.17	0.41*	

Phenotypic correlation below diagonal and genotypic correlation above diagonal. DSS: Disease severity scores, PAELA: Percent affected ear leaf area, IP: Incubation periods, LS: Lesion sizes, NL: Number of lesions, AUDPC: Area under disease progress curve, AIR: Apparent infection rate

*, ** Significant at 0.05 and 0.01, respectively

Chapter Five

Discussion

Artificial inoculation with the *Helminthosporium turcicum* conidia led to the development of leaf blight in all genotypes and hybrids under investigation. Though there was frequent rainfall during the crop season, there was intermittent short dry spells for disease development. The disease first manifested itself 8 to 14 days after inoculation in the different test materials. The diseases quickly became severe by the time of silking. Similar observations were made by De Leon and Pandey (1989) working on maize inbred lines. Some plants within the segregating F_2 populations were prematurely killed by leaf blight.

Six different types of disease resistance parameters were utilised for disease assessment. Besides disease severity scores which was based on general plant observation all other parameters were based on actual physical measurements and counting of lesions on individual plants. Assessment of percentage of infected ear leaf areas in a uniformly inoculated plot facilitated the evaluation of type I (resistance to initial infection) and type II (resistance to spread of disease after initial infection) resistance simultaneously.

Evaluation of the non segregating F_1 hybrids revealed that there were significant differences between the hybrids for their response to the disease as measured by the various disease resistance parameters. J34-1 and J34-3 showed the highest levels of resistance as shown by the significant lower values of resistance parameters when compared with the other hybrids. They tended to have a greater part of the foliage free of

disease (very few and small lesions) in spite of the disease pressure and favourable conditions. This kind of resistance was termed rate – reducing resistance by Freppon *et al.* (1994). A hypersensitive response defence mechanism was suspected to be involved in J34-3 as shown by the small lesions sizes and slow disease progress over time. In hybrids J34-2 and J34-4 resistance parameters indicated that they were more susceptible to leaf blight. Disease severity scores and percent affected ear leaf area had high values across assessment dates and they had significantly many and larger lesions. They also had higher values on apparent infection rates which measure the absolute rate of disease increase over time.

Benomyl fungicide application controlled leaf blight by minimising infection by the pathogen and slowing the rate of disease development. Grain yields of all hybrids on the sprayed trial were significantly greater than that those in the inoculated trial. These effects may be attributed to reduced photosynthesis due to reduced available photosynthetic areas on the leaves of the inoculated trial. This in turn leads to reduced movement of metabolites to developing seeds with seed size and weight reduction as the normal consequence. Similar observations were made by Dodd (1980) who was investigating the effects of foliar diseases in maize productivity. Yield losses were more pronounced on J34-3 (40.61%) and J34-2 (38.80%), a similar observation was made by Raymundo and Hooker (1981) and Levy (1997) who reported yield losses between 20 to 54%. Interestingly these hybrids (J34-3 and J34-2) were discovered to be more resistant and susceptible to leaf blight, respectively. This might be due to the fact that on the spray trial the hybrids were able to reveal their true genotypic potential in the absence of leaf

blight while on the inoculated trial they were limited due to the stress and thus low yields. They competed very well with the other hybrids on the sprayed trial as shown by the yields, but were severely affected on the inoculated trial. This may explain the non significance in yields between hybrids on the inoculated trial.

The analyses of the segregating F_2 populations revealed that variation in leaf blight resistance as measured by the various resistance parameters was clearly quantitative. The continuous distribution of the resistance parameters values in some populations indicated that the resistance was most likely controlled by a polygenic system as suggested by Sigh *et al.*(1995), Van Ginkel *et al.* (1996), Ban and Suenaga (1998).

Observed variation among plants within populations can be due to genetic differences or environmental causes. In all cases, genetic coefficient of variation was less than the phenotypic coefficient of variation, indicating the role of the environment (heterogeneity in soil fertility status or other unpredictable factors) in the expression of leaf blight resistance. The characters possessing high genetic coefficient of variation stand greater chances of improvement through selection and it is well known that high heritability improves selection based on phenotypic values. A broad sense heritability estimate is used to determine the portion of phenotype which is due to the genotype. According to Johnson *et al.* (1995) and Panse (1957), heritability estimates and genetic advance as percent of mean should be considered jointly. The genetic advance (as percent of mean) is a useful indicator of the progress in leaf blight resistance that can be expected as a result of exercising selection on the populations evaluated.

In the present study, most resistance parameters used to measure leaf blight resistance showed high broad sense heritability and high genetic advance (as percent of mean) values in populations J34-1, J34-2, J34-3 and J34-5. According to Sawar *et al.* (2004); (2005), high heritability coupled with high genetic advance (as percent of mean) is an indicator that the character under study is governed by additive types of genes. With these high heritability estimates and the predominance of additive genetic variance indicate that in these populations, progenies with improved leaf blight resistance could be selected. Conventional pedigree techniques like mass selection and full sib S_1 recurrent selection as recommended by Sprague and Eberhart (1977) could be effective for selecting resistant genotypes and thus positive achievements could be obtained in these four populations. Gandara (2005) working with sesame, suggested that selection could be made directly for the improvement of this character at an early stage.

Populations J34-4 and J34-6 exhibited a low broad sense heritability and genetic advance (as percent of mean) across all resistance parameters. Low broad sense heritability alone indicates a greater influence of the environment on leaf blight resistance and this means the character was not under genetic influence. When low broad sense heritability is coupled with low genetic advance (as percent of mean), then the character is controlled by non additive types of genes (dominance and epistasis) and high genetic by environmental interaction (Bayder, 2005). Leaf blight resistance in populations J34-4 and J34-6 as indicated by the various parameters was found to be under the influence of non additive gene action. This means there would be limited gains in selection for leaf blight resistance from these populations. Saravanan and Kumaru (2004) working with sesame

suggested that selection in such populations should be postponed and performed in the advanced/succeeding generations and in this case F_3 or S_2 generations. Sofi and Rather (2002) working on maize suggested that a character under non additive types of genes could be improved by development of hybrid varieties or isolation of transgressive segregates in a heterosis breeding programme and taking advantage of general and specific combining ability. According to Ceballos *et al.* (1991), the absence or small magnitude of non additive effects for leaf blight resistance could be attributed to the absence of deleterious genes that cause endogamic depression. The low heritability on the disease resistance parameters was being exhibited due to favourable influence of the environment rather than the genotype itself.

Phenotypic correlation coefficient values across all populations as measured by the various disease resistance parameters were not consistent; they varied from as low as 10.33 to 85.64 %. In population J34-4, they were generally low and were also associated with low heritability and genetic advance. A similar observation was made by Nehvi *et al.* (2004) working with maize downy mildew. They concluded that this low variation among the test material is indicative of less scope for improvement. This agrees with the present study as J34-4 was found to offer less gains in leaf blight resistance.

Broad sense heritability and genetic advance for the three agronomic traits, plant height, ear height and flowering dates were for most of the populations were generally high except in J34-4. This is in agreement with published reports (Sidwell *et al.*, 1978; Nanda *et al.*, 1981 and Teich, 1984). This means such agronomic traits in these populations were

under the influence of additive types of genes which can be exploited for positive gains. This statement excludes plant heights in population J34-2 and J34-6 which were found to be under non additive gene control as shown by their low heritability and genetic advance. Population J34-4 showed a clear cut non additive gene action in all agronomic traits. This means these traits in these populations can be hardly be improved if selection would be based on them.

In most cases genotypic correlations, in general, were higher than the corresponding phenotypic correlations. This indicates little influence of the environment on any inherent association among the traits studied. Correlation analyses between flowering dates and disease resistance were consistently negative, indicating that early maturing plants were more susceptible and late maturing resistant. The additive gene action influencing flowering dates can be exploited by selecting late maturing genotypes which can lead to gains in leaf blight resistance. This also indicates that maturity is an important factor when improving resistance to leaf blight. This is also in agreement with a study of Ceballos and Gracen (1989) and Smith and Cordova (1987) who reported a strong association between leaf blight resistance and late maturity.

Plant and ear heights were poorly correlated with all resistance parameters thus indicating limited use in breeding for leaf blight resistance. However the present study is not in agreement with that of Thakur *et al.* (1989) who found a strong association between the two. According to Van Ginkel *et al.* (1996) the poor relation between leaf blight resistance and plant height could be due to genetic effects (linkage or pleiotropic effects)

but more likely to the effects of microenvironment. Correlation analyses showed a strong relation between plant and ear heights which agrees with most published reports (Hallauer and Miranda, 1988; Hallauer and Eberhart, 1970).

Chapter Six

Conclusion

It should be emphasised that the magnitude of the genetic parameters estimated in this study are quite specific for the populations evaluated and the geographical area used, and in this case it applies only to the six populations evaluated at the Golden Valley Agricultural Trust in Chisamba only. This would be especially true for the correlations involving time of flowering.

The data from some populations indicated ample opportunity for improving leaf blight resistance as indicated by the relative types of variation. Selection could therefore be effectively based on leaf blight resistant genotypes and their phenotypic expression would be a good indicator of their genotypic potentiality.

The development of leaf blight resistant cultivars should be possible by phenotypic selection under disease pressure conditions at a population level, given sufficient number of plants. The correlation among some agronomic traits and leaf blight resistance may be exploited for by indirect selection by using the path coefficient analyses.

6.1 Recommendations

For plant breeding applications involving a large number of genotypes, the use of six resistance parameters to assess leaf blight resistance might not be practical. In this study all parameters were consistent across populations in the estimation of variability

parameters. This indicates that the use of one can be effectively used to assess the disease and obtain valid variability parameters. Assessment using the percent affected ear leaf area parameter is recommended because it also estimates other useful disease epidemic parameters like the area under disease progress curves and the apparent infection rates.

Among the six maize populations evaluated only four showed sufficient genetic variation for leaf blight resistance and these were J34-1, J34-2, J34-3 and J34-5. Resistant or tolerant genotypes can be selected directly in these populations for inbred line development or creation of another experimental population by interpopulation improvement strategies like the reciprocal recurrent selection (full-sib) where further selection could be done. Population J34-4 and J34-6 showed that the variation observed was not under genetic control and thus there would be less scope for improvement for leaf blight resistance since they were much under the influence of the environment. It is recommended that these populations should be allowed to advance to further generations to increase variability before selection could be done.

Future studies may consider the use of molecular markers on the populations showing sufficient genetic variation for leaf blight resistance. This would include identification, mapping and isolation of genes of resistance which can be introgressed into agronomical superior varieties. The use of markers can also assist the breeder in selection performed without artificial inoculation.

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Appendices

Appendix 1: ANOVA for disease severity scores (F_1 Hybrids)

		MS (Days after inoculation)					
Source of variation	df	16	23	31	39	48	56
Genotypes	5	0.082	0.089	0.17	0.173	0.194	0.21
Replications	2	0.041	0.0044	0.0038	0.011	0.005	0.005
Error	10	0.0137	0.0072	0.0067	0.018	0.02	0.03
Total	17	0.137	0.101	0.181	0.202	0.22	0.25
CV(%)		6.29	12.85	9.76	11.20	10.39	12.67
F-ratio		5.98**	12.36**	25.37**	9.61**	9.7**	7.0**

Appendix 2: ANOVA for percent affected ear leaf area (F_1 Hybrids)

		MS (Days after inoculation)					
Source of variation	df	16	23	31	39	48	56
Genotypes	5	2.066	4.33	5.64	8.29	10.56	12.99
Replications	2	0.193	0.027	0.412	0.036	0.041	0.078
Error	10	0.059	0.073	0.87	0.214	0.144	0.114
Total	17	2.32	4.36	6.922	8.54	10.75	13.182
CV(%)		2.24	11.76	5.18	12.40	11.48	4.18
F-ratio		35.02**	59.32**	6.48**	38.74**	73.33**	113.95**

Appendix 3: ANOVA for yield in sprayed and inoculated trials (F_1 Hybrids)

		MS	
Source of variation	df	Sprayed	Inoculated
Genotypes	5	1.96	0.51
Replications	2	0.446	0.90
Error	10	0.210	0.304
Total	17	2.62	1.714
CV(%)		6.49	
F-ratio		9.33**	1.68 ^{ns}

Appendix 4: ANOVA for disease resistance parameters (Population J34-1)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NL	AUDPC	AIR
Between	9	3.4	28.96	16.91	2.36	16.42	49.12	0.026
Within	20	0.43	8.03	1.16	0.59	8.92	2.44	0.002
CV (%)		17.53	6.37	9.79	11.29	22.15	35.40	7.71
F ratio		7.9**	3.61**	14.58**	4.00**	1.84 ^{ns}	20.13**	13.00**

Appendix 5: ANOVA for disease resistance parameters (Population J34-2)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NL	AUDPC	AIR
Between	9	0.72	17.7	212.55	5.29	12.44	32.47	1.88
Within	20	0.066	1.5	0.029	0.64	1.407	1.36	0.192
CV (%)		8.42	3.36	18.92	9.19	9.9	18.55	10.19
F ratio		10.64**	11.88**	212.55**	8.84**	23.88*	9.75**	9.75**

Appendix 6: ANOVA for disease resistance parameters (Population J34-3)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NL	AUDPC	AIR
Between	9	1.97	120.82	7.00	142.36	37.34	13.05	0.0378
Within	20	0.598	18.28	0.52	2.8	9.41	2.34	0.011
CV (%)		20.72	12.77	7.21	20.41	4.9	4.9	8.29
F ratio		1.84 ^{ns}	6.61**	13.46**	50.84**	90.24**	90.24**	3.41*

Appendix 7: ANOVA for disease resistance parameters (Population J34-4)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NL	AUDPC	AIR
Between	9	0.93	28.38	23.78	2.93	56.23	38.44	0.044
Within	20	0.51	12.3	9.6	0.816	19.67	18.7	0.038
CV (%)		20.72	8.62	33.70	9.38	27.78	7.2	35.44
F ratio		1.84 ^{ns}	2.31 ^{ns}	2.56 *	6.08 **	2.74*	2.056 ^{ns}	1.158 ^{ns}

Appendix 8: ANOVA for disease resistance parameters (Population J34-5)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NS	AUDPC	AIR
Between	9	1.51	219.38	10.73	8.75	7.84	122.62	0.0136
Within	20	0.43	25.1	3.26	1.31	0.31	11.44	0.0004
CV (%)		16.99	12.70	18.055	12.43	3.71	5.44	3.33
F ratio		3.51**	8.74**	3.29**	6.54**	25.29**	10.72**	3.4**

Appendix 9: ANOVA for disease resistance parameters (Population J34-6)

Source of variation	df	MS						
		DSS	PAELA	IP	LS	NL	AUDPC	AIR
Between	9	2.12	19.17	11.71	19.39	3.85	34.12	0.037
Within	20	0.95	34.51	8.56	1.81	1.6	9.82	0.019
CV (%)		31.75	26.61	29.25	12.34	9.035	36.9	21.20
F ratio		2.23^{ns}	2.26^{ns}	1.36^{ns}	10.71**	2.40*	3.47**	1.95^{ns}

Appendix 10: ANOVA for agronomic traits (Population J34-1)

Source of Variation	df	MS		
		Plant height	Ear height	Flowering dates
Between	9	3518.45	621.1	261.75
Within	20	31.27	31.27	12.24
CV (%)		5.88	5.88	5.88
F ratio		19.86**	19.86**	21.38**

Appendix 11: ANOVA for agronomic traits (Population J34-2)

Source of Variation	df	MS		
		Plant height	Ear height	Flowering dates
Between	9	4535.98	430.40	362.86
Within	20	2462.92	50.63	36.25
CV (%)		22.98	7.73	10.38
F ratio		1.84^{ns}	8.50**	10**

Appendix 12: ANOVA for agronomic traits (Population J34-3)

		MS		
Source of Variation	df	Plant height	Ear height	Flowering dates
Between	9	6065.1	598.07	2128.37
Within	20	709.53	47.81	908.30
CV (%)		12.56	7.86	51.96
F ratio		8.55**	12.51**	2.34 ^{ns}

Appendix 13: ANOVA for agronomic traits (Population J34-4)

		MS		
Source of Variation	df	Plant height	Ear height	Flowering dates
Between	9	6373.12	677.1	360.52
Within	20	613.33	408.72	189.91
CV (%)		11.52	21.74	24.18
F ratio		10.39**	1.66 ^{ns}	1.89 ^{ns}

Appendix 14: ANOVA for agronomic traits (Population J34- 5)

		MS		
Source of Variation	df	Plant height	Ear height	Flowering dates
Between	9	3843.23	919.17	318.47
Within	20	700.13	97.35	6.8
CV (%)		12.08	10.96	4.21
F ratio		5.49**	9.44**	46.8**

Appendix 15: ANOVA for agronomic traits (Population J34-6)

		MS		
Source of Variation	df	Plant height	Ear height	Flowering dates
Between	9	3691.09	696.05	142.32
Within	20	1523.26	11.84	10.2
CV (%)		18.49	3.82	4.9
F ratio		2.42*	58.79**	13.95**

Appendix 16: Growth stages of the maize plant

Vegetative growth stages	**Reproductive stages**
VE – emergence stage	R1 – silking stage
V1 – first leaf stage	R2 – blister stage
V2 – second leaf stage	R3 – milk stage
V3 – third leaf stage	R4 – dough stage
V (*n*) - *n*th leaf stage	R5 – dent stage
VT – tasseling stage	R6 – physiological maturity