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NAME: Mr. Daniel Abebe Geletu

THIS THESIS HAS BEEN ACCEPTED BY

Narong Singburaudom

THESIS ADVISOR

(Associate Professor Narong Singburaudom, M.S.)

S. Sangchote

COMMITTEE MEMBER

(Associate Professor Somsiri Sangchote, Ph.D.)

Ed Sarobol

COMMITTEE MEMBER

(Associate Professor Ed Sarobol, Ph.D.)

Somnuk Wongtong

PROGRAM CHAIRMAN

(Associate Professor Somnuk Wongtong, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON 18 May 06

Vinai Artkongharn

DEAN

(Associate Professor Vinai Artkongharn, M.A.)

THESIS

**MORPHOLOGICAL CHARACTERISTICS AND
PATHOGENICITY OF *EXSEROHILUM TURCICUM*
(PASS.) LEONARD AND SUGGS ISOLATES ON MAIZE
GENOTYPES IN ETHIOPIA**

DANIEL ABEBE GELETU

**A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of Doctor
of Philosophy (Tropical Agriculture)
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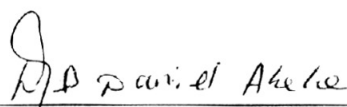
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Survey and collection of the isolates of the causal agent of northern corn leaf blight (NCLB) were carried out in maize growing areas of Gambella and Oromiya Regional States of Ethiopia in 2004 and these isolates were used for studying their cultural, morphological and pathogenicity variations. The results revealed that northern corn leaf blight was widely distributed in almost all the surveyed areas. Out of the seventy isolates that had been studied for morphology, most of the conidia shapes are to curved (27%), spindle (16%) and elongated (27%). The average size of the conidia was 93.97 μm in length and 13.11 μm in width. The observed number of septa was in a range of 2 to 7. The study of cultural characteristics showed that variation existed among representative of 28 isolates in colonial morphology color and pigmentation. Twenty representative isolates were selected and evaluated for pathogenicity on seedlings of 11 maize varieties and the result revealed that there was significant difference on disease reaction among tested isolates, varieties and isolates by varieties interaction ($P \leq 0.05$). The Dendrogram of disease reaction derived by cluster analysis using unweighted pair-group method (UPGMA) using an average with virulence similarity based on lesion size of *E.turcicum* isolates variation into indicated 5 groups.

The northern leaf blight evaluation field trials were made for 13 maize varieties planted at three locations of Gambella and Abobo (both in Gambella State) in 2003 cropping year, and at Bako (Oromiya State) in 2004 cropping year. Different blight epidemics were observed in different locations of the experimental plots. Percent of incidence was increased from 0.00 to 96.66% at Abobo and from 10.00 to 96.66% at Bako, in 2003 and 2004, respectively. However, at Gambella the incidence was as low as from 0.00 to 83.33%. Variations among maize varieties were observed for several disease variables such as area under disease progress curve (AUDPC), lesion size, lesion number and scale severity rating. Varieties Gussau, Abobako and Local- M had high AUDPC, large lesion size, fast onset of disease and many lesion numbers and categorized as highly susceptible varieties. The results also indicated that Kuleni was the most moderate resistance to northern leaf blight across three locations and had low rating score, lower AUDPC value, and smaller lesion sizes.

The correlation analysis indicated that there were significant positive relationship among disease assessments. The relation between AUDPC and disease severity with yield showed significant negative correlation ($r = -0.51$ and -0.82), respectively. Significant but negative correlation AUDPC and severity with yield mean that both contributed to most of the yield variations among varieties. A significant positive correlation among blight evaluation methods and their negative correlation with seed weight and yield, indicate that the use of any one of the methods can effectively measure the disease reaction and its effects on yield.


Student's signature


Thesis Advisor's signature 15-1 May 2006

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**MORPHOLOGICAL CHARACTERISTICS AND
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INTRODUCTION

Among food grain crops, maize ranks the third in terms of production and acreage in world cereal production. It is the widely grown of the major crop species, with more than 70 countries planting maize on at least 100,000ha, including 53 developing countries. It also grows productively throughout the world, covering the lowland tropical, subtropical and temperate zones wherever rainfall or irrigation is adequate and grows from sea level to over 300 meters above sea level (Dowswell *et al.*, 1996).

Maize is used in more ways than any other cereal for human consumption, as a feed grain, a fodder crop, and for hundreds of industrial purposes. Its grain, stalk, leaves, cobs, tassel and silk all have commercial value in some settings. On global basis, two thirds of all maize grown principally for animal feed in the world it is also important as a staple food crop for human consumption of several countries in Latin America, Africa, and Asia (Shuttleff, 1980).

Maize is an exotic crop in Ethiopia. Portuguese merchants introduced it into the country during 1600s-1700s (Haffangel, 1961). Currently, it is one of the most important cereal crops grown in areas range from moisture stress to high rainfall zones and from lowlands to the highlands of Ethiopia (Kebede *et al.*, 1993). The total annual production and productivity exceed all other cereal crops, though it is surpassed by tef (*Eragrostis tef*) in area coverage (Benti *et al.*, 1997). Therefore, considering its importance in terms of wide adoption, total production and productivity, maize is one of the high priority crops to feed the increasing population of the country (Mosisa *et al.*, 2001). Maize is widely produce in western, southern, and southwestern, and in some northern, northwestern and eastern part of the country

and over 90% of the maize produced is used as food especially in major maize growing area for low incomes group (Ferdu *et al.*, 2001). The leaf and stalk are used for animal feed and dried stalk and cobs are used for fuel. It is also used as industrial raw material for oil and glucose production. Relatively high productivity, favorable growing conditions, and the technological advances over the last decades have contributed to its increased area of production as compared with other crops. In 1980s, Ethiopia produced 20 million quintals of maize, while areas of planted to maize reached 1 million ha. Currently in 2001/2002, 1.3 million hectares of land is covered with maize and it is estimated that the annual maize production is not less than 28 million quintals (Central Statistical Authority, 2001).

Under optimum crop production management practice of Ethiopia, there is a potential to produce maize grain yields ranging from 5.0 to 6.6 t ha⁻¹. However, the current national average of maize yield in Ethiopia is very low (2.1 t ha⁻¹) on peasant farms as compared with, both national and world yield potential (CSA, 2001). The low productivity of maize in Ethiopia was associated with dimensional biotic and abiotic factors (Ransom *et al.*, 1993). Among the biotic stresses those are serious constraints to yield improvement throughout the country are diseases and insect pests and low yield potential of farmer's cultivars (Ransom *et al.*, 1993).

Maize, like other cereals, is subjected to a number of diseases. All parts of maize plant are highly susceptible to a number of diseases that can create significant problem for the maize growers and reduced yield potential and value of fodder, alter normal maturity, reduce grain quality and cause stand ability problems. These factors can slow harvest and increase field loss. World reduction in grain production caused by diseases is estimated to average 9.4% and annual losses in the corn belt of the USA ranges from about 7 to 17% (Shuttleff, 1980).

Users, mostly in developing countries, are not aware of the impact of diseases in the field and in stores; hence, low yields in developing countries are due to diseases and largely to ineffective control strategies (Ayab-Takem and Chheda.1982). Another

reason for low yields is the use of diseased seeds from previous season, which is in various stages of degeneration.

Puccinia sorghi the causal agent of common rust, *Puccinia polysora* caused lowland rust; leaf blight incited by *Exserhylum turcicum*, *Bipolaris maydis*, *Phaeosphaeria maydis* (leaf spot) , ear and kernel rots (*Diplodia spp.*, *Fusarium spp.*), head smut (*Sphacelothecia reliana*) and maize streak virus (MSV) were found the most important pathogen in Ethiopia ((Tewabech *et al.*, 2001). Although about 47 maize diseases have been identified to infect maize, very little is known about the major maize diseases incidence and severity, pathogen distribution, importance and occurrences, epidemiology, yield loss and physiological specialization of pathogen in the country (Tewabech *et al.*, 2001).

Northern corn leaf blight (NCLB), caused by fungus *Exserhylum turcicum* previously called *Helmithosporium turcicum*, can cause yield loss is potentially destructive disease in maize (*Zea mays* L.) in humid areas where maize growing region of the world (Shuttleff ; 1980 , Ceballose *et al.*, 1991). In the United States and parts of Europe, Asia and Australia, NCLB is one of the predominant diseases of sweet corn (Shuttleff, 1980; Pedersen, 1988). In Africa where maize is staple food crop, northern leaf blight is reported to be widespread and destructive disease of maize in warm and humid growing regions of Ethiopia, Tanzania and Uganda (Nkonya *et al.*, 1988; Adipala *et al.*, 1993; Tewbach *et al.*, 2001).

In Ethiopia, out of many diseases identified on maize, northern corn leaf blight caused by *E.turcicum* is the most important foliar diseases, which widely distributed and has high economic importance in the country (Assefa, 1995). The infections appear during both the minor and major seasons, but it is more serious during the major season in predominately wet and humid area. The recent increase in the distribution and severity of the disease has been associated with an increase in maize production area, monoculture of maize production system, and increase use of minimum or non- till farming practices (Tewbach *et al.*, 2001). Although the disease is expected to have long co-evolution with the crop in Ethiopia, high severity has been

recorded since 1952 in Kefa, Hararghe, Shewa and Sidamo provinces, and Stewart and Daganchew (1967) indicated as an important disease on maize. Kranz (1969) reported that the conidial state of *Trichmetasphaeria turtica* (currently *Setosphaeria turtica*) is a dangerous fungus to a number of maize varieties. Assefa *et al.* (1996) also considered it as economically important maize disease on maize and sorghum. A review by Teklemariam (1985) and checklist by Mengistu (1990) described northern corn leaf blight as the second most important disease of maize. Since most of the local and east Africa maize varieties were moderate resistance/resistant, much attention has increased enormously since mid 1980s. This could be attributed among others to the extensive use of improved varieties. Tewbach (1990) has shown its wider distribution in mid to high land areas where maize is extensively grown. Assefa and Tewbach (1993) in their review reported that as number one disease problem on maize for its wider distribution, frequent infection and severe intensity through the country. It prevailed in areas with an annual rainfall of 500 to 1500 mm and at altitude between 500 to 3000 meters above sea level but was more severe between 600 and 2000m (Assefa, 1995).

The magnitude of yield losses caused by northern leaf blight depends on two factors, the stage of maize growth when the infection occurs and the severity of the disease. If the disease established before silking and environmental conditions favor disease development, over 50% yield reduction is expected (Ullstrup and Miles, 1957; Raymundo and Hooker, 1981; Perkins and Pedersen, 1987). However, if infection is delayed by 6-8 weeks after silking, yield losses are maximum. *E.turcicum* infected maize in addition to grain losses and reduced forage value, plants are often predisposed to root and stalk rot infections (Raymundo, 1978). Foliar diseases have a direct influence on the amount of dry matter stored in the grain and leaf blight can reduce grain yield up to 50% (Ullstrup and Miller, 1957). There are some estimates of yield loss of maize due to this disease in some countries and they are varied from place to place. Yield loss of more than 50% reported in U.S.A Corn Belt areas (Lim *et al.*, 1974). In Uganda, maize yield losses due to northern leaf blight was estimated to be as high as 60% (Adipala *et al.*, 1993) and up to 75% leaf damage occurred in North Carolina in an epidemic caused by race I in 1985 (Leonard and Leatch, 1986). In

Ethiopia, according to Assefa *et al.* (1995), northern leaf blight caused the highest mean grain yield loss of 50% and 1000 kernel weight loss of 16.4% under artificial infestation condition using highly susceptible cultivars Pool 32c19. In other experiment conducted at Awasa (southern part of the country), yield loss of 34.08%, 29.05% and 2.21% were recorded for varieties Abo-bako, Beletch and BH660, respectively (EARO, 1999). Even in the resistance cultivar, yield was reduced by 29.8%. This indicating that the disease is potentially very important (Ullstrup and Milles, 1957; Chenula and Hora, 1962; Fisher *et al.*, 1976). Generally, the increased incidence and economic importance of the disease linked to environmental conditions, minimum tillage of maize and use of highly susceptible varieties.

Gambella Regional State is found at southwestern part of Ethiopia. The regional state is characterized by ample precipitation, high humidity and warm temperature. The maize crop is the most widely cultivated cereal in the region but it faced with major challenges. These include diseases, field and storage pests, weeds and low yield of farmers' cultivar. Most maize cultivation activities are done in the region manually. Maize being the major staple food crop, the major cropping pattern is a monocropping system of maize. The successive plantings of corn in the same area have resulted in an increase of inoculums of northern leaf blight epidemic that are typified by rapid disease progress and the late plantings of maize was more vulnerable to the disease. Hence, lack of appropriate farming system, the absence of crop rotation practice, and climatic condition in region increased the potential for northern leaf blight such that it became a major yield-limiting factor in the region. The surveys conducted in the past in order to determine the incidence of northern corn leaf blight on maize crop in Gambella was found that as high as 96% incidence and 8.52 % disease prevalence were recorded (Assefa, 1995). Current reports indicate a growing concern by framers, plant pathologists, and breeders over the potential of northern leaf blight disease of maize.

While the disease is known to be present under field conditions little is known about the reaction of several maize varieties to the disease. Moreover, there is no

information on the variability of the pathogen among the different isolates. Cognition to those facts, the present work addresses the following objectives:

OBJECTIVES

1. To evaluate the variations of *E.turcicum* using morphological characters and their pathogenic among isolates.
2. To evaluate the ability of the pathogen isolates to cause disease.
3. To examine the effects of maize varieties on northern leaf blight progress and to determine the level of resistance in several maize varieties under field condition.

LITERATURE REVIEW

1. Taxonomy and Etiology

Turcicum leaf blight (*TLB*), commonly known as northern leaf blight caused by anamorph *Exserhillum turcicum* (Pass.) Leonard and Suggs (= *Helminthosporium turcicum* Pass) telemorph *Setosphaeria turcica* Luttrell (= *Trichometasphaerium turcica* Lutterl). It is widely spread and most economically important disease of maize in the world.

The spores are olive-gray, spindle-shaped, slightly curved, three to eight septate, 20x105µm with protruding hilum, and germinating by polar germ tubes. The conidia are olive gray, spindle-shape, slightly curved, three to eight septate, and measure 50-144 x18-33µm long. They grow on olivaceous conidiophores, measuring 7-9 x150-250 µm. Sporulation can be induced in a moist chamber (Shurtleff, 1980).

T.turcica, the sexual stage, occurs rarely, if ever, in nature but produce black, globes pseudothecica in the laboratory. The asci are cylindrical with a short stripe and contain one to eight, but usually two to four ascospores, which are hyaline, straight or slightly curved, typically three septate, and 13-17x42-78µm,form in culture (Shurtleff, 1980;McGee,1990).

2. Morphology

The conidia of the fungi attack maize are olivaceous- gray, elongated and Spindle shape often less curved on one side compared with the conidia of *H.maydis*, which are more curved. Average size is about 20-150µ. Septation ranges from one in the shortest spores to 9 in the longest. A conspicuous spore feature of the conidia that distinguishes it from other of the more common species of *Drechselra* attacking corn is the protruding hilum (Shurtleff, 1980: McGee, 1990).

The perfect stage of *E.turcicum* pathogen was first discovered in 1958 (Lutterll, 1958) when *E.turcicum* has been isolated from various wild and cultivated species of Graminae, as well as from maize. The fungi are heterothallic and the perfect stage can be produced in culture by mating compatibility isolates morphological observations and a review of the taxonomic literature have lead to the conclusion that the perfect stage is most closely related to species in the genus *Trichometaspheria*

3. **Epidemiology**

Epidemic of northern leaf blight depend on the ability of *E.turcicum* to infect, grow, and sporulate on maize plants. The dependence of these processes on weather conditions has been well documented. The infection process is affected by light, temperature, dew period, plant age, and inoculums concentration. This information may be valuable for stimulating blight development and for making decisions about disease control. Providing the presence of inoculum, the most important factor influencing infection is dew duration. Five hours of dew at optimal temperature was the minimum for establishing infection. Infection peg formation and lesion development is more frequent at 20⁰ C, whereas appressoria formation is more frequent at 15⁰ C. The dew point temperature range in which infection occurred depends largely on the inoculums concentration and length of the dew period. Young plants are more highly susceptible than older plants to infection by *E.turcicum* owing to their stimulating effect on appressorial formation (Leavy and Cohen, 1983).

4. **Mode of survival and dispersal**

The fungus causing NCLB over- winters as mycelia and conidia on maize residues left on the soil surface. The conidia are transformed into thick-walled resting spores called chlamydo spores. During warm, moist weather in early summer, new conidia are produced on the old corn residue and carried by the wind or rain to lower leaves of young corn plants. Infection by germinating conidia occurs when free water

is present on the leaf surface for 6-18 hours and the temperature is between 66 and 80°F (18-27°C). Lesions develop within 7-12 days. Secondary spread within fields occurs by conidia produced on the leaf tissues (<http://www.ohioline.osu.edu/acfact/0020.html>).

5. **Symptom expression**

Northern corn leaf blight is essentially a leaf disease. Even under the most severe epidemics infection of kernels has not been observed but lesions may occur on leaf sheaths and husks. Symptoms usually appear at any stage of development of plant and appear first as a water-soaked spot on the leaf that develops into the elliptical gray green lesion between 3 and 15cm in length. Fully developed symptoms are long, elliptical gray to tan lesions with sizes ranging from 25-40mmx150-170mm. Mature lesions may form concentric zones and coalesce to cover the entire leaf. The whole leaf may be killed and entire field might turn dry. Symptoms were not observed on kernels. Secondary ears are sometimes severely affected. Symptoms may some times be confused with lesion of the leaf blight phase of bacterial wilt (*Erwinia Stewartii*) that develops after silking. The latter, however, are generally longer, narrower, irregular in width and show translucent margins when held to the light. Lesions of northern corn leaf blight can vary depending on the race present. Race 1 lesions are tan, oval to circular with concentric zones and are commonly 1/2 inch in width and 1 inch in length. Race 2 infections are rare. Lesions are oblong, dark brown to blackish in color and 1/8 inch in width and 1 inch in length. Race 3 lesions are most common in the maize belt. These lesions are narrow and linear in shape, with lengths less than 1 inch and widths less than 1/8 inches. Lesion shape and size may vary with the genotype of the plant. Lesions are grayish-tan and surrounded by a pigmented border. Northern corn leaf blight can cause premature death and gray appearance of foliage that resemble frost or drought injury. <http://ipm.uiuc.edu/disease/series200/rpd202/>).

6. **Cytology and histology.**

H.turcicum, the causal agent of northern leaf blight of maize where conidia are disseminated from infected plant to other plants by wind. It germinates 3-6 hours after inoculation. Germ tubes grow at an angle rather than parallel to the veins of the leaf and produced an appressoria, from which a penetration peg develops. Germ tubes develop more from apical than basal cells of the conidia. Anastomosis occurred occasionally between the germ tubes of two conidia (Hailu and Hooker, 1964). Penetration of *E.turcicum* was similar on resistance and highly susceptible leaves of both young and mature plants. Maximum penetration occurred 12-18 hours after inoculation. About 90 percent of all penetrations were direct and typically occurred over the juncture of vertical epidermal walls, whereas 10 percent was stomata. Elliptical appressoria, averaging 4.0 x 6.0, was associated with all penetration. In highly susceptible and resistance seedling, initial symptoms developed as minute chlorotic fleck 2-3 days after inoculation. Macroscopic symptoms appeared first as minute light green to whitish fleck. The fleck remained materially unchanged in size and within 5 weeks after inoculation lesion had not enlarged into mature lesion. Hyphal grew in the mesophyll cells in these flecks and in cell beyond the necrotic tissue of the flecks. Flecks enlarged either single or coalesce to form long elliptical, grayish- green tan lesions ranging from 2.5 to 15 cm in length (Hilu and Hooker, 1964).

7. Geographical distribution and economic importance

Northern corn leaf blight (NCLB) caused by the fungus *Exserohilum turcicum* previously called *Helmithosporium turcicum*, is one of the most important disease of maize (*Zea mays* L). It occurs worldwide and particularly in areas where higher humidity and moderate temperature prevail during the growing season (Shurtleff, 1980; Ceballos *et al.*, 1991). The disease was first reported in Passerine on maize in Italy in 1876. In the United States The disease was first reported in New Jersey in 1878 and a serious outbreak occurred in connected in 1889 (Drechsler, 1923), northern leaf blight is the major disease of maize (Perkins and Pederson,1987; Bowen and Pedersen,1988). In Africa where maize is the staple food, northern leaf blight was reported to be widespread and destructive disease of maize in warm and humid region

of Ethiopia, Tanzania and Uganda (Nkonji *et al.*, 1980; Adipala *et al.*, 1993; Tilahun *et al.*, 2001).

8. Conditions for development of disease

The northern leaf blight is favored by mild temperature, high rainfall, extended period of leaf wetness (rain or dew), high humidity, frequent light shower, minimum tillage of corn production and grows corn continuous during the growing season (Ullstrup, 1970; Shurtliff, 1980). Heavy dews, high humidity, and frequent rains are conducive environmental conditions for disease development (Jordan *et al.*, 1983). Northern leaf blight is generally known to be sporadic in occurrence, depending on the environmental conditions and the level of disease resistance of the plant (Perkins and Pedersen, 1987). In general, the increase in the prevalence and importance of the disease might be attributed to monocropping practice, high humidity, morning fogs and extended dew period, minimum tillage and the use of uniform and highly susceptible varieties; all factors lead the build up of the pathogen inoculums.

Plant age has been implicated in contributing to the onset and severity of the disease. Leavy and Cohen (1983) reported that the was more aggressive to in young highly susceptible plants than older plant. This phenomenon has also been observed in other plant pathogen and similar results reported on maize infected by *H.maydis* (Ying, 1976) and Hau and Rush (1982) found cultivar resistance in rice against *H.oryzae*.

9. Host range

Its natural hosts are maize, sorghum, teosinte and *Paspalum* sp. However, it can attack various grasses, barely, wheat, oat, sugarcane, rice and millet. It does not infect dicots, except tobacco seedling. Physiological specialization is present within the species. The race that attacks Johnson grass does not attack maize; like wise isolates from sorghum and Sudan grass do not infect maize. Recently a second

physiological race of *D.turcica*, capable of attacking genotype with the Ht-gene, has been identified in Hawaii. A new source of *D.turcica* has been identified in Africa. Reaction appears to be conditioned by a single dominant gene, which protects the host until after silking at which time lesions begin to enlarge (Shurtleff, 1980).

10. Yield losses

A key result of studies on crop yield in relation to disease is that time of plant infection has a major effect on the resulting yield (James, 1968); with decreasing impact of disease as time of infection is delayed yield losses in highly susceptible hosts are considerable when infection occurs before silking and the environment favors disease. According to Ullstrup and Milles (1957), quantitative information is needed especially on the relationship between disease development and yield loss. Ramonydo and Hooker (1981), in their study, measured the relationship between northern corn leaf blight and yield loss trial and they concluded that difference in disease development and subsequent loss in yield among the hybrids were determined primarily by the resistance mechanism in each hybrid. The magnitude of yield losses caused by northern leaf blight depend on two factors; the stage of maize growth when the infection occurs and the severity of the disease. If the disease established before silking, yield reduction up to 40% may occurs (Raymond and Hooker, 1981), but if infection delayed until 6-8 weeks after silking, yield losses are minimal (Raymundo, 1978). A fresh weight yield loss of 0.3 to 0.75% for every 1% of the leaf area affected was observed in sweet corn (Pataky, 1992). In general, the early maturing varieties of maize are more highly susceptible to *H.turcicum* infection than late varieties (Cebalbs *et al.*, 1991).

Position of leaves infected relative to crop growth is essential in assessing the disease loss relationship (Raymond and Hooker, 1986). Upper leaves contribute more photosynthesis to the plant than lower leaves. Leaf tissue blighted early in the grain – filling period has a great effect on yield than an equivalent amount of leaf tissue blighted later in the grain-filling period (Ullstrup and Milles, 1957). Yield reduction were less than 10% when disease severity was less than 10%, 20% or 30%, depending

upon the trail and method of measuring yield. These results ,which are based on evaluation of a great number of sweet corn hybrids than earlier yield loss trials, corroborate previous finding of Pataky (1992 and1994) which support the proposition of Hooker and Perkins (1980) that blight on the lower leaves of maize plants has a greater effect on hybrid appearance than on hybrid performance.

11. **Managements**

Current recommended control measurements of northern corn leaf blight approaches; disease management by resistance varieties in most preferred over the sole fungicides use that associates with economic and environmental risk. One- to two-years rotation away from maize is helpful because the disease tend to increase in continuous culture. Tillage to bury infected residue may also helpful where erosion is not a problem. However, significant yield losses still occur when environmental conditions are favorable for the disease. Efficient disease control was achieved through use of fungicide spray and fungicide sprays are recommended only for fresh market sweet corn and hybrid seed production fields. The spray schedule should start when the first lesion appear on the leaf below the ear (Berger, 1973). Several fungicides are available for use on maize for northern corn leaf blight control. Read labels for rates and follow application directions, which vary with each fungicide product fungicide including Maneb, Chlorothalonil and Propconzale offer the most consistent method of control northern leaf blight (Burnette and White, 1985). Maintaining high balanced fertility based upon a soil test is also helpful. Apply excessive nitrogen since this may increase infection levels.

12. **Cultural and Pathogenicity Variations**

Robert (1952) studied the cultural and pathogenicity variability among isolates of corn leaf blight fungus, *Helminthosporium turcicum*, and found them to differ in virulence, but the difference were not uniform from year to year. Robert has reviewed the variability in several of the more important species of *Helminthosporium* attacking graminicolous hosts also with conclusion that the frequency of variation

differs with each species and some species are highly variable. The fungus is known to be highly variable in cultural characteristics and pathogenicity. Knox-Darise and Dickson (1961) reported sufficient evidence of heterokaryons and their perpetuation through the conidia and suggested that the high variability in the fungus population might be related to heterokaryosis. Assefa (1995) indicated that there was significant difference among *E.turcicum* isolated in their virulence. Correlation analyses showed that means virulence rating was highly correlated with mean lesion number and lesion type. Mean virulence rating indicated that there was significant difference among *E.turcicum* isolated and highly significant correlated with spore length and rate of germination. *E.turcicum* required relatively fewer days to initiate lesion on maize variety; *E.turcicum* variation existed from isolate to isolate. The result obtained in study of Leavy (1991) showed that the *E.turcicum* in Israel, differences among populations from different location were significantly greater than difference among isolates from the same field population. Populations differ significantly in three feature affecting pathogenesis: lesion area, sporulation, and infection efficiency. Previous studies by Merele *et al.* (1957) had indicated that seasonal variability could be major influenced factor in the development of disease resistant variety. High seasonal variability may result in variation in the pathogenicity causal organism. The variations among isolates could be due to variations in the resistant of the host plant and differences in the environment or from interaction among these variables. This pattern of variation in cultural and morphological also observed on other *Helminthosporium* Spp (Siwila *et al.*, 2001).

13. **Effect of Temperature on Morphological Variation of *E.turcicum***

Temperatures not only influence the rate of development and abundance of spore produced, but also their morphology. These observations on morphology change confirmed earlier finding of Nisikado (1992) who proved and explained the difference of spore types commonly trapped above diseased maize in the field. Spore-trapped slides showed a predominance of elongate, curved, and many septated conidia, it was evident from temperature recorded that the preceding temperature had been relatively warmed. When spore showed short and straight with few or no septa,

it indicated that the preceding night temperature had been relative low. Leach *et al.* (1977) studied the importance of temperature, relative humidity and light in sporulation and spore release in *D.turcica*. The result provided a better understanding of the relationship of environmental condition to spore germination, infection, and lesion development. He concluded that temperature influenced the rate of development of conidia and also their morphology and septation. The lower limit of sporulation was approximately 9⁰c, the upper limit 3⁰c, and the optimum range was about 22-26⁰c. From the other studies, Luttrell (1958) observed that morphology of *H.turcicum* isolates and found that they produced conidia abundantly on the exposed barely straw, and most isolates produced numerous tiny, black, sclerotium-like knots of hyphae on the straws above and below the agar surface.

14. **Reaction of Maize Cultivars to NCLB**

The variations among isolates could be due to variations in the resistance of the host plant and differences in the environment or from interaction among these variables. Adipala *et al.* (1993) observed a similar response among NCLB resistant Uganda maize cultivars. Reactions to NCLB were clearly different in field trials in Uganda when NCLB was severe, but the reaction of the most resistant cultivars could not be different when condition was less conducive for the development of NCLB. Jenkins *et al.* (1957), in his work on leaf blight infection of 58 inbred lines for 4-6 years under artificially induced epidemic, determined that the difference in environmental constituted a major source of variation. Previous studies of Merle *et al.* (1957) were designed to appraise a procedure of utilizing disease highly susceptible to evaluate the potential breeding value of inbred lines of corn resistance to the leaf blight caused by *Helminthosporium turcicum*. He concluded that analyses of variance of the data indicated that there were highly significant differences in leaf blight reaction, both among resistance and among the highly susceptible testers. Difference in response to northern leaf blight among cultivars was apparent between adult plant test and seedling test. Low correlation was existed between disease assessment on seedling and adult plant result for *E.turcium* on maize (Perkins, 1986). Based on the correlation between seedling and adult plant result for some variables, seedling test

can help identify source of chlorotic resistance and identifying highly susceptible genotypes, but field tests are necessary to fully characterize rate-reducing resistance. On seedling, incubation period and lesion number, and on adult plants, percentage of leaf area affected and lesion number seem to be useful for assessing rate-reducing resistance to northern leaf blight (Adipala *et al* 1993).

15. **Expression Resistance and Pathogenic Race.**

There are two types of resistance to *E.turcicum* in maize: monogenic and polygenic resistance (Hooker and Perkins, 1980; Lipps *et al.*, 1997; Pataky *et al.*, 1998). Monogenic or race specific resistance is single dominant genes and characterized by formation of chlorotic lesion, reduced pathogen sporulation, delays the onset of epidemics and lesion formation (Leonard, 1989). The polygenic resistance or rate reducing piratical resistance are normally characterized genotypes by reduced number of lesion and decrease in lesion size and amount of sporulation (Ullstrup, 1970).

Robert and Sprague (1960) found that the resistance and susceptible of maize is determined by genetic differences in the host, spore germination, infection and lesion development. Hooker (1961) reported that several source of resistance are available to maize breeders. The highly susceptible cultivars characterized by rapid developing necrotic, highly susceptible lesion with abundant sporulation. The resistance cultivars with type of lesion chlorotic, reduction in size, delay of necrosis, and in habitation of fungus sporulation. The lesion is in contrast to wilted and necrotic lesion expressed by highly susceptible maize. This phenomenon has also been observed in other plant pathogen. Kinsey (1975) reported similar results and for infection of maize by *H.maydis*, which showed monogenic chlorotic-lesion resistance in maize to *H.maydis* race O was expressed in the form of lesion type, lesion size, and reduced sporulation with lesions. These results are in agreement with those of Craig and Daniel-Kailo (1968) working with the original source of resistance and an African isolate of *H.maydis*.

The fungus is known to be highly variable in cultural characteristics and pathogenicity. Knox-Davies and Dickson (1961) reported sufficient evidence of heterokaryons and their perpetuation through the conidia and suggested that the high variability in the fungus population might be related to heterokarysi. At least four race of *E.turcicum* are reported (Leonard *et al*, 1989). Race designation is done according to the resistance genes in maize that their virulence matches (Table 1). There are at least three known races of northern corn leaf blight. Race 1 is highly pathogenic to some inbred lines; Race 2 is much less pathogenic; Race 3 is primarily a problem in seed production fields. It can also produce lesions on commercial hybrids but does no economic damage. There is also evidence that a fourth race may occur or it may be a biotype of one of the other races. There are two types of resistance to *E.turcicum* in maize: monogenic and polygenic resistance.

Monogenic or race specific resistance, controlled by *Ht1*, *Ht2* and *Ht3* genes characterized by formation of chlorotic lesion and reduced sporulation that delay onset of epidemic, or as in *HtN* genes, delays lesion formation in such way that plants in the field with *HtN* gene remain free from lesion until shortly after pollination (Leonard, 1989).The *Ht1* gene is used more extensively in commercial maize hybrids in the United State where as *Ht2*, *Ht3*, and *HtN* are limited only to experiment use (Leonard,1989). The occurrence of genes in *E.turcicum* matching the four resistance genes in maize is an indication potential for many more pathogenic races to be identified.

Table1 Race of *E.turcicum* and matching resistance genes in maize(adapted from Leonard (1989)

Race designation	Resistance genes			
	Ht1	Ht2	Ht3	HtN
0	R	R	R	R
1	S	R	R	R
23	R	S	S	R
23N	R	S	S	S

R=resistant, S= susceptible

MATERIALS AND METHODS

1. Study on Morphological and Cultural Characteristics

1.1. Site description

The survey sites included two-regional states namely, Gambella and Oromiya Regional States. Gambella Regional State is in the southwestern part of Ethiopia adjacent to the Republic of Sudan. This region represents the low altitude sub humid zones (500m) and grows maize, sorghum, lowland oil crop, pulses and horticulture crops. The area located at 7°-8°37' latitude and 33°-35°2' longitude at an altitude of 500-1500m with average annual temperature of 20-25°C, mean maximum temperature at 38°C and annual rainfall of 900-1400mm. The regional state is characterized by ample precipitation, high humidity, and warm temperature.

Oromiya, where the study was conducted, is the largest regional state of Ethiopia with reference to its economy size as indicated by population, geographical area and production. Oromiya has a wide range of agro-ecological diversity that is favorable for producing several varieties of crops. Most of the arable and cultivated land is between the altitude of 500 and 2,500 meters. This range of climate is suitable for growing tropical and sub-tropical crops as well as crops of temperate climate. Most parts of the region receive sufficient and reliable rainfall during the main rainy season. Due to its favorable climate, Oromiya grows diverse of the crops such as cereals, pulses, oilseeds, vegetables and root crops. Cereals occupy an important position in the agrarian economy of Oromiya accounting for 82 percent of each of the cropped area and production (CSA, 2001). The three administration zones of Oromiya, namely IlluAbbaBoraa, east Wollega and west Shoa, where the survey was conducted, maize based cropping system have been widely practiced. These zones located in the western and southwestern part of Oromiya generally receive heavy rainfall up to 2600 mm (Girma *et al.*, 2001). The position of the two regional states in the map of Ethiopia is presented in Figure 1.

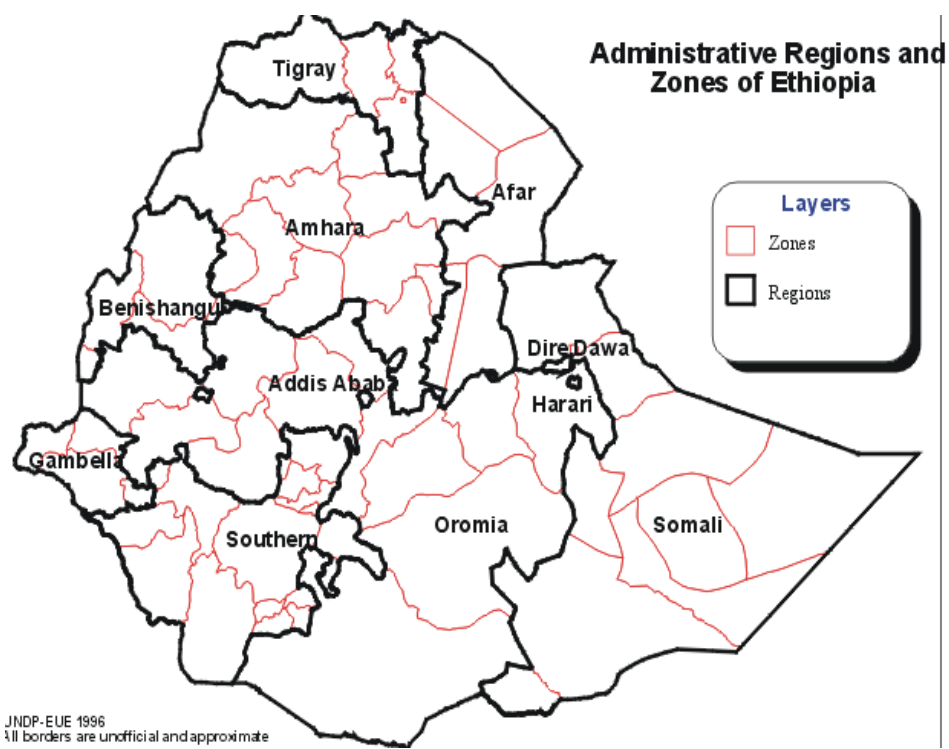


Figure 1 Position of Gambella and Oromia Regional States in Ethiopia.

1.2. Collection of isolates

In 2004, roadside surveys were carried out and diseased maize leaf samples were collected from maize growing farmers' of western and southwestern part of Ethiopia. Seventy leaf samples isolates were collected and put in paper bags. These leaves were finally air-dried with herbarium and kept in room temperature for laboratory investigations at Holetta Agricultural Research Center (HARC).

1.3. Isolation of the fungus

Single conidial isolations were made by incubating infected leaves in moist chamber for 72 hours under fluorescence and single conidia was isolated with sterile

blood lancet using dissecting microscope and transferred to slant agar, maintained on PDA, and then, kept in refrigerator.

1.4. **Morphological characteristics study**

Seventy isolates were used in this study. Each isolate of *Exserhilum turcicum* was culturing on PDA for 9 days, and investigated for morphological variations such as shape, size of conidia, and the number of septa. The size (length by width) of conidia was also measured (μ) with ocular micrometer using binocular compound microscope. In addition, 3 conidia of each isolates were measured.

1.5. **Cultural characteristics study**

The variations in cultural characteristics of 70 isolates *E.turcicum* were investigated. Each isolate was cultured on potato dextrose agar (PDA) with three replications for each isolates and randomized on the incubating bench. The qualitative feature such as colony color and pigmentation of the culture were recorded after 9 days of incubation. The colony color or pigment was described using a standard color chart (Kornerup and Wanscher, 1967).

2 **Evaluation of Pathogenic Variation of isolates**

2.1 **Maize varieties**

Eleven maize varieties that are different in reaction to northern leaf blight were obtained from Bako National Maize Research Center and Gambella Research Center and evaluated for their resistance to *E.turcicum* in the laboratory at room temperature at Holleta Agricultural Research Center. Totally, eight varieties namely, BH140, BH540, BH660, BH670, Gibe, Gutto, Kuelni and QPM were obtained from Bako National Maize Research Center, and the rest, three, varieties namely; Abobako, Gussau and Local-M were obtained from Gambella Research Center. The description of these maize varieties shown in table 2.

Table 2 Description of tested maize varieties with their agro-ecological adaptation, agronomic characters and NCLB reaction.

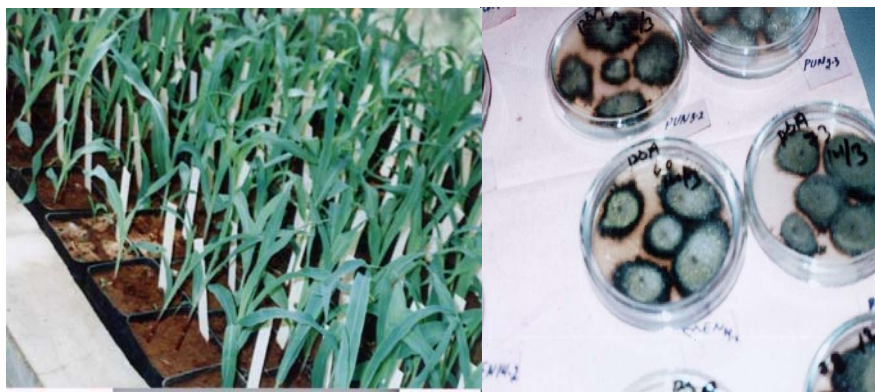
Variety	Altitude (m)	Rainfall(m m)	Plant height(cm)	Days to maturity	reaction to nclb
BH660	1600-2200	1000-1500	255-290	160	R
BH540	1000-2000	1000-1200	230-260	145	MR
BH140	1000-1800	1000-1200	240-255	145	MR
BH530	1000-1300	1000-1500	200-300	140	MR
Kuelni	1700-2200	1000-1200	240-265	150	R
Abobako	500-1000	1000-1200	240-265	150	MR
Gutto	1000-1700	800-1200	165-190	126	MR
Gibe-comp	1000-1700	900-1200	240-260	145	MR
Gussau	500-1000	1000-1200	240-265	150	MR
Local-m	500-1000	1000-1200	265-270	190	S

R=resistance; MR= moderately resistances; S= susceptible

Source; Second National Maize Workshop of Ethiopia (2002).

2.2. Experimental Design

The treatment was arranged in completely randomized block design (CRBD) with three replications; one-pot and a pretreatment four seeds were raised into sterile soil in plastic pot. The seedlings were grown in the greenhouse until the fourth leaf stage or 4 weeks old (Figure 2A). The seedling in each pot was thinned to two plants per pot. Fertilizer and irrigation by tap water were applied as needed to maintain optimum moisture for seed germination, emergence and growth of seedling in the green house.

**A****B**

Figures 2 Four weeks old plants (A) and three weeks old culture of *E. turcicum* (B)

2.3. Disease assessment

Once a week after inoculation and every day thereafter, plants were examined for presence of symptoms. Disease reactions were evaluated at 15 days after inoculations Hooker 1961 and 1963; and Pratt *et al.* (1993). The reactions were classified as resistance or highly susceptible according to the size of lesion. Lesion length and width of inoculated plants were measured in centimeters; two-selected lesions per test plants as conducted follow by Leath and Pedersen (1986). Disease reactions were categorized according to lesion size; 1 = highly resistance (HR) with lesion size of 0.0-0.5cm²; 2 = resistance (R) with lesion size of 0.6-1.0 cm²; 3 = moderately resistance (MR) with lesion size of 1.1-2.0cm²; 4 = highly susceptible (S) with lesion size of 2.1-3.0cm²; 5 = highly susceptible (HS) with lesion size >3.0cm²

2.4. Inoculation

Pure cultures of these isolates were grown potato dextrose agar in 90 mm petri dishes for three weeks (Figure 2B). Concentrated inocula were prepared by washing conidia including the agar medium of 21 days old culture. The plates were moistened with a few drop of sterile water using a sterile pipette. Conidial concentrations were not standardized since a qualitative difference reaction among isolates was the principal criterion to determine. The suspension was filtered through double folded

cheesecloth. Inocula were added with 2-3 drops of Tween 20 per liter as suggested by Warren (1975).

Fourteen days after planting or plants reached four to five leaves stage, inoculum was sprayed to the leaves runoff in the green house at when the plant was ready for inoculation stages (Jeffers and Chapman, 1995).

2.5. Data Analysis

The data were analyzed using Duncan multiple range test at $P \leq 0.05$ (SAS, 1989, Institute, Inc, Cray, NC). Moreover, fifteen days after inoculation, lesions sizes were measured and subjected to statistical analysis. The average lesions sizes were then, categorized into 1-5 scales in order to do a cluster analysis to identify the similarity of virulent pattern among isolates. For this analysis, a similarity matrix was derived with the simqual program (NTSYS 1993 pc, version 1.7) using simple matching coefficient of similarity. A dendrogram was produced by the unweighted pair group method for arithmetic average (UPGMA) in the SAHN program.

3. Reactions of Maize Varieties to Northern Corn Leaf Blight under Field Conditions.

3.1. Experimental sites

The northern leaf blight evaluation field trials were made for 13 maize varieties planted at two locations of Gambella Regional State at Gambella and Abobo in 2003 cropping year, and one location of Oromiya Regional State at Bako National Maize Research Center in 2004 cropping year. These locations included farmers' field and research plots. Gambella and Abobo are situated about 777 and 816 km from Addis Ababa (capital city of Ethiopia), respectively, in the southwest part of the country. Both locations are an ideal for evaluation of maize varieties for genetic variability as the temperature and relative humidity are optimum for disease development. Bako National Maize Research Center is situated about 257 kilometers away from Addis Ababa in west showa zone of Oromiya regional state at 37°09'E, 09°06'N and at altitude of 1650 m above sea level.

3.2. Test Materials

Twelve realseed maize varieties were evaluated for reaction of northern leaf blight under field condition and one highly susceptible local maize genotypes included in this study used as a susceptible check because the local variety grow in all region and highly susceptible to disease.

3.3. Field plots management for disease epidemic

Plants were infected by *E.turcicum* under natural conditions because this disease occurs naturally on maize and makes it unnecessary for inoculation. Field plots were established in fields previously planted with maize and plants were kept weed free by hand weeding. To avoid the influence of rust disease and stock borer (insect pest) on yield, recommended pesticide and insecticide were applied when it

necessary. These varieties were planted in July 23 and 24, 2003 at Gambella and Abobo and 11 June 2004 at Bako.

3.4. **Experimental design**

The varieties was arranged in the completely randomized block design with three replications was used. Each replicate consisted of four rows. Rows were 75cm apart, and two plant per hill were spaced every 30cm within rows. Plot size was 10.8m² (3.00x3.60). Diseases were recorded and yield data were taken from ten randomly selected and tagged plants from the central two rows.

3.5. **Disease assessments**

3.5.1. Field disease assessment at each location was assessed 6 times throughout the growing season from onset of the disease until the maize reached the dent stage (Ringer and Grybauskas, 1995). Ten randomly selected plants in the central rows were tagged and used for successive disease assessments. The progress of percentage incidence of disease in maize was quantified in staggered plant at 10 days intervals starting for onset of disease to dent stages. The percentages of disease were calculated by using the following formula.

$$\% \text{ incidence} = \frac{\text{No of diseased plants} \times 100}{\text{Total no of plants}}$$

3.5.2. Variables such as lesion number and lesion size were used to compare the epidemic in different areas recorded similarly percentage of disease incidence. Disease severity was recorded at maturity time once average of 10 plants- calculated using a rating on a scale recommended by CIMMYT methods, 1-5scale, where 1 = no symptoms; 2 = trace of lesions below the ear, none above; 3 = many lesions below the ear, trace above; 4 = several lesion development below the ear, all leaves above the ear with lesions and 5 = all leaves dry and dead.

3.5.3. Lesion size and number

Diseased plants were scored at 10-day interval, number of lesion on the ear leaf and second leaf above the ear leaf was counted on each tagged two leaves per plants. Lesion size in centimeters of two lesions on randomly selected 10 plants in the center row were measured at 10- day interval to determine the rate of lesion expansion .The monitored lesion were marked with marker so that same lesion would be measured in each week and total 20 lesions per experiment were recorded.

3.6. Agronomic Characteristics

Agronomic parameters such as stand count per plot at emergence and harvest, and plant height were recorded from 10 randomly selected plants in each plot. At maturity, two central rows at each tagged plant were hand harvested grain yield and 1000 seed weight (TSW). The total grain yield at 15% moisture were determined for each plant and converted to kilograms per hectare at harvest.

3.7. AUDPC analysis

Disease progress curve, which consist of proportions of diseased plats (i.e. disease incidence) recorded at ten days interval starting from the onset of disease 6 times in each location through out the growing period. To ensure consistent disease evaluation in the field, a disease progress curve was made. This curve was developed from 10 days percentageseverity reading in different locations. By constructing a curve, symptom development and disease severity was compared over years and locations. The area under disease progress curve (AUDPC) is used to quantify repressing of the beginning of the epidemic and the time until the blight reached peak. Leaf blight for whole plant was converted to AUDPC to compare relative level of resistance and highly susceptible varieties. The derived disease parameter, AUDPC was calculated according to the equation of Shaner and Finney (1971) using the following formula:

$$\text{AUDPC} = \sum_{i=1}^n (x_{i+1} + x_i) (t_{i+1} - t_i) / 2$$

Where

x_i = disease severity (percent disease leaf area) at the i^{th} observation

t_i = time (days) at the i^{th} observation

n = total number of observation.

Analysis of disease development could be performed when greater quantification is needed for resistance evaluation. The disease progress curve represent an integration of all host, pathogen and environmental effects occurring during disease development and provides an opportunity for greater in depth analysis, when comparing small difference among cultivars.

3.8. Data Analysis

Analysis of variance was conducted for AUDPC, disease incidence, lesion number and lesion size at all plant growth stages, yield data were subjected to analysis of variance, and means were compared using a Duncan Multiple Rang Test at $p \leq 0.05$ (SAS, 1989).

3.9. Correlation and regression analysis

Correlation analysis was performed using SAS PROC CORR (SAS, 1989) to determine relationship among disease assessment parameters such as disease incidence, lesion number, severity, lesion size and area under disease progress curve (AUDPC). Regression analysis was undertaken to examine the response of relationship between AUDPC and severity score (1-5) on yield of 13 maize varieties. The goodness of fit for regression equation models was determined by evaluating the indictors; coefficient of multiple determinations (r) that explains proportion of the total variation of the dependent variable (yield) associated with independent variable,

F-statistics that test the over all- significant of the regression equation at defined probability level; (Teng *et al*, 1979)

RESULTS AND DISCUSSION

1. Morphological, Cultural and Pathogenicity Variation of *Exserhillum turcicum*

1.1. Occurrence of NCLB in Gambella and Oromiya (2004)

The survey result on the occurrence, distribution and importance of NCLB in maize growing zones of two separate Regional State of Ethiopia are presented in (Table 3). Seventy isolates which had been collected from infected leaf samples revealed that NCLB was widely distributed in Gambella Regional State and western and southwestern zones of Oromiya Regional State. The altitude where the survey was conducted ranged from 500 m at Gambella plain to 2500 m at west Showa zone. Slight difference in severity and incidence was found between the two regional states. The severity and incidence were slightly higher at Gambella. The widely distribution and the high severity of NCLB in Gambella could be attributed to its ample precipitation, high humidity and warm temperature during the survey season, and the no-tillage system cultural practices and lack of crop rotation in the region that are favorable for stimulating the growth of the fungus. Boosalis *et al.* (1967) and Levy and Cochen (1983) showed that out break of northern leaf blight are associated with high humidity and certain cultural practice. Cultural practices, as no-tillage system is a common practice in the region. This trend may also contribute to the supply of initial inoculums in previous crop and the build up inoculum from subsequent cropping systems. Rotem (1988) emphasized and summarized the influence of weather on occurrence of epidemic and illustrated an importance of understanding the effect of environmental variation on disease development. Our finding also indicated that the importance of temperature in disease development is consistent with the geographic distribution of severely disease in low land area.

Table 3 Occurrence and severity of NCLB in different maize growing zones and districts of Gambella and Oromiya regions (2004).

Region/Zone	District	No. of Collected sample isolates	%	Nclb severity*
Gambella /Zone I	Jikawo	8		High
	I tang	5		High
	Sub total	13	(18.6%)	
Gambella/Zone II	Abobo	5		High
	Gambella	5		High
	Pundgo	10		High
	Sub total	20	(28.6%)	
Oromiya/ Illuababor	Bure	2		High
	Sibu	2		High
	Uka	2		High
	Gore	2		High
	Metu	1		High
	Urumu	2		High
	Yayo	1		High
	Chora	2		High
	Dedesa	4		moderate
	Bedele	2		Moderate
	Sub total	20	(28.6%)	
Oromiya/East Wollega	Nekemte	5		High
	Sire	1		Moderate
	Sayo	1		High
	Sub total	7	(10.0%)	
Oromiya/West Showa	Bako	4		High
	Bako-Tibe	2		Moderate
	Gudar	1		Low
	Ambo	1		High
	Dandi	2		Moderate
	Sub total	10	(14.3%)	
	TOTAL	70		

* Turcicum leaf blight severity rating by % infected leaf = (No of infected leaf x 100) /Total leaf

>40% = incidence high, 20-30% = moderate and ≤ 10% = low severity

1.2 Variation on morphological characteristics

Shape and size of conidia

Morphological characteristics of *E.turcicum* as described by its shape and size of conidia showed variation among the 70 isolates collected from the survey at different areas (Table 4). The result indicated that the shape of conidia was distinctively elongated for some while others were curved and spindle shaped. In the present study, the curved (38.57%) and elongated (38.57%) shaped of the conidia dominated in the population of the fungus (Figure 3). Variation on conidial size (length and width) and number of septum of 210 conidia samples, 3 for each isolates from 70 isolates of *E.turcicum* indicated that an average size of conidia was 92.68 μ (range 71.12-109.22) in length, 15.46 μ (range 12.70-17.78) in width, and 5.3 (range 2-7) in number of septum. The normal distribution of conidial width and length, and number of septum and value of standard deviation (SD) and variance (S^2) indicated that the variation on morphological characteristic had been existed among 70 isolates of *E.turcicum* (Table 5).

Table 4 Percent conidial shape of 70 isolates of *E.turcicum*

Conidial shapes	Number of isolates	Percent of total Isolate
- Elongated	27	38.57
- Curved	27	38.57
- Spindle	16	22.86
Sub total	70	100

1.3. Variation on cultural characteristics

The results of pure cultures of 28 selected isolates showed that the colonial growth raised and appraised and the colony density was sparse and tiny. About 21.4, 35.7, 21.4, and 21.4% of total isolates belonged to sparse, spraced, raced and tiny respectively (Table 6). The colonial color of the isolates generally belonged to the

olivaceous color group including olivaceous gray and olivaceous brown. On the reverse side of the fungus pigments varied from gray to green white in the agar center.

Table 5 Average mean conidial width, length and septum of 70 isolates of *E.turcicum*.

Statistical indicator	Width (μ)	¹ Length (μ)	Number septum
Range	12.70- 17.78	71.12-109.22	2-7
Mean	15.46	92.6	5.3
Median	15.24	91.44	5.0
Mode	15.24	91.44	2.7
SD	1.946	9.334	0.938

¹Average mean conidial obtained from total 210 conidia from 70 isolates, three conidia for each isolates were observed

Table 6 Proportion of colonial gross and colonial color of 28 *E.turcicum* isolates collected from different locations of western and southern Ethiopia in 2004.

Character types	No of isolates	Percent of total Isolate
- Raised	6	21.42
- Apprised	10	35.74
- Sparse	6	21.42
- Tiny	6	21.42
Sub total	28	100
Obverse side		
- Olivaceous gray	8	29.03
- Olivaceous brown	8	29.03
- Olivaceous	12	41.49
Sub total	28	100
Reverse side		
- Gray	15	53.20
- Green white	13	46.80
Sub total	28	100



Figure 3 Conidia of *Exsrohilum turcicum* from infected leaf lesion.

1.4. Variation on Pathogenicity

Selected twenty isolates that were tested for pathogenicity on eleven maize varieties in the greenhouse were exhibited highly significant difference among isolates, maize varieties and isolates by variety interactions (Table 7). Two contrasting lesion types of disease reaction were observed. In green house inoculation at 4-5 leaf stage, initial symptoms appeared as minute white to light green flecks 7-10 days after inoculation on both resistance and highly susceptible leaves. On resistant leaves, lesions were smaller and slightly elongated with chlorotic resistance type of lesions (Figure 4a). Highly susceptible plants expressed lesion that were elongated grayish green, white spot to tan necrotic lesions with no evidence of chlorosis (Figures 4b, c, d, f, and g). Among the improved maize varieties, highly susceptible varieties like Abobako, Gusaw, Gibe Comp-1, and Local-M showed significantly ($p < 0.05$) higher lesion size (average range of 2.62- 2.91 cm²). Significant ($p < 0.05$) smaller lesion size was recorded for seedling of variety of Kuleni (0.69 cm²) and BH540 (0.93 cm²). The rest tested maize varieties such as Guto, BH660, BH670 and

BH-QP-542 recorded average lesion size range of 0.91-1.29 cm² (Table 7). Among the isolates, GIN-98, PUN-1.3, GIN-100 and GAM-1 had caused higher lesion size of 1.71, 1.6, 1.57 and 1.56 cm², respectively. The isolates ELI-4 produced the least lesion size (0.58 cm²) among all the isolates. The results indicated that there were five virulence patterns exhibited on 11 maize varieties after inoculating with 20 isolates of *E.turcicum* (Table 8). Maize varieties exhibited different reactions to different isolates. Gibe-comp-1 was highly susceptible to isolates GIN-100 and highly susceptible to other six isolates. Followed Gibe-comp-1, local-M and Gusawu were highly susceptible to six and five of the isolates respectively. Abobako was highly susceptible and highly susceptible to two and one of the isolates respectively. However, Kuleni was highly resistance and resistance to six and 12 of the isolates respectively. Moreover, Kuleni was found highly susceptible to none of the isolates. This showed that Kuleni was the most stable variety to 20 isolates of *E.turcicum* as indicated by lowest scale values. Similarly, BH540 was highly resistance to five of the isolates and highly susceptible to none of them.

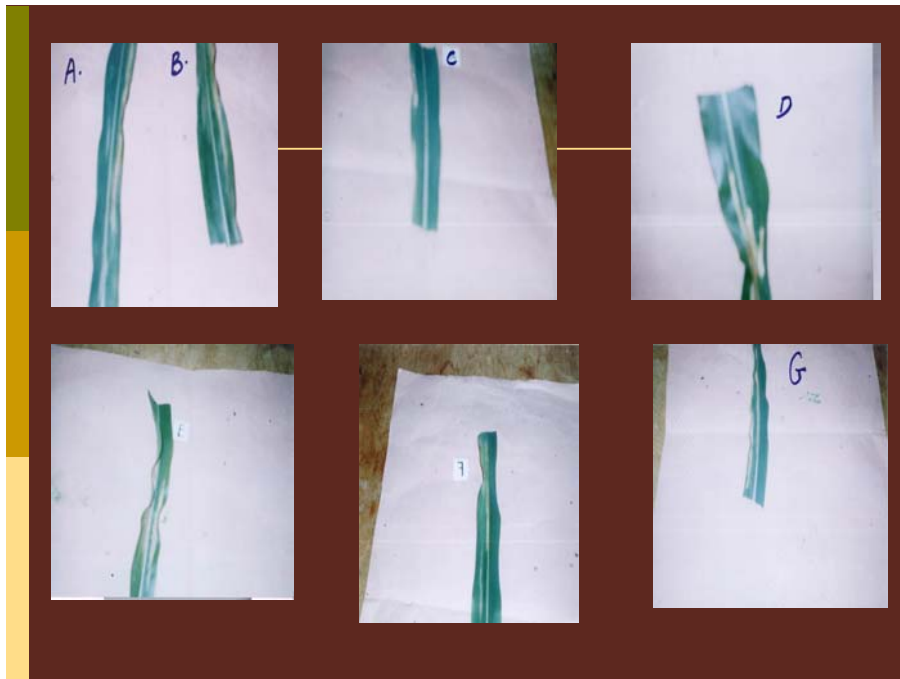
The impact of the isolates on maize varieties was also remarkably different among isolates (Table 8). The impact of isolate PUN-1.3 was highly aggressive on Abobako and BH140 than that on the other maize varieties. GIN-100 was very aggressive on Gibe-comp-1 and aggressive on other two varieties. GOR, BAT and GIN-98 isolates had aggressive impact on four, three and three varieties respectively. On the contrary, isolates ELI-4, PUN-1.5, PUN-2.1 and BUR were non-aggressive or weak pathogen to all varieties. Isolate BAD3.2 was less aggressive on varieties BH140 and Abobako than isolate PUN 1.3 on the same variety. However, BAD3.2 and PUN 1.3 were caused less aggressive on varieties BH660 and Kuleni. Isolate BAD3 and PUN-1 were moderately aggressive on most varieties.

Cluster analysis

Dendrogram showing virulence similarity and successive clustering of the twenty isolates of *E.turcicum* on 11 maize varieties is presented in Figure 5 and Table 9. Cluster analysis using UPGMA (unweighted pair group method based on arithmetic

mean) was done based on the data of lesion size of *E.turcicum* isolates on 11 maize varieties, with similarity coefficient of 0.57. Cluster I comprised one isolate, NEW, causing an average lesion size of 1.39 cm². Cluster II consisted of isolate GOR that caused average lesion size of 1.36 cm² and most aggressive to larger number of maize varieties. Cluster III contained four isolates. Cluster IV, the largest cluster, contained 12 isolates. Cluster I, II and III were identified as virulent pattern. Cluster IV that collected from different locations was classified as moderately virulent. There was wide variation in lesion size, which range from 1.01 cm² to 1.71 cm² in the cluster IV and classified into subgroups. Isolates BED -70 and PUN1.1 separates from others at the similarity coefficient of 0.91 because its virulent reaction showed to all maize varieties except Kuleni. Cluster V classified as avirulent isolates that contained BURE-1 and ELI-4 that exhibited pattern on most varieties.

These experiments have demonstrated that most isolates obtained from high altitude areas like GOR and GIN had high pathogenicity when compared with isolates from low altitude of Gambella such as BURE-1 and ELI-4 that exhibited as avirulent. Data from this study as well as from previous reports (Merle *et al.*, 1957) on work with this fungus supported the hypothesis that variability existed in pathogenicity of the causal organism. Our result also supported by Warren (1975) reported showed that the difference of pathogenicity due to difference in environment constituted a major source of variation.



Figures 4 Characteristics of symptoms of NCLB produced on some maize after 7-10 days

inoculations

A. Kuleni (R) × PUN1.5;

B. BH660 (S) × PUN2.1;

C. Gusaw (S) × PUN2.1;

D. Abobako (S) × GAM-1;

E. BH-QP-542 (M) × PUN1.1;

F. Gibe Comp-1 (S) × NEK-1;

G. Gusaw (S) × BAD3.2

Table 7 Average lesion size (cm²) produced by 20 *E.turcicum* isolates on eleven maize varieties after 14 days inoculation.

ISOLATE	Varieties											
	Abobako	BH140	BH540	BH660	BH-670	GIBE COMP-1	GUSAW	GUTO	LOCAL-M	KULENI	BH-QP-542	MEAN
BURE-1	1/ 0.73a-j ^{1/}	0.73a-j	0.33a-f	2.20p-w	0.13ab	0.10a	2.20p-w	1.11a-k	1.66c-n	0.89a-k	0.68a-j	0.97 ^{2/}
PUN2.1	2.00j-w	0.70a-j	0.75a-j	1.50a-m	1.30a-l	1.63b-w	1.97a-m	1.33b-n	2.00a-k	0.58a-j	0.86a-k	1.32
BUR	0.78a-k	0.80a-j	0.93a-k	1.13a-k	1.00a-k	1.13a-k	1.86a-n	1.23a-n	1.86a-n	0.66a-k	0.90a-k	1.11
NEW	3.00 wx	2.66v-x	0.86a-m	0.53a-j	0.20a-i	0.23a-d	1.66a-m	0.20a-i	1.66a-k	0.77a-k	1.66a-m	1.39
PRE	0.86a-m	2.66v-x	0.86a-n	0.66a-k	0.66a-k	1.36a-k	1.77a-k	2.00j-w	1.83a-n	1.06a-m	0.66a-j	1.24
GIN-98	2.00j-w	2.00j-w	0.86a-k	0.66a-k	2.40a-m	3.00w-x	1.73d-w	2.06d-n	1.80e-w	0.72a-m	1.68a-m	1.71
BED-70	2.00j-w	1.26a-m	1.60a-m	1.68a-k	1.68a-k	2.36r-w	1.54a-m	1.33a-m	1.33a-m	0.58a-k	1.66a-m	1.28
PUN1.1	1.16a-l	1.13a-k	1.33a-m	1.33a-m	1.06a-k	2.36r-w	1.49a-m	0.66a-k	1.33a-k	0.54a-k	1.86a-m	1.29
PUN1.5	1.40a-m	0.88a-k	1.69d-n	0.45a-i	1.11a-w	1.46a-m	1.49a-m	1.50a-m	1.50a-m	1.62b-w	2.00l-w	1.37
BAD3.2	0.93a-k	0.94a-m	1.45a-i	0.46a-k	0.46a-m	0.93a-s	1.50a-m	1.46b-n	1.46b-n	0.60a-k	0.96a-k	1.01
GAM-2	1.78e-n	0.86a-k	1.62b-n	1.18a-m	1.33a-m	1.00a-k	1.52a-m	1.50a-m	1.80e-w	0.73a-j	0.73a-k	1.44
NEK-81	1.40a-m	0.70a-j	0.86a-k	1.56a-n	1.93h-w	2.16u-y	1.50a-m	0.16a-c	1.50a-m	0.54a-j	0.97a-k	1.20
GAM-1	3.73y	0.66a-j	0.90a-k	1.00a-k	2.60t-x	1.60a-n	1.58a-l	1.16a-l	2.13n-w	0.66a-i	1.24a-m	1.56
ELI-4	0.40a-g	1.04a-k	0.36a-f	0.63a-g	0.26a-d	1.60a-k	0.26a-d	0.56a-j	0.50a-j	0.33a-f	0.49a-j	0.58
ITA1.2	2.00j-w	1.10u-k	0.52a-f	0.33a-f	0.93a-s	0.66a-j	2.16u-y	1.10 a-u	2.16 0-w	0.64a-i	0.74a-k	1.12
BAT	0.30a-e	0.53a-j	1.53a-m	0.73a-k	0.93a-s	2.66v-x	2.16u-y	1.37a-m	2.160-w	0.84a-k	0.80a-k	1.27
PUN3.1	1.33a-m	2.00l-w	1.06a-k	0.36a-g	1.10a-u	0.93a-s	1.84f-w	1.40a-v	2.10e-w	0.85a-k	0.80a-k	1.25
PUN1.3	3.83xy	3.83xy	0.36a-k	0.60a-g	1.10a-u	1.16a-u	1.71f-n	1.33a-m	1.80e-w	0.30a-e	1.63b-w	1.60
GIN-100	1.80a-k	1.06a-k	0.36a-g	0.73a-j	1.00a-k	4.50y	2.16u-y	1.10a-u	2.160-w	0.44a-i	2.00j-w	1.57
GOR	0.43a-j	0.70a-j	2.00j-w	0.41a-h	2.44s-w	2.64u	2.16u-y	0.80a-k	2.160-w	0.38a-g	0.93a-k	1.36
MEAN	2.89	1.29	0.93	0.91	1.19	2.89	2.91	1.17	2.62	0.69	1.12	

^{1/} In a column, means followed by common letters are not significantly different at 5% levels of probability by DMPRT. the lesion size data measured in the greenhouse represent the mean of three replications.

Table 8 Virulent phenotype pattern of 20 *E.turcicum* isolates on eleven maize varieties after 14 days after inoculation.

ISOLATE	varieties											
	Abobako	BH140	BH540	BH660	BH-670	Gibe Comp-I	Gusaw	Ghtto	Local-M	Kuleni	BH-QP-542	Mean
BURE-1	2 ¹	2	1	4	1	1	4	3	3	2	2	2.27
PUN2.1	3	2	2	3	3	3	3	3	3	2	2	2.64
BUR	2	2	2	3	2	3	3	3	3	2	2	2.45
NEW	4	4	2	1	1	1	3	1	3	2	3	2.27
PRE	2	4	2	2	2	3	3	3	3	3	2	2.64
GIN-98	3	3	2	2	4	4	3	4	3	2	3	3.00
BED-70	3	3	3	3	3	4	3	3	3	2	3	3.00
PUN1.1	3	3	3	3	3	4	3	2	3	1	3	2.82
PUN1.5	3	2	3	1	3	3	3	3	3	3	3	2.73
BAD3.2	2	2	3	1	1	2	3	3	3	2	2	2.18
GAM-2	3	2	3	3	3	2	3	3	3	2	2	2.64
NEK-81	3	2	2	3	3	4	3	1	3	1	2	2.45
GAM-1	5	2	2	2	4	3	3	3	4	2	3	3.00
ELI-4	1	2	1	2	1	3	1	2	1	1	1	1.45
ITA1.2	3	3	1	1	2	2	4	3	4	2	2	2.45
BAT	1	1	3	2	2	4	4	3	4	2	2	2.55
PUN3.1	3	3	3	1	3	2	3	3	4	2	2	2.64
PUN1.3	5	5	1	2	3	3	3	3	3	1	3	2.91
GIN-100	3	3	1	2	2	5	4	3	4	1	3	2.82
GOR	1	2	3	1	4	4	4	2	4	1	2	2.55
MEAN	2.89	1.29	0.93	0.91	1.19	2.89	2.91	1.17	2.62	0.69	1.12	2.57

¹ = Virulent phenotype pattern were categorized according to lesion size: 1 = highly resistance (HR) with lesion size 0.0-0.5 cm²; 2 = Resistance (R) with lesion size 0.6 – 1.0 cm²; 3 = moderately resistance (MR) with lesion size 1.1 – 2.0 cm²; 4 = Highly susceptible (S) with lesion size 2.1 – 3.0 cm² highly susceptible (HS) with lesion size more than 3.0 cm².

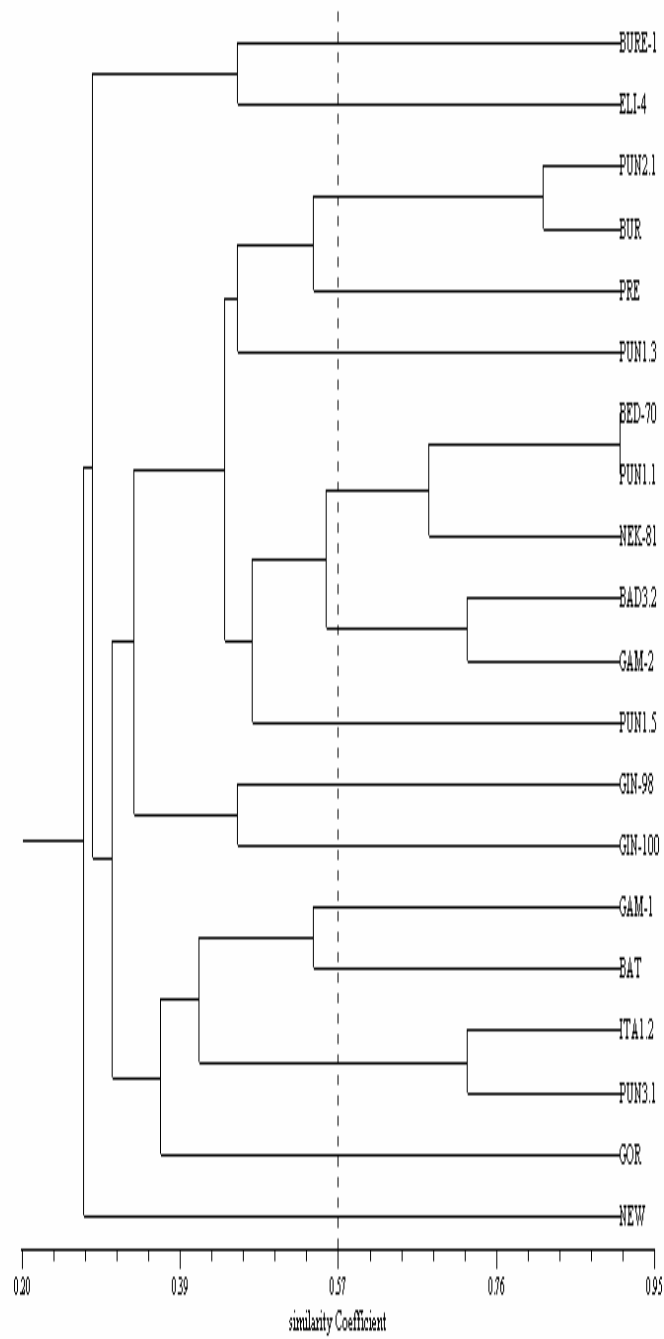


Figure 5 Dendrogram showing virulence similarity and successive clustering 20 isolates of *E. turcicum* on 11 maize varieties.

Table 9 Average lesion size of isolates corresponding to cluster analysis

<i>Cluster group</i>	<i>isolates</i>	<i>Average of lesion size on 11 maize varieties.</i>
I	NEW	1.39
	Avr. group	1.39
II	GOR	1.36
	avr	1.36
III	PUN3.1	1.25
	ITA1.2	1.12
	BAT	1.27
	GAM-1	1.56
	Avr. group	1.3
IV	PUN2.1	2.64
	BUR	2.45
	PRE	2.64
	PUN1.3	2.91
	BED-70	3.00
	PUN1.1	2.82
	NEK-81	2.45
	BAD3.2	2.18
	GAM-2	2.64
	PUN1.5	2.73
	GIN-98	3.00
	GIN-100	2.82
	Avr. group	3.23
	V	BURE-1
ELI-4		1.45
	Avr. group	1.86

2 Evaluations of NCLB Reaction on Maize Varieties under Field Conditions

2.1. Disease onset of development

The disease symptom appeared on the highly susceptible variety earlier than in all locations. Disease onset (DA) days after crop planting of northern leaf blight appeared early at 30 days at Gambella. The same trend was observed in 2003 cropping season at Abobo (Table 10). Disease appearance in 2004 cropping season at Bako was between 78-85 days after planting. The dry weather at Bako at planting time most probably delayed the onset of northern leaf blight. Disease onset on the highly susceptible variety Abobako was 15 and 13 days earlier than Kuleni and BH660, respectively at Gambella. The same result was obtained in 2003 at Abobo that the symptom was observed 9days earlier on Abobako than Kuelin and BH660. Whereas it was earlier by 7 days compared with the latter two varieties at Bako. The initial earliest report of symptoms expression for both location related to ultimate crop losses. Early onset of the disease is generally a key to high potential damage in a given season, provided climatic conditions are favorable for disease development.

Table 10 Disease onset (DO) recorded for northern leaf blight under field conditions of different locations.

Genotype	Disease onset (days after emergence)		
	Bako	Gambella	Abobo
BH-QP-	78	36	36
Local-m	78	36	30
Abobak	78	36	30
Gusaw	78	30	30
BH-541	84	36	39
Kuleni	85	45	45
BH660	85	30	43
BH-530	78	42	36
BH140	84	41	39
BH540	84	36	38
Guto	78	36	36
Gibe	78	36	36
BH-670	84	36	36

2.2. Disease development

The mean value of the disease assessment for north corn leaf blight varied considerably among locations due to environment and varieties the disease reached maximum severity in mid October at both locations and no increase was observed until end of October (Figures 6, 7 and 8). Within each experiment, disease development was differed markedly among varieties. Variety Kuleni had no infection at the first rating date at Gambella and Abobo. At Abobo, varieties such as BH140 and BH530 had no infection at the first rating date. In the five moderately moderate resistance varieties, their final severity reached 88.88% (Figure 6). While, on moderate resistance varieties disease increase very slow and reached maximum of 75.55% at the end of growing period (Figure 7). However, the disease growth on highly susceptible varieties, disease increase was very fast and reached maximum at 94.44% on variety Gusawu (Figure 8).

Average disease development rate for different locations is presented in Figure 9. The development of the disease in different locations differed remarkably. In the most of the plot first disease incidence low. These low levels of initial infection not continue to be low, gradually developed to the level of moderate or high epidemic. For instance, at Abobo average growth ranged from 8.1 to 77.69%. While at Bako, high level of epidemic and growth rate was very fast and reached 80.25% and at Gambella, the disease increase to 63.58% (Tables 11, 12 and 13).

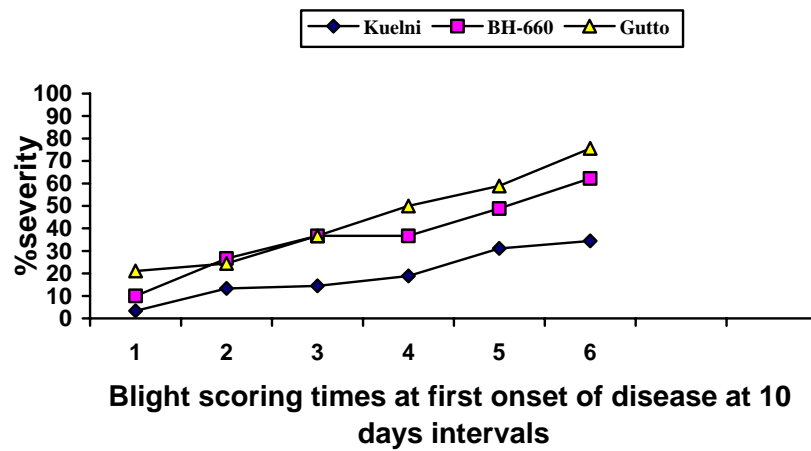


Figure 6 Disease progresses for northern corn leaf blight that recorded for six times on the moderately moderate resistance maize varieties at Gambella, Abobo and Bako in 2003 and 2004.

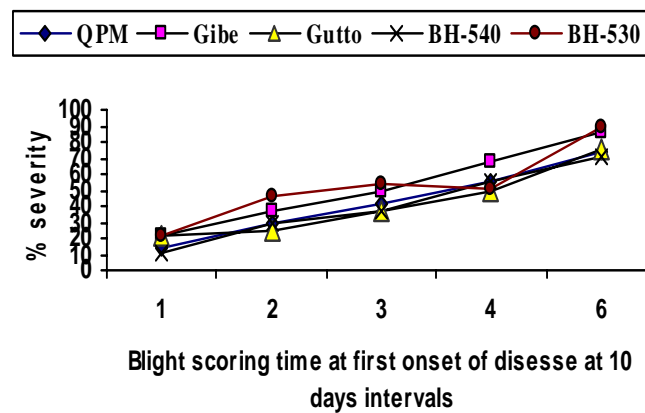


Figure 7. Disease progress of northern leaf blight recorded six times at different growing period on moderate highly susceptible varieties in 2003 and 2004 at Gambella, Abobo and Bako locations.

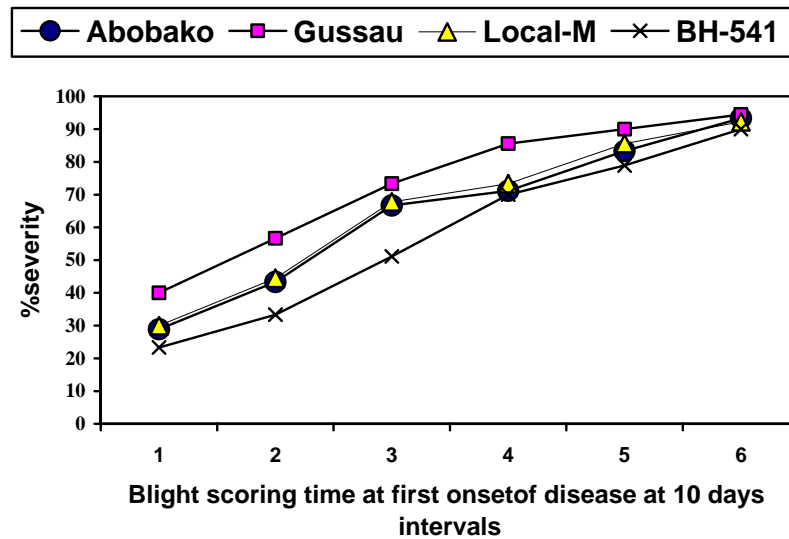


Figure 8 Disease progresses for northern corn leaf blight recorded six times on very highly susceptible varieties at Gambella, Abobo and Bako n 2003 and 2004

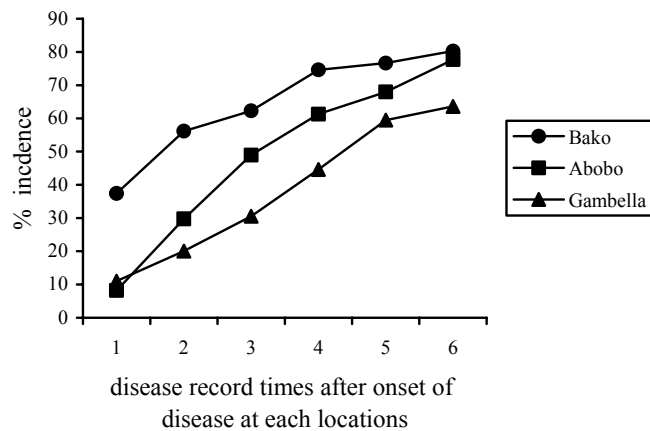


Figure 9 Disease progress development of northern leaf blight at each recorded time at three locations in 2003 and 2004.

Table 11 Progress of disease development of northern leaf blight of corn on 13 maize varieties in relation to recorded time at Abobo in 2003.

Variety	% of incidence at recorded time for 10 days intervals ¹					
	1	2	3	4	5	6
BH-QP-542	3.33a	13.33cd	40.00b	53.33cd	60.00d	76.66ad
Gibe Comp-1	20.00a	36.66ab	66.67a	76.66ab	76.66ab	90.00ab
Guto	3.33a	13.33cd	30.00bc	40.00d	40.00e	60.00d
BH-670	10.00a	30.00ac	40.00b	46.66cd	60.00d	80.00ac
BH540	10.00a	30.00ac	36.66b	63.33bc	73.33ad	73.33bd
BH140	0.00a	13.33cd	30.00bc	43.33b	53.33cd	63.33bc
BH-530	0.00a	30.00ac	53.33ab	76.66ab	80.00ac	80.00ac
BH660	10.00a	30.00ac	36.67b	53.33cd	60.00d	66.66d
Kuleni	0.00a	6.66d	10.00c	16.66e	23.33f	30.00ac
BH-541	10.00a	23.33ad	53.33ab	63.33bc	70.00bd	90.00ab
Gusaw	20.00a	40.00a	76.66a	86.66a	90.00a	96.66a
Abobako	10.00a	20.00ad	73.33a	80.00ab	83.33ab	93.33ab
Local-M	10.00a	26.66ac	76.66a	80.00ab	90.00a	93.33ab
P=0.05	NS	* ^b	*	*	*	*

^a means of four replications, ^b Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹ Recording time 1. before tasseling; 2. After tasseling; 3. before silking; 4. after silking; 5. before dent stage; 6. at dent stage

Table 12 Progress of disease development of northern leaf blight of corn on 13 maize varieties in relation to recorded time at Gambella in 2003.

Variety	% of incidence at recorded time for 10 days intervals ^L					
	1	2	3	4	5	6
BH-QP-542	3.33bc	13.33ce	23.33de	40.00ce	56.66b	66.66d
Gibe Comp-1	20.00a	26.66de	40.00ce	56.66ac	70.00ac	90.00ab
Guto	3.33bc	10.00be	23.33ce	40.00ce	56.66ac	83.33ac
BH-670	10.00ac	16.66be	26.66ce	40.00ce	53.33b	83.33ac
BH540	10.00ac	20.00bd	30.00bd	40.00ce	53.33b	63.33bd
BH140	6.66bc	13.33ce	23.33de	40.00ce	53.33b	66.66d
BH-530	16.66ab	26.66de	30.00bd	40.00ce	50.00b	80.00ac
BH660	3.33bc	16.66be	23.33de	30.00ef	43.33b	73.33bc
Kuleni	0.00c	3.33e	30.00bd	16.66f	26.66b	30.00c
BH-541	10.00ac	20.00bd	33.33bd	66.66a	76.66a	86.66ab
Gusaw	20.00a	36.66a	50.00a	75.53a	83.33a	93.33a
Abobako	20.00a	30.00ab	36.66a	50.00bd	73.33a	90.00ab
Local-M	20.00a	30.00ab	43.33ab	50.00bd	73.33a	86.66ab
P=0.05	* ^b	*	*	*	*	*

^a mean of four replications, Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

^L Recording time 1. before tasseling; 2. after tasseling; 3. before silking; 4. after silking; 5. before dent stage; 6. at dent stage

Table 13 Progress of disease development of northern leaf blight of corn on 13 maize varieties in relation to recorded time at Bako in 2004.

Variety	% of incidence recorded time for 10 days intervals ¹					
	1	2	3	4	5	6
BH-QP-542	36.67bc	63.33ad	70.00ac	73.33abb	73.33ab	73.33ab
Gibe Comp-1	23.33ce	56.67cd	60.00ce	70.00ac	76.67ab	80.00ac
Guto	40.00ce	46.67cd	56.67ce	70.00ac	80.00a	83.33ac
BH-670	16.67de	43.33ce	43.33ef	60.00bc	66.67ab	70.00bc
BH540	13.33e	40.00ce	43.33ef	63.33ad	73.33ab	76.67ab
BH140	36.67be	56.66bd	66.67bd	83.33ab	83.33a	86.67ab
BH-530	50.00ad	80.00ab	80.00ac	83.33ab	96.67a	96.67a
BH660	13.33e	33.33de	40.00ef	43.33cd	43.33cd	56.67cd
Kuleni	10.00e	13.33e	20.00d	23.33d	43.33cd	43.33d
BH-541	50.00ad	56.67bd	66.67bd	83.33ab	90.00a	93.33ab
Gusaw	80.00a	93.33a	93.33a	96.67a	96.67a	96.67a
Abobako	56.66a	80.00a	90.00ab	90.00ab	90.00a	96.67
Local-M	60.00ab	66.66ac	80.00ac	83.33ab	83.33a	90.00ab
P=0.05	* ^b	*	*	*	*	*

^a mean of four replications, ^b Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹. Recording time: 1. before tasseling; 2. after tasseling; 3. before silking; 4. after silking; 5. before dent stage; 6. at dent stage

2.3. Lesion number and lesion size

Variation occurred in lesion number and size of northern leaf blight different varieties from different locations was high at the end of growing season. At beginning of onset disease, lesion number was not significantly different at Abobo and Gambella due to the low level of disease incidence and a small lesion number on leaves. While, at the end of growing period final lesion number significantly different ($P \leq 0.05$) for all locations (Tables 14, 15 and 16). For highly susceptible and moderate resistance varieties, the increment of lesions was consistent. Moderate resistance variety Kuleni showed small lesion number (1.24 at Gambella, 2.04 at Abobo and 0.53 at Bako) for all locations (Figure 10). Highly susceptible variety Gusau recorded high lesion number at Gambella, Abobo and Bako (5.70, 5.00 and 7.56, respectively) when compared with other varieties (Figure 11). Lesion number for highly susceptible varieties increased by 0.057%, while on moderate resistance varieties remained virtually unchanged, which was consistent with the finding of Hilu and Hooker (1964) that disease level increase increased the number of lesions for highly susceptible varieties. The average increase of lesion number in each location is presented in Figure 12. At Bako, lesion number was increased from 0.35 to 2.41, at Abobo location the increment of lesion number was 0.16 to 3.56, and 0.44 to 3.33 at Gambella.

Similar result like was found for lesion size in all locations and among all varieties (Tables 17, 18 and 19). Gusawu had significantly large lesion size in all locations whereas for Kuleni variety lesion size was small and consistent in all locations. The development of lesion size was different in each location. At Gambella and Abobo, average lesion size development was small and reached 9.93 cm^2 and to 8.09 cm^2 at the end of growing season, respectively. While, at Bako lesion size expansion was very fast compared with the two locations and reached 14.68 cm^2 (Figure 13). Varieties considered as moderate resistance such as Kuleni and BH660 produced small lesion finally reached 2.56 and 5.05 cm^2 respectively. Varieties that considered as highly susceptible such as Gusaw, Local-m and BH-140 recorded the largest lesion size at finally reached 15.01 and 19.31 cm^2 . Lesion of northern leaf blight on highly susceptible varieties easy to recognize large in size and width and

many in lesion number. Our present data was in agreement with the finding of Populer (1978) who reported that lesion size is one of the components of highly susceptible of host to a fungus. Hence, highly susceptible lines of maize produced longer and wider lesion. Lesion sizes depend partially on the number of infection site, genotypes, environmental conditions and aggressiveness of the pathogen. Traut and Warren (1993) reported that highly susceptible lines of corn produce longer and wider lesions than those with partial or full resistance.

The average level of resistance or mean lesion area, the rate of increase in lesion size, and the shape of the lesion and size was influenced by host genotype as determined by contributions of each parents (Singules *et al*, 1988). Other reports indicated that toxin-producing pathogens are some races that produce large in size and fast developing lesion on hosts (Xiao *et al*, 1991, 1992, Traut and Warren1993). General resistance to NCLB is expressed as reduced disease severity due to reduction in number of lesions and lesion size.



Figure 10 Symptoms of NCLB on leaves of resistance varieties.



Figure 11 Symptoms of NCLB on leaves of susceptible maize varieties.

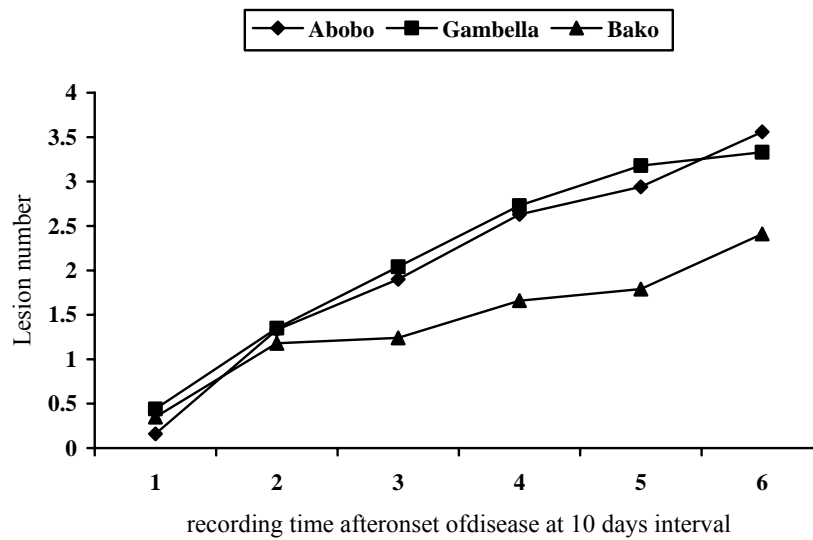


Figure 12 Progress of lesion number development recorded times at three locations in 2003 and 2004.

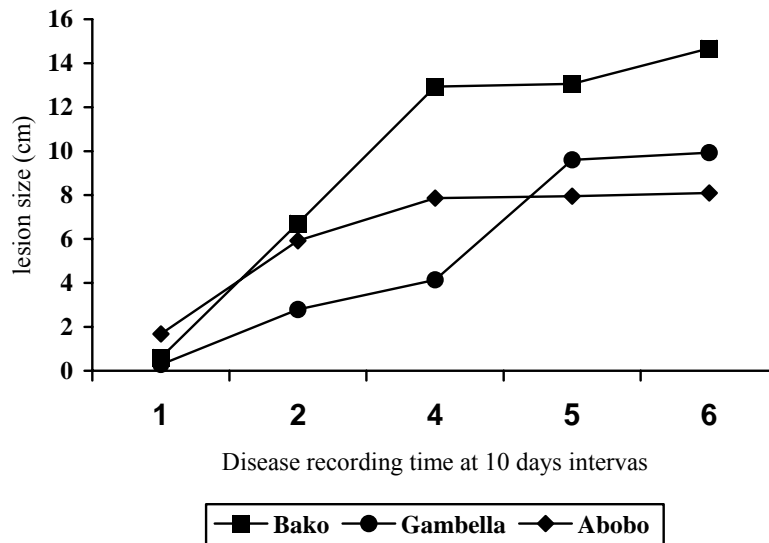


Figure 13 progress of lesion size development of northern leaf blight on 13 maize varieties at three locations

Table 14 Progress of increased lesion number development on 13 maize varieties at six recording times at Abobo in 2003.

Variety	Lesion number recording at 10 days intervals ¹					
	1	2	3	4	5	6
<i>BH-QP-542</i>	0.00a	1.16bc	1.33de	1.83de	2.16de	3.26de
Gibe Comp-1	0.00a	1.00bc	1.46ce	2.66cd	3.06bd	3.79bd
Guto	0.00a	1.23ac	1.43ce	3.06bc	3.21bd	3.28de
BH-670	0.00a	1.16bc	1.36ce	2.00de	2.07de	3.09e
BH540	0.00a	1.00bc	1.46ce	2.00de	2.05de	3.07e
BH140	0.00a	2.00ab	2.03bd	2.66de	3.06bd	3.79bd
BH-530	0.00a	1.16bc	1.83be	2.50cd	2.70ce	3.40ce
BH660	0.00a	1.50ac	1.46ce	2.00de	2.09ce	3.11e
Kuleni	0.00a	0.48c	0.81e	1.08e	1.66e	2.04f
BH-541	0.00a	1.83ab	2.40ac	3.90ab	4.05ab	4.06b
Gusaw	1.07a	2.30a	3.33ad	4.35a	4.41a	5.00a
Abobako	0.93a	1.66ab	3.23a	3.33bc	4.01ab	4.16b
Local-M	0.13a	1.33ac	2.63ab	2.83cd	3.75ac	4.33b
P=0.05	NS	* ^b	*	*	*	*

^a mean of four replications, ^b Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹ Recording time; 1. before tasseling; 2. After tasseling; 3. before silking; 4 after silking; 5. before dent stage; 6. at dent stage

Table 15 Progress of increased lesion number development on 13 maize varieties at six recording times at Gambella in 2003.

Entry	<i>Lesion number recorded at 10 days interval¹</i>					
	1	2	3	4	5	6
BH-QP-542	0.00a	1.33a	1.16bc	2.46cd	2.83ef	2.93c
Guto	1.01a	1.66b	2.20ab	2.43cd	3.46ce	3.80bd
BH-670	0.02a	1.01bd	1.03bc	1.28d	1.70fg	2.10ef
BH540	0.00a	0.34d	1.36bc	1.43cd	1.80fg	1.96ef
BH140	0.00a	1.06d	1.40bc	2.00cd	2.10ef	2.23ef
BH-530	0.35a	1.00bd	1.80bc	2.00cd	2.10ef	2.23ef
Gibe	0.01a	1.66ab	2.63ab	3.00bc	3.91bd	3.95bc
BH660	0.68a	1.06bd	1.80bc	2.00cd	2.10ef	2.23ef
Kuleni	0.00a	0.56cd	0.93c	1.00d	1.22g	1.24f
BH-541	0.70a	1.56ac	2.90a	4.16ab	4.78ac	4.86ab
Gusaw	1.33a	2.33a	3.16a	5.00a	5.46a	5.70a
Abobako	0.68a	2.00ab	3.23a	4.50a	5.27ab	5.35a
Local-M	1.01a	2.00ab	2.46ab	4.30a	4.73ac	4.83ab
P=0.05	NS	* ^b	*	*	*	*

^amean of four replications, ^b Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹. Recording time 1.before tasseling; 2. After tasseling; 3.before silking; 4 after silking; 5.before dent stage; 6.at dent stage

Table 16 Progress of increased lesion number development on 13 maize varieties at six recording times at Bako in 2004.

Varieties	<i>Lesion number recorded at 10 days intervals¹</i>					
	1	2	3	4	5	6
BH-QP-542	0.43ad	1.06b	1.16bc	1.40b	1.53b	1.56b
Gibe Comp-1	0.33ad	0.96b	1.33bc	1.60b	1.66b	1.67b
Guto	0.20bd	0.33b	0.96bc	1.76b	1.85b	1.88ab
BH-670	0.23ad	0.36b	0.60c	0.86b	1.00b	1.13ab
BH540	0.33ad	0.53b	0.80bc	1.26b	1.31b	1.30ab
BH140	0.33ad	0.80b	0.26bc	1.20b	1.28b	1.36ab
BH-530	0.53ab	3.70a	3.70a	1.60b	1.67b	1.70ab
BH660	0.06dc	0.56b	0.96bc	0.90b	0.95b	1.16ab
Kuleni	0.00d	0.13b	0.33c	0.50b	0.50b	0.53b
BH-541	0.40ad	0.46b	0.57c	1.20b	1.28b	1.36ab
Gusaw	0.66a	2.00ab	2.50ab	5.46a	5.58a	7.56a
Abobako	0.50ab	1.06b	1.33bc	1.46b	2.23b	2.30ab
Local-M	0.66a	1.83ab	2.00bc	2.40b	2.45b	3.00ab
P=0.05	* ^b	*	*	*	*	*

^a mean of four replications Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹. Recording time; 1.before tasseling; 2. After tasseling; 3.before silking; 4' after silking; 5.before dent stage; 6.at dent stage

Table 17 Progress of lesion size development of northern leaf blight on 13 maize varieties at six times recorded at Bako in 2004.

Varieties	<i>Lesion size (cm²) recorded at 10 days intervals¹</i>					
	1	2	3	4	5	6
BH-QP-542	0.71 ^{ac}	4.27 ^b	11.84 ^{ad}	13.66 ^{bd}	17.02 ^{ac}	17.20 ^{ac}
Gibe Comp-1	0.69 ^{ac}	4.27 ^b	7.12 ^{ad}	10.72 ^{bd}	11.82 ^{bd}	11.96 ^{bd}
Guto	0.19 ^{bc}	5.02 ^{ab}	12.36 ^{ad}	13.36 ^{bd}	13.87 ^d	13.91 ^{bd}
BH-670	0.27 ^{bc}	1.96 ^b	4.01 ^d	6.85 ^{cd}	7.80 ^{cd}	8.00 ^{cd}
BH540	0.68 ^{ac}	8.33 ^{ab}	6.37 ^{bd}	9.17 ^{bd}	11.64 ^{bd}	11.81 ^{bd}
BH140	0.62 ^{ac}	11.32 ^{ab}	11.08 ^{ad}	13.23 ^{bd}	14.23 ^{bd}	14.31 ^{bd}
BH-530	0.95 ^{ab}	6.34 ^{ab}	7.46 ^{ad}	9.02 ^{bd}	11.97 ^{bd}	14.36 ^{bd}
BH660	0.09 ^{bc}	2.32 ^b	4.37 ^{cd}	6.77 ^{cd}	8.10 ^{bd}	8.20 ^{cd}
Kuleni	0.00 ^c	2.08 ^b	2.29 ^d	3.97 ^d	4.31 ^d	4.35 ^d
BH-541	0.87 ^{ac}	7.29 ^{ab}	14.19 ^{ac}	18.13 ^{ac}	20.30 ^{ac}	20.11 ^{ac}
Gusaw	1.22 ^a	14.51 ^a	16.16 ^a	26.10 ^a	27.34 ^a	27.40 ^a
Abobako	0.84 ^{ac}	10.14 ^{ab}	14.35 ^{ac}	20.02 ^{ab}	20.87 ^{ab}	21.05 ^{ab}
Local-M	0.91 ^{ac}	9.39 ^{ab}	16.12 ^{ab}	17.30 ^{ac}	17.92 ^{ac}	18.18 ^{ac}
P=0.05	*	*	*	*	*	*

^a mean of four replications

Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹. Recording time 1.before tasseling; 2. After tasseling; 3.before silking; 4. after silking; 5.before dent stage; 6. at dent stage

Table 18 Progress of lesion size development of northern leaf blight on 13 maize varieties at 6 times recorded at Abobo in 2003.

varieties	Lesion size (cm ²) recorded at 10 days intervals ¹					
	1	2	3	4	5	6
<i>BH-QP-542</i>	0.00a	9.16bc	10.13a	13.33a	13.36b	13.51b
Gibe Comp-1	0.00a	4.83d	4.96d	5.33b	5.42c	5.48c
Guto	0.00a	1.70ef	4.00d	4.50b	4.71c	4.06c
BH-670	0.00a	1.83ef	4.00d	4.50b	4.71c	5.10c
BH540	0.00a	3.36de	4.16d	4.21b	4.25c	4.43c
BH140	0.00a	3.83de	4.66d	4.78b	4.78c	4.20c
BH-530	0.00a	4.00de	4.50d	4.55b	4.55c	4.60c
BH660	0.00a	3.66de	3.66de	3.86b	4.41c	4.48c
Kuleni	0.00a	1.00f	1.08e	1.29c	1.29d	1.32d
BH-541	0.00a	7.32c	9.06c	13.39a	13.41b	13.46b
Gusaw	9.27b	14.00a	14.83a	15.63a	16.37a	16.50a
Abobako	4.80b	12.00a	12.83ab	13.20a	13.43b	13.80b
Local-M	7.77b	10.42ab	13.33ab	13.66a	14.20b	14.25b
P=0.05	*	*	*	*	*	*

^a Mean of four replications

Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

¹. Recording time 1. before tasseling; 2. After tasseling; 3. before silking; 4. after silking; 5. before dent stage; 6. at dent stage

Table 19 Progress of lesion size development of northern leaf blight on 13 maize varieties at 6 times recorded at Gambella in 2003.

variety	Lesion size (cm ²) recorded at 10 days intervals ^L					
	1	2	3	4	5	6
BH-QP-542	0.00c	1.00ac	1.33bd	1.83bd	7.93bc	8.33b
Gibe Comp-1	0.33bc	1.00ac	1.03cd	1.26de	9.68ab	9.76ab
Guto	0.10c	0.56bc	1.16bd	1.66cd	12.39ab	12.52ab
BH-670	0.04c	1.01ac	1.39bd	1.83bd	12.20ab	12.26ab
BH540	0.00c	1.00ac	1.58ac	2.16ac	12.24ac	12.33ab
BH140	0.00c	0.64bc	1.50ac	2.33ac	8.55ab	9.20b
BH-530	0.40ac	1.20ab	1.57ac	1.93bd	7.12c	8.80d
BH660	0.37ac	1.00ac	1.23bd	1.70bd	2.38d	2.46c
Kuleni	0.00c	0.74bc	0.75d	0.80e	1.95d	2.03c
BH-541	0.37ac	0.86ac	1.65ab	2.00ab	12.35ab	12.46ab
Gusaw	0.80a	1.33a	2.00a	2.83a	13.20a	14.05a
Abobako	0.80a	1.10ac 1.13ac	1.70ab	2.50ab	12.31ab	12.39ab
Local-M	0.47ac	1.13ac	1.67ac	2.03bd	12.43d	12.60ab
P=0.05	*	*	*	*	*	*

^a mean of four replications

Asterisks indicated significant level by Duncan Multiple Range Test at $p \leq 0.05$ probability.

^L 1. Recording time 1. before tasseling; 2. After tasseling; 3. before silking; 4' after silking; 5. before dent stage; 6. at dent stage

2.5 **Yielding ability under disease incidence.**

The mean yield and 1000seed weight at Gambella location was significant ($p < 0.05$) greater than that of Bako when mean disease severity significantly ($p < 0.05$) lower (Table 20). This is because the disease at Bako started late but later at the growth stage of the crop followed by fast disease progress before the crop reached dent stage. Whereas, at both location (Gambella and Abobo) highest yield and Tsw was obtained in 2003 due to in both locations the disease symptoms was observed earlier and the development of the disease very slow and reached maximum when the crop reached at maturity stage. Hence, the disease effect on yield was relative lower on both locations. Yield and Tsw of the relative highly susceptible varieties was generally low with all locations. The yield of moderate resistance varieties was generally better yield and Tsw were at all locations when the disease severities score also very low. Variety Gusawu, which considered as highly susceptible variety had high severity value 4.33 at Abobo and Gambella, and 4.66 at Bako location. The least severity of blight score on Kulen indicated the better resistance of this variety particularly under the severe epidemic. The rank of varieties reaction to disease severity was not affected by locations. This means that a variety that was moderately moderate resistance or highly susceptible in one location acted similarly in other locations indicating there was no difference in virulence in the pathogen among locations.

The result of the regression analysis (Figure 14) between severity and yield showed that there was strong but negative relationship between yield and severity ($r = -0.82$; $P = 0.0001$) which, means that under these conditions severity explains most of the yield variations.

Table 20 Average of severity (Sev), yield (q/ha) and thousand seed weight (1000g) of three locations.

Varieties	Abobo			Gambella			Bako		
	sev	Yield	Tsw	sev	Yield	Tsw	sev	yield	tsw
BH-QP-542	3.33a	31.04a	0.30bd	2.66ce	30.98b	0.33b	3.00ce	22.39ad	0.28d
GIBE-OMP-1	3.00	34.50a	0.35bc	2.66ce	36.39a	0.35b	3.33bc	20.57bd	0.39c
GUTTO	3.66a	33.30a	0.36b	3.66ac	32.23a	0.32bc	3.33bc	17.48cd	0.27d
BH-670	3.66a	29.19a	0.31bd	2.33df	27.42b	0.34b	2.00e	20.46bd	0.42ab
BH-540	3.33a	35.06a	0.33bc	3.33ad	33.04a	0.37b	3.00c	28.78a	0.48a
BH-140	3.33a	31.89a	0.32bc	3.00be	30.89a	0.32bc	3.33bd	25.10ac	0.39bc
BH-530	3.33a	32.69a	0.34bc	3.66ae	32.69a	0.34b	4.00ac	20.14bd	0.33cd
BH-660	3.66a	24.71a	0.34bc	2.00ef	19.62d	0.33b	2.33bd	24.85ac	0.41ac
Kuelni	1.33c	37.34a	0.57a	1.33f	41.19a	0.55a	2.00e	25.60ac	0.46ab
BH-541	3.66a	19.46c	0.32bc	4.00ab	21.81c	0.35b	3.33bd	28.22ab	0.46a
Gussawu	4.33a	12.42d	0.25d	4.33a	13.06e	0.23c	4.66a	14.34d	0.28d
Abobako	4.00a	22.83b	0.30bd	4.33a	18.53d	0.28bc	4.33ab	14.90	0.25d
Local	4.00a	19.45b	0.28cd	4.00a	23.81b	0.31bc	4.33ab	16.68d	0.30d
MEAN	3.17	27.79	0.33	3.17	27.82	0.34	3.55	222.86	0.32

* Means followed by the common letters within a column are not significantly different at 5 %probability level by DMRT.

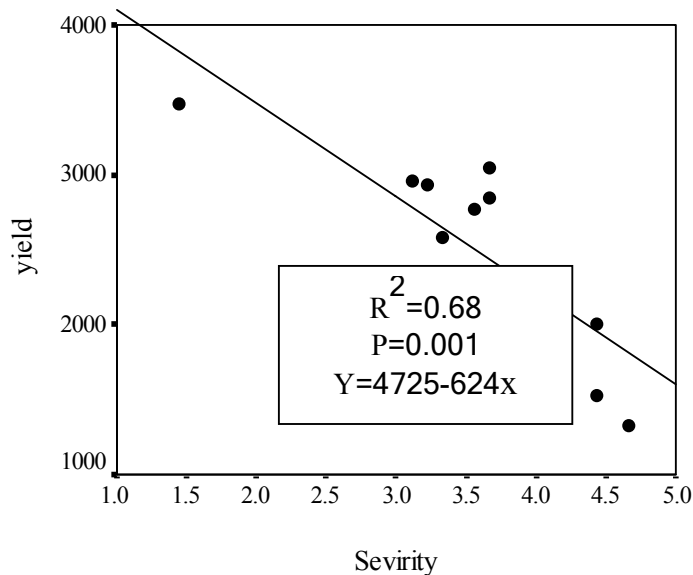


Figure 14 Linear relationship between severity of northern leaf blight and yield of 13 maize varieties

2.4. Area under disease progress curve (AUDPC).

Percent of disease incidence expressed as area under progress curve (AUDPC) was significantly different among varieties and locations (Table 21). Highly susceptible varieties resulted in consistently higher area under progress curve recorded for all varieties as compared at all locations. While variety such as Kuelni recorded, lower AUDPC value such as 1617, 784 and 1332.5 for Gambella, Abobo and Bako locations respectively. On the other hand, variety Abobako were the most highly susceptible varieties recorded AUDPC value high and considered as highly susceptible varieties and their AUDPC value reached as high as 4805 at Gambella and 3470.8 and 5159.2 at Abobo and Bako locations respectively.

The relation between northern corn leaf blight expressed, as AUDPC and yield were similar to the relations obtained for severity and yield. These relation were showed a highly significant negative correlation between AUDPC with yield and

thousand seed weight ($r=-0.51$; $P=0.001$ and $r=-0.69$; $P=0.05$ respectively) (Figures 15 and 16). Highly significant but negative correlation between AUDPC with yield and 1000 seed weight means that AUDPC contributed to most of yield and seed weight variations among varieties. Our present study Yield reduction due to northern corn leaf blight are affected by disease severity and AUDPC similarly supported by Raymond and Hooker, 1981; Hooker and Perkins, 1986

Table 21 Area under disease progress curve of 13 maize varieties recorded for three locations

Varieties	Gambella	Abobo	Bako
BH-QP-542	2783ab	3217.5ac	3034cf
Gibe Comp-1-2	4010ab	4124.5ab	3040.8cf
Guto	4093ab	2164.2ac	4059.0ad
BH-670	3578ab	3707.7ac	2289.3eg
BH540	3127ab	3127ab	2879.3eg
BH140	2871ab	2871ab	3450.8be
BH-530	3056ab	3056ab	4674.0ac
BH660	2959ab	2959ab	1650.8fg
Kuleni	1617b	784.0e	1332.5g
BH-541	4187a	4679.5a	4339.2ad
Gusaw	4010ab	3571.8ac	3571.8ac
Abobako	4805a	3470.8ac	5159.2a
Local-m	3410ab	2090.7ce	5015.5ab

* Means followed by the common letters within a column are not significantly different at 5% probability level DMRT.

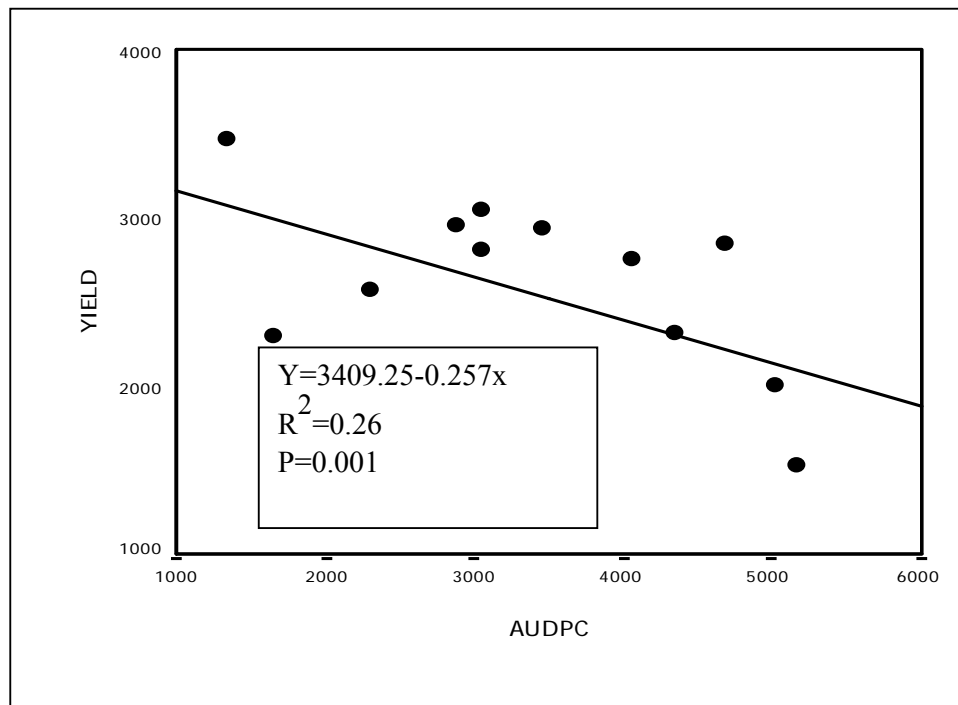


Figure 15 Linear relationship of area under disease progress curve (AUDPC) of northern leaf blight to yield of maize.

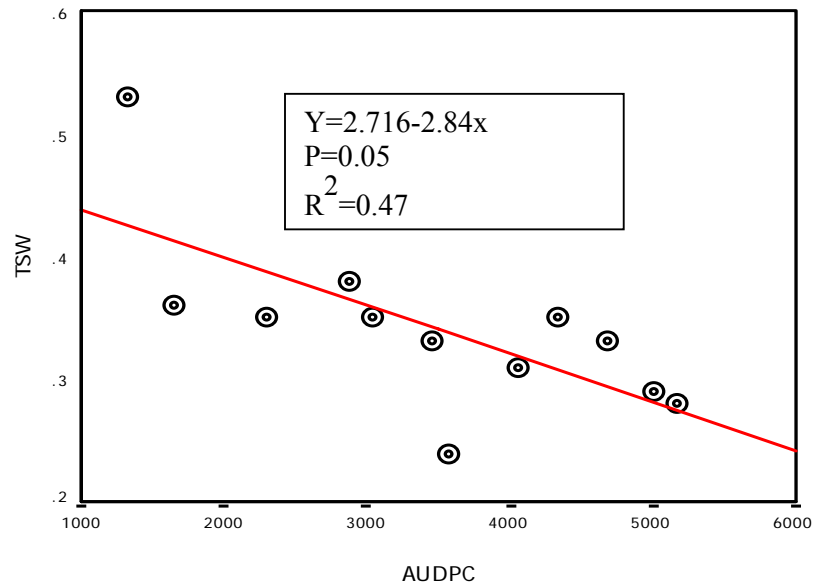


Figure 16 Linear relationship of area under disease progress curve (AUDPC) of northern leaf blight to thousand seed weight of maize.

2.6. Correlation analysis

The result of Pearson correlation analysis indicated highly significant and positive relationship between all disease assessments observed at Abobo and Bako locations due to high disease pressure in that areas. However, we observed non-significant correlations observed between the disease indices with AUDPC only at Gambella. The reason for non-significant correlated in Gambella was probably due to low disease pressure (Table 22).

Correlation among the various northern leaf blight evaluations with yield and Seed weight were determined (Table 23). There were significant negative correlation between severity to TSW and yield in all locations. This result indicated that severity had significant effect on this yield parameter in all locations. Area under disease progress negatively correlated with seed weight at Gambella and Abobo. However, did not significantly correlated to yield in all locations. Blight incidence score, lesion number and, lesion size with blight incidence score correlated with seed weight and

yield at Gambella and Abobo locations respectively. This indicating that all those parameters effectively measured the disease progress and had effect on yield and seed. At Bako location, most of the disease assessments Parameters not correlated with yield and seed weight. The reason for non-significant correlation between them probable due to the disease started late growth stage of the crop followed by fast disease progress after the crop reached close to maturity. Hence, the disease some of parameter effect on yield and seed weight was relatively lower than other locations.

This study indicated that the use of relative resistance cultivars has a pronounced positive effective. Moreover, the management of the disease. The significant effect of varieties indicated the recommendation on the management of the disease is highly influence by varieties. A significant positive correlation among northern leaf blight evaluation methods and their negative relation with seed weight and yield indicated that the use of any one of the methods could effectively measure the disease and its effects on yield.

Yield was significantly affected by variety ($P \leq 0.01$) but no significant different was observed among varieties for thousand seed weight. (Table 24). This means that varieties with low blight values had high TSW. On the other hand, there is a good relation between all disease assessment such lesion size, lesion number, severity 1-5 scale, AUDPC, blight incidence score were affected by variety. Significant reaction of variety by disease indicated that reduction in grain yield due to this disease varied from variety to variety, and that specific variety showed difference amount of yield depression due to disease. In generally, the study show that northern leaf blight attack on maize resulted in significant reduction of yield and yield components in the absence of any control measure. The result of this study demonstrated that the use of resistance varieties effectively reduce the incidence the disease and minimize yield loss. Adequate levels of host resistance can prevent reduction in Yield.

Table 22 Pearson correlation (r) among diseases assessments used to quantify northern leaf blight reaction at Abobo, Gambella and Bako.

<i>Disease assessments</i>	<i>Disease assessments</i>				
Gambella ^a	AUDPC	Blight incidence	severity	Lesion number	Lesion size
AUDPC ^r		0.60	0.52	0.55	0.52
Blight incidence			0.73*	0.74*	0.73*
Severity				0.75**	0.71*
Lesion number					0.85**
Abobo ^a					
AUDPC ^r		0.85*	0.73***	0.66*	0.61*
Blight score			0.81*	0.67*	0.79**
Severity				0.81**	0.82**
Lesion number					0.63*
Bako ^a					
AUDPC		0.91**	0.85**	0.74**	0.76**
Blight score			0.81*	0.78**	0.71**
Severity				0.70*	0.89**
Lesion number					0.85**

* Asterisks indicated significant level: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

^a tested locations

^r AUDPC= area under disease progress curve, Blight score =last blight incidence.

Table 23 Pearson correlation among the difference northern leaf blight assessments with yield and seed weight at different locations

<i>Disease assessments</i>	<i>yield</i>	<i>Seed weight</i>
Gambella		
severity	-0.58*	-0.72**
AUDPC	0.52	-0.58*
LBI	-0.64**	-0.86**
Lesion number	-0.68*	-0.67*
Lesion size	0.40	-0.68*
Abobo		
severity	-0.72**	-0.94**
AUDPC	0.40	-0.66*
LBI	-0.67*	-0.89*
Lesion number	0.14	0.18
Lesion size	-0.83**	-0.65*
Bako		
severity	-0.64*	-0.73**
AUDPC	0.56	0.66
LBI ^a	0.17	0.40
Lesion number	-0.64*	0.65
Lesion size	0.49	0.60

** and * correlation is significant at $p < 0.01$ and $p < 0.05$ levels, respectively.

^a LBI= last blight incidence

Table 24 Main effects varieties on northern leaf blight disease assessment, yield and thousand seed weight at three locations.

Source of variation	AUDPC	Last incidence	Lesion size	severity	yield	TSW
Variety	**	**	***	***	***	NS

NS, *, ** and *** non-significant, significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ probability level.

CONCLUSION

Growth Chamber Experiments

Variation was observed among isolates of *E.turcicum* fungus, the causal agent of NCLB, in appearance, morphological characteristics, and the pathogenic variation within and among locations. The results obtained from this study showed that the differences among population *E.turcicum* from different locations were significantly greater than differences among isolates from population of the same field. Different variations existed in existing pathogenicity. Aggressive and non- aggressive for pathogenicity were found. Most of the isolates, grouped as non- aggressive belonged to the isolates collected from low altitudes of Gambella (500 masl).

The variations among isolates such as, conidial shape and size, morphological characteristics exhibited dependent on geographical areas and specific locations. This result can be explained by many factors including different in environments and the pathogens. The significant interaction of genotypes and isolates may suggest some kind of specialization in the fungus population, because there were variations both in the resistance level of maize varieties and in the aggressiveness of the pathogen isolates. The identification of physiological specialization of a pathogen is an important step for the development of disease resistance cultivars in many host pathogen system where major genes control resistance. Although the resistance was predominately polygenic, the significant genotype x isolate interaction (different effect) presented some evidence for major gene resistance in maize genotypes and *E.turcicum* isolates (Vanderplank, 1963).

From this study, we concluded that there was a wide variation among the fungus population of *E.turcicum* under studied and that the variation in pathogenicity among isolates due to variation in the resistance host plant, variation in environment, or from interaction among this variables. The relative importance of each item must be considered in planning any specific breeding program. Hence, this information will

be necessary to maize breeding program and pathologist involved in developing resistance genotypes using the virulent isolates, together with a mix of isolates, in order to test the disease interaction and select for a maize genotypes. Moreover, further investigations are recommended to complete map of the variations of *E. turcicum* in each agro ecological zones.

Field Experiments

There was significant variation among varieties for AUDPC, disease severity, lesion number, lesion size, yield and 1000 seed weight. The percentage incidence of the disease (AUDPC) however varied from location to location depending on the difference in the environmental conditions, appearance of disease and other related factors. The % incidence of the disease was the highest at Bako even though the appearance of the disease was delayed because of the dry period at the time of planting. After early dry period, environmental conditions were generally favorable for northern leaf blight development during the remaining crop season. At Gambella, the low rainfall in cropping season was not suitable for disease development. Thus, the percentage incidence (AUDPC) was less than that of at Bako and Abobo. Varieties with low AUDPC, lesion number, lesion size were considered as moderate resistance to the disease. In this study, variety Kuleni exhibited low AUDPC value at all locations, here it was considered resistant variety. Variety Gusaw, Abobako and Local-M have high AUDPC values at all locations considered as highly susceptible. Varieties moderate resistance or highly susceptible showed a similar reaction at all locations; this means that there was no difference in virulence in the pathogen populations at all locations.

Among the experimental varieties, we have tested in green house or observed in field plots the reaction of maize varieties to northern corn leaf blight from highly susceptible and moderate resistance. However, majority of them were moderately tolerances, no immune (no lesion) and resistance (fleck-type of lesion). Gusawu and Abobako maize varieties have been recently released in Gambella region for resistance of maize streak virus (MSV) and farmer's varieties, collected from this region, were highly susceptible to northern leaf blight. Generally, all the released varieties in all tested locations showed highly susceptible reaction to northern leaf blight with the exception of Kuleni and hybrid of BH660 were relatively moderately resistance to the disease.

Variety contributed significantly to the differences in disease assessment and yield. This study indicated that the use of relative resistance cultivars has a pronounced positive effective. Moreover, the management of the disease's significant positive correlation among northern leaf blight evaluation methods and their negative relation with seed weight and yield indicated that the use of any one of the methods could effectively measure the disease and its effects on yield.

In conclusion, in our field-tests the performance of some varieties generally was consistent with result of previously seedling tests experiment. In this case, highly susceptible varieties such as Abobako, Gusaw and Local-M under field conditions have shown similar host highly susceptible reaction. On the optimistic side, variety Kuleni showed promise for northern leaf blight moderate resistance in the field and, have demonstrated moderate resistance response to the aggressiveness isolates of the pathogen under greenhouse condition too.

Our present data also indicated that maize variety such as Kuleni showed good level of moderate resistance under field conditions and in greenhouse studies. The resistance was of quantitative type. High-type of resistance may also depend on a single gene that, as has happened in other host-parasite relation, could be overcome by a single –gene mutation in the pathogen. For this reason, the modern concept of breeding for resistance looks more favorably toward a broader, polygenic basis- which, although usually not as high a degree of resistance, may be more stable. Therefore, the national breeding program must incorporate greenhouse testing of lines to determine and develop plant resistance.

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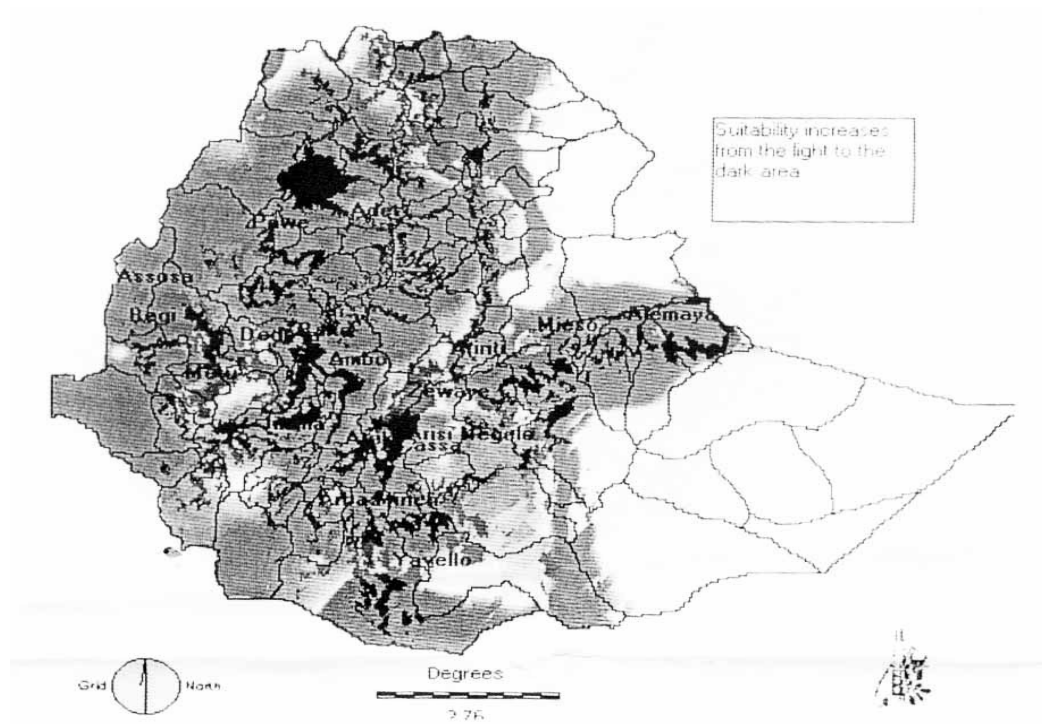
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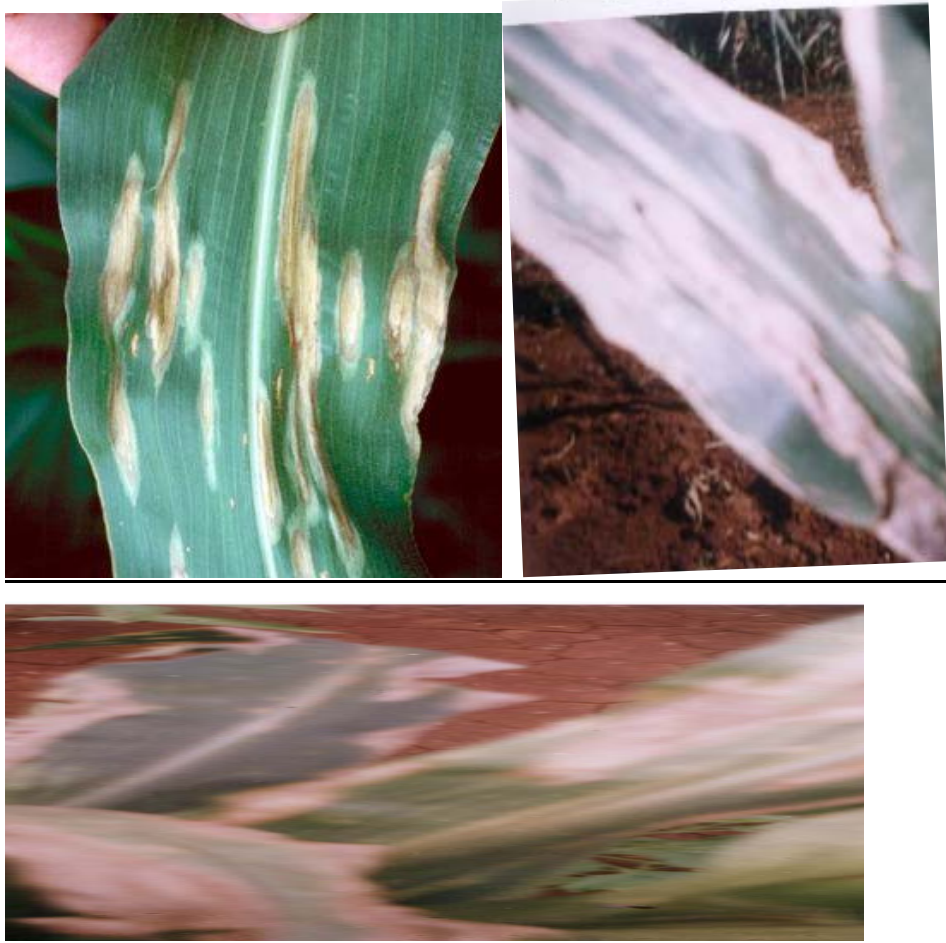
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APPENDICES

Appendix Figure 1 Maize growing zones of Ethiopia.



Appendix Figure2 High severity of northern leaf blight lesions.

Appendix Table 1 Estimation of area, production and yield of cereal crops for private peasant holding in 2000.

Crop type	Area ('000ha)	Total production ('000q)	Yield (q/ha)
Cereals	6747	77412	11.47
Teff	2123	17175	8.09
Maize	1407	25254	17.95
Barely	794	7419	9.34
Wheat	1025	12126	11.83
Sorghum	995	11811	11.87
Millet	360	3195	8.87
Oats	41	430	10.33

Source: CSA 2001

Appendix Table 2 Estimation of area, production and yield of maize, 1990-2000.

<i>Year</i>	<i>Area ('000ha)</i>	<i>Production ('000q)</i>	<i>Yield (q/ha)</i>
1990	1277	20556	16.09
1991	908	11589	12.75
1992	751	12344	16.44
1993	808	13915	17.20
1994	902	11127	12.33
1995	1104	16732	15.15
1996	1851	31053	16.80
1997	1688	29277	17.30
1998	1448	23443	16.20
1999	1303	24166	18.55
2000	1407	25254	17.95

Source: CSA, 1990-2001

Appendix Table 3 A broad classifications of maize growing zones in Ethiopia.

<i>Production Zones</i>	<i>Elevation (m)</i>	<i>Rainfall (m)</i>
Mid-altitude sub humid	1000-1800	1000-1250
Moisture stress	500-1800	<800
High altitude sub humid	1800-2400	1200-2000
Low altitude sub humid	<1000	1200-1500

Appendix Table 4 Analysis of variance for 9 days growth rate for 28 isolates.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Isolates(A)	27	295.343	9.527	1.911	**
error	55	304.097	4.985		

CV=:19.6%

** Significant at <0.001 probability level

Appendix Table 5 Analysis of variance for 11 days growth rate for 28 isolates.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Isolates(A)	27	298.206	995.573	1.27	**
error	55	4.985	4.988		

%CV=:18.96

** Significant at <0.001 probability level

Appendix Table 6 Analysis of variance of inoculation of 20 isolates on 11 maize genotypes.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Isolates(A)	19	39.866	2.124	4.61	*
Genotypes	10	67.912	6.791	14.92	*
(B)					
A XB	190	229.045	1.205	2.65	*
Error	440	200.290	0.455		

%CV=17.99

*Significant at <0.05 probability level

Appendix Table 7 Analysis of variance of first severity blight (FSB) at Abobo.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Fsb(A)	12	3607.459	300.621	0.79	NS
error	26	9933.755	382.067		

%CV=14.8

NS=not significant

Appendix Table 8 Analysis of variance of first severity blight (FSB) at Gambella.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Fsb(A)	12	10656.410	888.034	2.08	*
error	26	44488.888	427.777		
%CV=11.4					

*Significant at <0.05 probability level

Appendix Table 9 Analysis of variance of Average lesion size at Gambella.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Average lesion size (ALS)	12	346.801	28.900	1.9	*
error	26	385.556	14.829		
%CV=0.64					

* Significant at <0.05 probability level

Appendix Table 8 Analysis of variance of last severity blight (LSB) at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	155.555	77.777	0.62	NS
Last severity(B)	12	44488.888	2478.490	19.73	***
AXB	24	5355.555	223.148	1.78	*
Error	78	1480.000	189.743		

*, ***Significant at <0.05 and 0.0001 probability level; NS=non-significant
%CV= 23.12

Appendix Table 9 Analysis of variance of first lesion number (FLN) at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	0.939	0.469	1.70	NS
First lesion number(B)	12	10.916	0.909	3.26	**
AXB	24	5.507	0.229	0.83	NS
Error	38	21.579	0.276		

**Significant at <0.001 probability level; NS=non-significant
%CV= 20.12

Appendix Table 10 Analysis of variance of onset of disease (DO) at Abobo.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
DO (A)	12	390.308	32.525	2.25	NS
Error	26	174.666	66.717		
%CV=21.84	16.4				

NS= non-significant

Appendix Table 11 Analysis of variance of first lesion number (FLN) at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	0.939	0.469	1.70	NS
First lesion number(B)	12	10.916	0.909	3.26	**
AXB	24	5.507	0.229	0.83	NS
Error	38	21.579	0.276		
%CV=20					

Appendix Table 12 Analysis of variance of first severity blight (FSB) at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	1975.555	987.777	52.06	***
First severity blight (FSB)	12	1057.777	881.481	4.65	***
AXB	24	1002.222	417.593	0.83	*
Error	38	1480.000	189.743		

*, ** and *** significant at $p < 0.05$, 0.01 and 0.0001

%CV=8.1

Appendix Table 13 Analysis of variance of area under disease progress curve (AUDPC) at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	1450.850	725.425	6.11	*
AUDPC (B)	12	913.119	760.843	6.41	**
AXB	24	485.483	2023.061	1.70	*
Error	78	246.952	189.743		

* and ** significant at $p < 0.05$ and 0.01

%CV=8.1

Appendix Table 14 Analysis of variance of severity 1-5 scale at three locations.

<i>Source of variation</i>	<i>df</i>	<i>Sum square (ss)</i>	<i>Mean square(ms)</i>	<i>f-value</i>	<i>Prob</i>
Location (A)	2	7.299	3.649	7.36	***
Severity (B)	12	75.529	6.044	12.19	***
AXB	24	20.700	0.862	1.74	*
Error	78	38.666	16.00		

* and ** * significant at $p < 0.05$ and 0.001

%CV=20

Appendix Table 15 Released Years of improved maize varieties that used for green house and field experiments

Improved varieties	Released years
BH-660	1993
Kuelni	1995
Gutto	1988
Gussau	2002
Gibe Comp-1	2001
BH-140	1988
BH-541	2002
BH-QP-542	2002
Abobako	1986
BH-540	1995
BH-530	1995

Source: The Second National Maize Workshop

Appendix Table 16 World largest maize producing regions and the productivity of field maize in the region.

Region	Hectare ('000ha)	Yield (t/ha)
North America	41,064,000	6.48
South America	19,612,000	2.88
Europe	13,251,000	4.78
Asia	42,203,000	3.82
Africa	24,878,000	1.63
Oceania	83,000	6.22

Source: USDA, 1988

Appendix Table 17 Importance and prevalence of maize diseases in Western and Northwestern Ethiopia.

<i>Common name</i>	<i>Causal pathogen</i>	<i>Prevalence*</i>
Gray leaf spot	<i>Cercospora zae-maydis</i>	++++
Turcicum leaf blight	<i>Exserohilum turcicum</i>	++++
Leaf spot	<i>Phaeosphaeria maydis</i>	+++
Leaf spot	<i>Pellucid leaf spot</i>	+++
Leaf spot	<i>Curvularia spp</i>	+
Leaf blight	<i>Helminthosporium maydis</i>	+
Common leaf rust	<i>Puccinia sorghi Schw</i>	++
Sorghum leaf rust	<i>Puccinia polysora</i>	+++
Streak virus	Maize streak virus	+++
Mosaic Virus	Sugar cane mosaic virus	+
Dwarf stripe	Maize dwarf stripe virus	+
Bacterial leaf spot	<i>Pseudomonas spp</i>	++
Corn smut	<i>Spiroplasma Kunkel</i>	+
Crazy top	<i>Sclerospora macrospora sacc.</i>	++
Down mildew	<i>Sclerospora sorghi</i> (Weston)	++
Late wilt	<i>Cephalosporium spp</i>	+
Head smut	<i>Sphacelotheca reilliana</i>	++
Red ear rot	<i>Gibberella zae</i> (Schw.)petch	++
Giberella ear rot	<i>Fusarium monlifforme</i> sheld	+++
Diplodia ear rot	<i>Diploids maydis</i>	+++
Aspergillus ear rot	<i>Aspergillus flavus</i> (LK)exfries	+++
Nematodes	<i>x.americanum</i>	+
Bacterial stalk rot	<i>Erwinia caratovora</i>	++
Stalk rot	<i>Fusarium spp.</i>	+
Root rot	<i>Fusarium spp.</i>	+

* The intensity increase with '+' sign :+(0-10%), ++ (11-30%), +++ (31-50%), +++++ (over 51%).

Source: Assefa (1999)

1. Name: **Daniel Abebe Geletu**
2. Birth date: **14 February 1968**
3. Birth place: **Jimma, Ethiopia**

4. Educational background and Academic Award:

Academic Award	Place and Country	year
Master of Science in Crop Protection	Ukrainian State of Agricultural University, Ukrain	1994
Ethiopian School Leaving Certificate	Ambo High School Ethiopia	1988
5. Professional Status:	Agricultural Research	
6. Office	Gambella Agricultural Research Institute P.O.BOX 62 dlabebe@yahoo.co.uk	