EVALUATION OF BARLEY GENOTYPES FOR RESISTANCE TO NET BLOTCH AND DETERMINATION OF PATHOGEN DIVERSITY

BY

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DECLARATION

Declaration by Student

This is to declare that this thesis is my original work towards the Masters of Science Degree and has not been presented for award of any degree in any other institution and due acknowledgement has been made in the text to all other sources of information or materials used. No part of this work should be reproduced without prior permission of the authors and / or University of Eldoret.

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DEDICATION

This work is dedicated to my father (Joash), mother (Monicah), brothers, sisters and cousin (Jasper), who have been there for me and sacrificed their interests for my academic benefit.

ABSTRACT

Net blotch caused by *Pyrenophora teres* is one of the most destructive foliar diseases of barley (*Hordeum vulgare*) in the world, Kenya included. Frequent evolution of net blotch pathotypes, continuous overcoming of resistance and changes in environmental factors require screening of advanced barley genotypes to identify more sources of host resistance and different strains of the pathogen for crop improvement. Twenty advanced barley genotypes from Moi University-East Africa Malting Limited collaborative project (2 susceptible, 2 resistant checks and sixteen with various attributes) were screened to evaluate their reaction to net blotch in the field (Purko sheep ranch in Mau Narok and Chepkoilel) and screenhouse (Chepkoilel). Disease assessment was done on a scale of 0-9. Net blotch infected leaves were collected and the fungus isolated to assess both morphological and physiological diversity. Data collected from the field and screenhouse were subjected to analysis of variance computed on Genstat version 12.2. Genotypes Cerise Laurel recorded the least level of disease severity while the susceptible checks gave the highest disease severity. Other groups of genotypes (HKBL, Six row and Syngenta) gave moderate resistant and moderate susceptible responses respectively. On the other hand, six row barley genotypes (54CAN, 77Aloe, Gloria Bar, NBD Petunia) had the highest ear infection. Five morphologically diverse *P.teres* groups were identified. The grouping of isolates was based on colony colours, texture and conidia characteristics. The different barley genotypes gave different responses to the five groups of isolates indicating that the genotypes had different levels of resistance. Approximately 5 isolates were identified indicating that different strains of the pathogen exist in the growing areas and that the virulence of *P. teres* is influenced by these strains. There is therefore need to confirm the molecular basis of the observed responses of the genotypes and the five morphological groups.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Barley (*Hordeum vulgare* L.) is an important cereal grown in most parts of the world. It belongs to the genus *Hordeum* and family, Poaceae also known as Graminea. It is cultivated in diverse environments with greater concentration in temperate areas and high altitudes areas of the tropics. It is cultivated as a summer crop in temperate areas and as a winter crop in tropical areas. It is ranked fourth cereal crop in terms of quantity produced and the area under cultivation (FAOSTAT, 2011) after maize, rice and wheat. The total world production in 2010 was 123,477,192 tonnes, with Kenya contributing about 64,219 tonnes (FAOSTAT, 2011). This has been estimated to be 75% of the Kenyan production potential, showing that full production potential has not been achieved. Kenya's barley growing area is estimated to be 85,000 hectares, however, only 20,000 hectares is under barley production, thus 65,000 hectares has not been utilized. In Kenya it is majorly grown in high and medium altitude areas with consistent annual rainfall of more than 635mm (EPZA, 2005).

Originally, barley was mainly cultivated and used for human food (Fischbeck, 2002), but after dominance of rice and wheat as alternative food, it is now used primarily to produce malt and as animal feed, with small amounts used for seed and direct human consumption as health foods. However, it is a major food source for some cultures in areas of North Africa (Newman and Newman, 2006). The main purpose of incorporating barley in most diets has been reported to be due to its health benefits such as lowering of blood cholesterol (Behall *et al*., 2004). It is also used in soups and stews, and in barley bread in various cultures. Barley is also used to produce starch, either for food or

chemical industry as a sweetener or binder respectively. In addition, barley has some useful by-products, the most valuable being straw which is used mainly as livestock bedding in developed countries but also for animal feed in developing countries. In Kenya it is mainly grown as cash crop for base malt and the by-products such as screenings and straws used as animal feed (Moi University /East Africa Breweries Limited, 2011).

Barley production is hindered by many constraints including biotic factors such as low yielding varieties, weeds and foliar diseases, and abiotic constraints such as unfavourable climatic conditions, use of traditional techniques and infertile (acidic) soils. Fungal diseases such as rusts, net blotch, powdery mildew, scald, scab, loose smut, dwarf bunt, head blight have been reported to reduce seed production of some barley varieties such as Karne; especially by the Kenya Malting Limited (KML) (EPZA, 2005). The degree of crop loss due to fungal diseases depend on variety resistance, abundance of the pathogen and conducive weather conditions. Nutrient deficiencies or adverse weather conditions can also cause abnormalities on barley leaves.

Net blotch of barley caused by *Pyrenophora teres* (Drechs), is a major production problem for the barley industry around the world (Campbell and Crous, 2003; EPZA, 2005; Gupta *et al.*, 2003.; Leišova *et al*., 2005). The disease was first named because of the typical netting symptom produced by the pathogen on leaves of susceptible lines (Shipton *et al*., 1973). In addition, a spot-like symptom has also been observed and associated with *P. teres*; the causal agents for these two symptoms cannot be differentiated morphologically (Crous *et al.*, 1995). Based on the symptoms the net

blotch has been differentiated into two forms; net form (NFNB) caused by *P. teres f. teres* Dreschler and spot form (SFNB) caused by *P. teres f. maculata*.

Net blotch is severe in areas of high humidity of 95 -100 % and high rainfall and is associated with high severity levels in regions where susceptible varieties are cultivated. The disease causes premature leaf death and poor grain development resulting in a reduction in seed weight and density as a result of incomplete grain fill. This affects subsequent germination and crop development in the field.

Both net and spot forms of blotch occur in Kenya (MU-EABL, 2010). In spot form blotch symptoms are most commonly found on leaves, but can occasionally be found on leaf sheaths. They develop small circular or elliptical dark brown spots becoming surrounded by a chlorotic zone of varying width. These spots do not elongate to the net - like pattern characteristic of the net form. The spot may grow from 3-6 mm in diameter (McLean *et al*., 2009). Older leaves will generally have a larger number of spots than younger leaves. Spot form produces both asexual (conidia) and sexual spores (ascospores) in the pseudothecia. The net form of blotch starts as pinpoint brown lesion which elongates and produce fine, dark brown streaks along and across the leaf blades, creating a distinctive net- like pattern. Older lesions continue to elongate along leaf veins, and often are surrounded by a yellow margin.

The management practices used such as reduced or zero tillage, intensification of barley cultivation and the absence of stable resistant varieties, are among the factors favouring net blotch incidence. A number of resistant cultivars have been developed in different countries, however there is usually a considerable loss of resistance due to changes in virulence patterns of the pathogen populations (Gupta and Loughman, 2001). Thus there

is need to establish cultivars with stable resistance, for further breeding and production purposes.

1.2 Problem Statement

Pyrenophora teres is the pathogen that causes severe losses in yield and quality of grain in most barley producing areas of the World (Bekele *et al*., 2001). Among factors contributing to this severity, is the susceptibility of the barley cultivar. Different control measures have been put in place to reduce the spread of this disease including crop rotation, proper nutrition, stubble destruction, application of foliar fungicides, planting resistant varieties and healthy seeds (Brennan and Jayasena, 2007; Carmona *et al.,* 2008; Krupinsky *et al*., 2004). However, the disease still remains a challenge in production. In as much as some of these measures have reduced the disease to lower levels, they are also associated with different problems.

The use of fungicides has helped in control of this disease but they are expensive and besides they pose risk to human health and the entire environment (Bekele *et al.*, 2004). Pathogens might also develop resistance to these fungicides when used over a long time. Crop rotation, on the other hand, is limited by the large proportion of barley growing area and increased land pressure in the production areas forcing all - season - round use of the same land; hence the crops are prone to infection by the stubble borne inoculum. Therefore, the only reliable option left is the development of resistant and tolerant cultivars.

Due to high pathotype variations of net blotch populations in different environments, effective management of this disease is still a problem. Even with the use of resistant

cultivars, disease cases have been reported. This is because the use of host resistance is reliant on the identification of suitable resistance sources, thus the need to identify genotypes with stable resistance in different environments and pathotype diversity.

1.3 Justification of the problem

Barley is an important cereal crop grown in most parts of the world for its numerous domestic and industrial uses. Yield losses ranging from 10-40% and up to 100% in extreme cases of susceptible cultivars (Mathre, 1997; Murray and Brennan, 2010) have been associated with effects of net blotch disease during barley production. Due to the increasing demand for barley majorly for industrial uses there is need of finding possible ways of managing this disease to avoid the high losses. Many resistant cultivars have been developed, but they lose resistance due to changes in virulence patterns of the pathogen species, among other reasons. Therefore, more sources of resistance are needed to aid in development of cultivars with more durable resistance.

Additionally, there is inadequate knowledge on diversity of pathogenic races of the causal agent of barley net blotch in Kenya, yet this is vital in formulation of breeding objectives towards management of this disease. Understanding the different factors that contribute to loss of resistance in resistant cultivar and how these factors relate to each other will lead to information that will assist breeders in setting appropriate breeding objectives toward development of resistant varieties.

1.4 Study Objectives

1.4.1 Overall objective

To investigate the response of advanced and genetically stable barley lines to *Pyrenophora teres* and establish diversity of the pathogen in production areas of Kenya.

1.4.2 Specific objectives

- To investigate the response of advanced barley lines to *P. teres* in production areas Mau Narok and Chepkoilel, Kenya.
- To assess morphological diversity of *P. teres* from barley produced in different production areas – Mau Narok, Njoro, and Chepkoilel, Kenya.
- To assess the pathogenicity and virulence of morphologically diverse isolates of *P. teres* on the barley genotypes.

1.4.3 Hypotheses

H_O: Barley genotypes do not differ significantly in their response to *Pyrenophora teres* isolates in the field.

H_A: Barley genotypes differ significantly in their response to *Pyrenophora teres* isolates in the field.

H_O: The isolates of *P.teres* from barley production areas are not different morphologically.

H_A: *Pyrenophora teres* isolates from barley production areas are morphologically different.

H_O: Isolates of *P.tere* show similar pathogenicity and virulence on barley genotypes.

H_A: Isolates of *P.teres* show significant differences in pathogenicity and virulence on barley genotypes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Net blotch disease of barley

Pyrenophora teres is a necrotrophic fungal pathogen responsible for net blotch disease in barley. It is a prominent foliar disease that occurs in most barley growing areas of the world including Kenya (EPZA, 2005; Gupta *et al.*, 2003.; Leišova *et al*., 2005). Net blotch can cause yield losses of 10 - 40%, however 100% yield losses can occur in susceptible cultivars under extreme environmental conditions (Murray and Brennan, 2010). The disease affects the foliage of barley and under extreme conditions it reduces the photosynthetic capacity of the plants, reducing the carbohydrate levels in the kernel (Horsley and Hochhalter, 2004). Extreme losses are experienced when the disease infects or damages the flag leaf or the top two leaves, this is because flag leaf sits at the top of the canopy and receives more sunlight than other leaves and is more efficient in producing photosynthetic nutrients (carbohydrates) that are needed to fill the kernels. If the flag leaf or top two leaves are infected or damaged by the disease, their ability to manufacture carbohydrates is compromised, thus, the kernels are not filled properly (Jebbouj and Youfsi, 2009). The reduction in carbohydrate levels in the kernels results in proportional increase in protein levels and low yields as a result of low kernel weight (Horsley and Hochhalter, 2004). Other than yield losses, the disease also has a corresponding effect on malt quality and feed quality (Grewal *et al*., 2008) Excess grain protein is undesirable in malting as it causes a delay in germination and upon germination, excessive growth occur. This causes a reduction in the amount of extracts and beer quality causing malting losses (Edney, 1996). Reduced kernel weight also influences germination of seeds negatively and affects the eventual crop vigor.

2.2 *Pyrenophora teres* (The pathogen)

2.2.1 Identification of *P. teres*

Pyrenophora teres f. teres and *P. teres f. maculata* have been described collectively due to the similarities in their morphology and life cycle. The asexual stage of the *P. teres* has conidiophores that are solitary or in groups of two or three and are slightly swollen at the base. Conidiophores are mid-brown to olivaceous - brown in colour (Liu *et al*., 2011; Steffenson, 1997). It produces conidia that are of variable lengths, between 25-300 μm long and 7-11 μm thick, which are straight, cylindrical in shape, smooth with rounded ends, invisible hilum and have 1-14 pseudosepta, but usually 4-6. The conidia are sub-hyaline to yellowish – brown in colour (McLean, 2011; Smedegard-Petersen, 1971). The sexual stage produces dark, globosely shaped pseudothecia - dot-like structures covered by dark hair-like setae that are 1-2 mm in diameter on surface of barley residue. The pseudothecia contain asci with three to eight ascospores that are between 20-23 μm long and 10-13 μm thick (McLean *et al*., 2009; Steffenson, 1997).

2.2.2 Taxonomy and the relatedness to other blotch diseases of barley

Pyrenophora teres belongs to the phylum: Ascomycotina, subphylum: Pezizomycotina, class: Dothideomycetes, family: Pleosporacea (Liu *et al*., 2011). The imperfect stage of this pathogen was first placed in the genus *Helminthosporium*, however, upon revision *Helminthosporium teres* Sacc. was later placed into a new genus *Dreschlera*. This was on the basis of its cylindrical and not curved conidia, germinating from every cell. This was also due to its association with the *Pyrenophora* teleomorph (Liu *et al*., 2011).

Other *Pyrenophora* species *P. graminea* (anamorph *Dreschlera graminea*) and *Pyrenophora japonica* (*Dreschlera tuberosa*) are morphologically very similar to

P.teres and also cause foliar disease on cultivated barley. They are considered as three different species based on small differences in morphology (shape, size and colour) of ascocarp, conidia and conidiophores (Liu *et al*., 2011). However, the symptoms on barley leaves as demonstrated by the three species are quite different, thus disease phenotyping is mostly used in differentiating between the three fungi (Bakonyi and Justesen, 2007). *Pyrenophora graminea* causes long and extended necrotic stripes on barley leaves, and is a seed borne pathogen (Mathre, 1997). *Pyrenophora teres* is known to produce two different symptoms (McDonald, 1967), and based on the symptoms, Smedegård-Petersen (1971) differentiated the forms of *P .teres as P. teres f.teres* which produces brown netted lesions and *P. teres f. maculata* which produces brown spot lesions. The morphological characters of the spores overlap between the forms of *P. teres* and therefore it is difficult to identify the forms based solely on morphology (Crous *et al*., 1995). Originally the spot form of net blotch was named as a new form of *Pyrenophora* species: *P. japonica* (Ito and Kuribayashi, 1931). However *P. japonica* has been recognized as being *P.teres* (Crous *et al*., 1995). The spot form has also been referred to as *P. hordei* (Wallwork *et al*., 1992). Both forms of *P. teres* can cause variable symptoms according to environment and host genotype (Williams *et al*., 2001). In another study McDonald, (1967) identified an isolate which had descriptions similar to that of *P. japonica,* and this isolate could be crossed with a known *P. teres* isolate and was referred to as a mutant strain of *P. teres.* The capability of *P. teres* and *P. japonica* to interbreed was also observed by Smedegård - Petersen (1971); therefore, he proposed intraspecific taxa: *P. teres f. maculata* and *P .teres f.teres* for those inducing spot-type symptoms and typical net-type symptoms respectively. The rejection of *P. japonica* as an independent species was disputed in South Africa (Scott, 1991). However, later reports

from South Africa suggested that *P. japonica* was not a separate species, and should be treated the same as *P. teres* (Crous *et al*., 1995; Louw *et al*., 1994).

Studies using different DNA markers and mating type gene sequences have shown that *P. teres f. teres* and *P. teres f. maculata* are closely related; however, multiple studies indicate that the two forms differ from each other genetically and are phylogenetically independent (Bakonyi and Justesen, 2007; Campbell and Crous, 2003; Lehmensiek *et al*., 2010; Rau *et al*., 2007; Sirenius *et al*., 2005). Campbell and Crous (2003) suggested that recombination between the two forms can occur under field conditions to form a genetically stable progeny. However, studies by Rau *et al*., (2007) suggested that hybridization is rare or absent under field conditions.

2.2.3 Net blotch disease symptoms

Based on symptom development *P. teres* species have been separated into two forms designated as *P. teres f. maculata* and *P. teres f. teres* for isolates inducing spot-like and net-like symptoms, respectively (Smedegard-Petersen, 1971). The spot-like symptoms consist of dark-brown, circular to elliptical lesions measuring 3 mm × 6 mm, surrounded by chlorotic zones of varying width (McLean *et al.*, 2009). On resistant varieties, the symptoms are small pin - point dark brown necrotic lesions that do not increase in size, while moderately resistant varieties may develop moderate sized necrotic lesions and a small necrotic halo (Wallwork, 2000). The net-like symptoms on the other hand starts as small circular and elliptical dot-like lesions that soon grow along and vertical to the leaf vein, these later develop into dark-brown blotches with longitudinal and transverse striations creating a net-like pattern. This makes it difficult to distinguish the two symptoms at initial stages of infection (Liu *et al.*, 2011). On susceptible cultivars however, water-soaked or chlorotic areas may appear around the dark-brown, net-like

necrotic lesions. In conditions of severe infection, complete death of leaves may occur with a dry appearance (Mathre, 1997).

Differences in symptoms between *P. teres f. teres* and *P. teres f. maculata* have been reported to be due to different infection patterns exhibited by the two forms. *P. teres f. teres* establishes more extensive sub-epidermal growth with cell death more distant to the point of infection whereas, *P. teres f. maculata* establishes a more confined and slower extracellular growth within the epidermal cells and localized cell death (Lightfoot and Able, 2010).

2.2.4 Management of Net blotch disease of barley

Different methods have been suggested and used in management of net blotch. These include: cultural practices (rotation, destruction of previous crop residue, proper crop nutrition, delayed sowing), fungicides, biological control and host resistance. The use of rotation helps in elimination of primary inoculum in the field. Previous studies show that the risk of leaf diseases on barley could be effectively reduced when barley followed *Brassica napus*, *Triticum aestivum*, *Pisum sativum* (Krupinsky *et al*., 2004).

Due to reduced land sizes in most barley producing areas, there have been closer rotations of barley on the same fields, consequently most barley crops are grown on fields with barley stubble residue from a previous crop. Moreover, stubble is increasingly being retained in the fields to improve soil health, moisture retention capacity and soil carbon content. These farming practices have increased the development and inoculum survival of stubble-borne diseases including net blotch. Ensuring proper nutrition of the crops through proper fertilization helps improve the ability of plants to reduce infection by the pathogen (Brennan and Jayasena, 2007). Delayed sowing on the other hand help

in reducing the period in which disease epidemic can establish and pathogen reproduction can occur (Arvidsson *et al*., 2000). Cultural control measures might not guarantee the absence of net blotch disease in farmers' fields; this is because the inoculum can be moved by wind among neighboring fields. Due to the polycyclic nature of this pathogen, a small amount of inoculum can enable the disease to reach high level of epidemics given conducive environmental and host conditions.

The use of fungicides on seeds also help to reduce primary inoculum (Carmona *et al*., 2008). Foliar fungicides have been recommended for use in susceptible varieties and several active ingredients such as propiconazole, tebuconazole, azoxystrobins, procloraz and epiconazole are already in use. Application of foliar fungicide is most effective at stem elongation and flag leaf emergence (McLean, 2011). Use of foliar fungicides on upper leaves to reduce tissue loss during grain filling is also applicable (Liu *et al.*, 2011), however, in severe cases, several applications are recommended during growing season to reduce production loss and increase grain yield and quality. The use of foliar fungicide is however not justified due to its adverse effect on environment and increased cost of purchase (Bekele *et al*., 2004). Moreover, the pathogen can develop resistance towards the fungicides upon repeated use.

The use of biological control methods have not been exploited in the control of this disease, however, two strains of bacterium *Pseudomonas chlororaphis* have been reported to reduce infection by *P.teres f. teres* (Khan *et al.,* 2010). No biological control for *P. teres f. maculata* has been reported although, it is suggested that the two strains may be effective against *P. teres f. maculata* due to the similarities in their lifecycle.

Effective long term control of the disease should be based on growth of resistant varieties; however the virulence of *P. teres f. teres* and *P. teres f. maculata* is highly

variable (Cromey and Parks, 2003; Wu *et al*., 2003) and this poses great challenges to the breeders breeding for resistance.

2.1.4 Host range

Hordeum vulgare and *H. vulgare* sub - species *spontaneum* are considered to be the primary hosts for *P. teres*. However, wild species of *Hordeum* and other related species from the genera *Triticum, Avena* and *Bromus* are also infected by the disease (Shipton *et al*., 1973).The disease also infect a wide range of gramineous species in the genera *Cynodon, Elymus, Agropyron* and *Stypa* (Brown *et al*., 1993). These wild species majorly act as alternate hosts, however, their role as source of primary inoculum is reduced due to their high level of resistance to the disease (Liu *et al*., 2011).

2.1.5 Sources of inoculum

The main source of primary inoculum or initial infection is infected seeds and infected crop residues. Several reports have indicated that the infected seed is an important means by which *P. teres* survives, spreads, and initiates primary foci in net blotch epidemics (Carmona *et al*., 2008; Hampton, 1980; Jordan, 1981; Shipton *et al*., 1973). In Denmark, it has been reported that seed borne net blotch has been the greatest threat to organic seed production and exceeds the recommended 15% contamination threshold in barley seed lots. A positive correlation of net blotch contamination of seeds with net blotch severity at early grain filling stage was reported by (Pinnschmidt *et al*., 2005). Seed infection has been found to be contributing much on the severity and incidences of net blotch, coupled with high yield losses. Carmona *et al*., (2008) also reported a higher disease severity, incidence and reduced yields when untreated seeds were used. This shows that the seed - borne inoculum is a major problem in net blotch management. However, seed to crop infection is known to be influenced by the seeding rate, the amount of infected seeds

brought into the soil, edaphic and climatic conditions (Carmona *et al*., 2008). It has also been reported that infected residue also contributes to the early disease epidemics in the fields (Bekele *et al.*, 2004). The pathogen can survive for up to two years on infected crop residues. Initial crop infection occurs with up to 6 hours of moist conditions at temperatures between 10°C and 25°C. Primary inoculum is derived from airborne or splash dispersed spores from infected stubble of the previous crop. Air borne inoculum (conidia) are thought to travel up to seven metres from the point of dispersal within the field (Piening, 1968).

The formation and dispersal of secondary inoculum (conidia) takes between 14- 20 days after primary infection. Secondary infections can occur repeatedly throughout the growing season whenever conditions are favourable. As the barley plant begin to senesce, the fungus grows into the stem and can survive on stubble for long periods, producing the spores until the stubble is broken down. Stubble breakdown occur readily in warm wetter climates than in cooler drier climates, thus, the longevity of spore production by fungus on stubble vary in different climatic condition (Bhathal and Loughman, 2001).

Pyrenophora teres infect volunteer barley plants as well as grasses such as wheat and oat, as well as a wide host range of other grass species such as *Cynodon*, *Elymus*, *Agropyron* and *Stypa* (Brown *et al*., 1993). Thus, volunteer barley plants and the susceptible grass species may provide inoculum for the next season's barley crop.

2.1.6 Life cycle

Both forms of *P.teres* survive on the surface of infected barley residues, on barley seed (Liu *et al.*, 2011) or on alternate host plants (Brown *et al*., 1993). The resting structures produced on barley residue are pseudothecia and may exist in seeds as mycelia. The

pseudothecia are seen as dark dots on the surface of barley residue, measuring 1-2 mm in diameter and are covered by dark, hair-like setae (McLean *et al*., 2009). Bitunicate asci form within mature and fertile pseudothecia (Liu *et al*., 2011; Mathre, 1997). Each ascus contains eight ascospores that are light-brown in colour with three or four transverse septa. The ascospores are 18-28µm × 43-61µm in size (Mathre, 1997). At the beginning of a growing season mature ascospores are discharged by wind causing primary infection on the newly growing barley. The ascospores form the sexual reproductive structures (Van den Berg and Rossnagel, 1991). Seed-borne mycelium or conidia released from alternative hosts may also cause primary infection on newly established plants (McLean *et al*., 2009).

After colonization the fungus produces a large number of conidia that cause secondary infection. The conidia are smooth cylindrical and straight, round at both ends, sub - hyaline to yellowish brown with four to six septa (Mathre, 1997; McLean *et al*., 2009). The conidia are produced throughout the growing season and dispersed by strong winds and rains causing secondary infections. The production of conidia occurs on wet leaf surface when the relative humidity is high with temperature ranges of 6°C - 25°C. The conidia acts as the asexual reproductive structures (Van den Berg and Rossnagel, 1991). At the end of the growing season, the fungus colonizes the senescent tissues, producing the resting structures- pseudothecia.

Pyrenophora teres reproduces mainly asexually under field condition (Jalli and Robinson, 2000), asexual reproduction is important in the life cycle of *P. teres*. It is presumed that asexual reproduction the main form of spore dispersal during the growing season (Lehmensiek *et al*., 2010). The sexual spores are however produced only once, at the beginning of the growing season (Statkevičiūtė *et al*., 2010). The formations of the

sexual reproductive structures require isolates of different mating types and specific environmental conditions (Sirenius *et al*., 2005; Statkevičiūtė *et al.*, 2010). This is because *P. teres* is heterothallic and self sterile in nature (McDonald and Linde, 2002)

2.1.7 The role of sexual and asexual reproduction in *P.teres*.

Pyrenophora teres has a mixed reproductive system with one generation of sexual reproduction occurring on the stubble between crops and several generations of asexual reproduction occurring during the growing season of the crop (Peeve and Milgroom, 1994). Sexual reproduction facilitates the generation of genotypic diversity thus enabling the pathogen to adapt to the changes in genetic makeup of the host population (Peeve and Milgroom, 1994; Rau *et al*., 2003; Statkevičiūtė *et al*., 2010). The existence of both sexual and asexual reproduction cycles in *P. teres* poses a greater risk for overcoming resistance genes compared to strictly asexual or strictly sexual pathogens (McDonald and Linde, 2002). The mixed reproduction system thus impacts negatively on disease control and management efforts given that the pathogen has high chances of evolving.

2.1.8 Infection process

The infection process starts with the germination of ascospores or conidia upon landing on the surface of the leaves. This occurs after a few hours provided that environmental conditions are conducive (Van den Berg and Rossnagel, 1990). Once conidia land on the leaves the germ tube arises from the terminal cells. The hyphae form germ tubes that are able to grow to varied lengths. The germ tubes form appresoria- penetration structures used by fungi. *P. teres* penetrates directly through the cuticle into the epidermal cells (Jørgensen *et al*., 1998). This penetration occurs by enzymatic hydrolysis of the cuticle and cell wall as well as by pressure generated from the appresoria (Liu *et al*., 2011). The

plant host cells in some cases form a papillae-like structure to inhibit the entry of the fungus (Jørgensen *et al*., 1998).

If a successful penetration of the outer epidermal cell wall occurs, the fungal hyphae develop into a large intracellular vesicle. A secondary intracellular vesicle then develops within the epidermal cell causing functional disruption of the infected cells and adjacent epidermal cells (Keon and Hargreaves, 1983). Intracellular hyphae emerge from the intracellular vesicle and grow through the lower epidermal cell wall into the mesophyll tissue. Within two days of inoculation, host cell attached to the intercellular hyphae becomes necrotic. As the infection continues, chlorotic areas appear around the initial necrotic lesions (Keon and Hargreaves, 1983). Lightfoot and Able, (2010) demonstrated that *P. teres f. teres* rarely forms intracellular vesicles, it feeds as a necrotroph during the infection process by growing intercellularly and is therefore able to affect cells that are not near the mycelium. *P. teres f. maculata* however, is able to form haustoria-like intracellular vesicles, feeding similarly to a biotroph. This is followed by an immediate switch to necrotrophic growth. On the basis of this description, *P. teres f. teres* can be classified as a necrotrophic pathogen and *P. teres f. maculata* as a hemibiotroph (Liu *et al*., 2011)

2.1.9 Population diversity and genetic structure of *Pyrenophora teres*

The knowledge of population diversity and genetic structure is important in management of most fungal diseases, especially for the successful use of host resistance and effective use of fungicides. It indicates how rapid a pathogen can evolve and can be used to predict how long a control measure or resistance source can be effective (Campbell *et al*., 2002; Sirenius *et al.*, 2007). Different studies have shown that *P. teres* from different regions or within an area can vary greatly morphologically (McDonald, 1967; Shipton *et*

al., 1973). The use of different molecular markers, such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) have been employed to study the population diversity and genetic structure of *P. teres* isolates and to find out the possible cause of changes in the fungal population. Investigations performed on *P. teres* populations from different geographical regions have shown a high level of variability, even on a small sampling area compared to other fungi (Campbell *et al*., 2002; Lehmensiek *et al*., 2010). A high genetic differentiation has also been shown between populations that are separated by a long distance (Lehmensiek *et al*., 2010). Studies have shown that genetic divergence is slightly higher among the *P.teres f.teres* populations than among the *P. teres f. maculata* populations (Lehmensiek *et al*., 2010; Rau et al., 2003; Sirenius *et al*., 2005). Phylogenic studies have also shown that *P. teres f. teres* and *P.teres f. maculata* isolates are separated into two divergent groups, indicating that the two forms of *P. teres* should be treated separately (Rau *et al*., 2007).

Due to the ability of *P. teres* to reproduce both sexually and asexually, its population structure would be dependent on these two modes of reproduction. A random sexual reproduction has been shown to be important in many *P. teres* populations (Rau *et al*., 2003). However, other studies have suggested that reproduction in some *P.teres* populations occur mainly asexually (Campbell e*t al*., 2002; Jalli and Robinson, 2000; Lehmensiek *et al*., 2010). In another study of two mating type genes of *P. teres*, the ratio of two mating type genes was found to be 1:1, suggesting that sexual reproduction is the major force driving the population structure (Rau et al., 2005). It was reported that the existence of an equal distribution of both mating types, indicate that sexual reproduction

occurs regularly among *P. teres*, although asexual reproductive component was also observed (Bogacki et al., 2010; Khan and Boyd, 1969).

2.1.10 *Pyrenophora teres* specialization and virulence diversity

Several studies using large sets of differentials have been performed in attempts to show the diversity in physiological races for both forms of *P. teres* (Gupta and Loughman, 2001; Jalli and Robinson, 2000; Khan, 1982; Khan and Boyd, 1969; Tekauz, 1990; Tuohy *et al.*, 2006; Wu *et al*., 2003). Several known differential lines have been used in these studies to compare virulence diversity all over the world and within different study areas.

Khan and Boyd, (1969) differentiated four different races using two barley lines, showing the existence of a distinct host - pathogen interaction. They also reported that all *P. teres f. teres* isolates from West Australia were virulent to the common Australian cultivar 'Beecher'. However, *P .teres* isolates collected after 1976 were shown to have lost virulence on the cultivar Beecher (Khan, 1982).Gupta and Loughman, (2001) reported that *P. teres f. teres* remained avirulent on Beecher up to 1990s. All *P. teres f. teres* isolates from New Zealand were avirulent on Beecher (Cromey and Parks, 2003). Although Beecher and Harbin-barley lines have been used in most virulence studies, another set of nine differential lines have been proposed for use in the characterization of the global *P. teres* populations in barley producing areas provided that similar environmental conditions are used (Afanasenko *et al.*, 2009).

Resistance to *P. teres f. teres* and *P. teres f. maculata* has been reported to be under different gene control, studies to investigate the virulence of *P. teres f. maculata* have

used similar lines as used for *P. teres f. teres* (Gupta and Loughman, 2001; Wu *et al*., 2003).Some of these studies have reported that *P. teres f. teres* has a higher virulence diversity compared to *P. teres f. maculata* (Gupta and Loughman, 2001; Wu *et al*., 2003). Tekauz, (1990) evaluated a set of Canadian lines and identified 20 different pathotypes based on the reaction of a12-barley-line set. These studies showed that the virulence of *P. teres f. teres* and *P. teres f. maculata* is diverse. This is shown by the fact that differential lines with resistance can be effective against virulence in an entire population in an area, but ineffective against a population collected in another area.

2.1.11 Production of toxins by *P. teres* during an infection

The symptoms that occur on barley leaves after inoculation with *P.teres* occur due to the production of toxins by the fungus. The severity of these symptoms appears to be dependent on the amount of toxins produced (Sarpeleh *et al*., 2007). The toxins produced by this pathogen disrupt the normal physiological processes that sustain the plant, thus causing damage or death of plant cells. Some toxins produced by pathogens are toxic to only a given plant species or varieties and are completely or may be completely harmless to others. Three toxins have been found to induce toxic reactions in the host-barley during inoculation with *P. teres*. These toxins have been named A, B and C (Smedegård-Petersen, 1977). The toxins were reported to have a similar chemical structure to that of aspergillomarasmine A. Toxin A was identified to be N-(2-amino-2-carboxiethyl) aspartic acid, B as anhydroaspergillomarasmine A and C as aspergillomarasmine A. The toxins were found to differ in their biological activity on the host, with C being the most active (Weiergang *et al.*, 2002).

Phytotoxic proteinaceous metabolites from culture filtrate of *P. teres* have been reported. These metabolites were found to be dependent on light and temperature, and this indicates that they may target the chloroplasts in case of an infection (Sarpeleh *et al*., 2007; Sarpeleh *et al*., 2008). Lower molecular weight compounds (LMWCs) that cause chlorosis and necrosis on the host leaves were also identified. Further studies indicated that these LMWCs produced probably have similar chemical structures to the toxins A, B and C. The activity of these LMWCs were found to be non-host selective - cause disease symptoms not only on the host plants, but also on other plant species that are not attacked by the pathogen in nature (Sarpeleh *et al*., 2009).

2.2 Host factors influencing the response to *P. teres*

2.2.1 Host resistance to *P.teres*

Host resistance has been shown to be the effective method for management of net blotch disease (Østergárd *et al*., 2008). It also helps in reducing the use of fungicides that are a threat to the environment as well as reducing the cost of production for farmers. The resistance of barley to *P .teres* has been reported to be both quantitative (Robinson and Jalli, 1997; Steffenson *et al*., 1996) and qualitative (Friesen *et al*., 2006). Breeding for resistance could be challenging as resistance sources can either have quantitative or qualitative effect and are usually conferred by a number of genes located on different chromosomes (Liu *et al*., 2011). Quantitative resistances are only effective for part of crop's growth and developmental stages and only provide moderate resistance. On the other hand, qualitative resistances are effective throughout a crop's life. The presence of qualitative resistance suggests the potential for a gene -for- gene interaction occurring in this pathosystem.

Three major, incompletely dominant genes were found to be effective against *P.teres* isolates collected in California. These genes were designated Pt_1, Pt_2 and Pt_3 (Mode and Schaller, 1958). Single dominant genes were later reported by (McDonald and Buchanon, 1962). Most studies have used seedling data to evaluate inheritance of resistance. The major effects for seedling resistance to net form of net blotch (NFNB) have been reported to be on chromosome 6H near the centromere. Other seedling resistance genes were also identified at chromosomes 7H,4H and 2H in breeding lines (Williams *et al*., 2003). Effective seedling resistance has been mapped on chromosome 7H,6H,4H and 3H in two Canadian varieties (Grewal *et al*., 2008). In comparing seedling and adult plant resistance, Cakir *et al.,* (2003) and Grewal *et al*.,(2008) reported that 6H locus was effective at both plant stage, showing that seedling resistance is also effective at adult plant stage. Plant age as a factor in net blotch resistance has been reported and different studies indicate that resistance can be maintained or improved as the plants mature (Gupta *et al*., 2003.; Tekauz, 2000) especially in lines that were either initially susceptible or resistant. However, Douiyssi *et al*, (1998), observed different results using two isolates, where resistance to one isolate tended to increase with plant age, while resistance to the other isolate tended decrease with plant age.

Recent studies on net blotch resistance have shown that SFNB resistance genes are genetically distinct from those effective against NFNB (Grewal *et al.*, 2008; McLean *et al.*, 2009). Major genes for SFNB resistance have been identified on barley chromosomes 4H,5H and 7H, with other minor QTLs. This suggests that different host genes confer resistance to different forms of the pathogen, thus the two forms should be considered separately in breeding for resistance.

2.2.2 Host - pathogen interaction

In many plant – pathogen interactions, disease response result from the expression of a resistance (R) gene in the plant and a corresponding avirulence gene (Avr) in the pathogen. When a pathogen with an Avr gene comes into contact with the corresponding R gene during the initial infection, the Avr gene in the pathogen encode proteins, which when detected by the host, a resistance response is triggered (Lai *et al*., 2007). This interaction may activate a signal transduction pathway and finally lead to active resistance (Agrios, 2005; Lauge *et al*., 1998). An inverse gene-for-gene interaction for host susceptibility also occurs (Liu *et al*., 2011). The susceptibility symptoms produced by barley are produced by necrotrophic effectors from the pathogen which are of protein origin and can be host or pathotypes specific. Host death only occurs in the presence of corresponding susceptibility genes and pathogen effector proteins (Tan *et al*., 2010).

The expression of R and Avr genes is induced by plant signal molecules such as jasmonic acid (JA), Salicylic acid (SA), ethylene (ET) and nitric oxide. In several host – plant interactions, plants react to attack by pathogens with an increased production of these plant signal molecules. Consequently a set of gene -to- gene resistance defence – related genes is activated in attempts to block the infection (Agrios, 2005). A MAPK (PTK 1) gene has been cloned from *P.teres* and shown to be important in the formation of the appresoria at infection. The strain resulting from the disruption of the target gene - PTK1 in *P.teres* produces no appresoria, leading to loss of pathogenicity (Ruiz-Roldan *et al*., 2001). Loss of pathogenicity also occurs due to the production of antimicrobial compounds or generation of signal molecules that act at the point of infection to establish systemic acquired resistance (Agrios, 2005). During early stages of spore germination, it has been reported that several genes are involved in signal transduction (Dilger *et al.*, 2003).

In a gene- for- gene type interaction currently known as effector – triggered immunity (ETI), reactive oxygen species (ROS) are often produced in a resistance response to pathogen attack. A correlation between ROS accumulation and pathogen growth using a single barley line and two *P.teres f.teres* isolates, one virulent and one avirulent has been reported (Able, 2003). Host genes involved in compatible and incompatible interactions have been studied. A host gene HvS40 which appears to play a role in *P.teres*-barley host – pathogen interaction has been identified (Krupinska *et al*., 2002). Expression of HvS40 was found to be induced by jasmonic acid and salicylic acid. Increased levels of expression were observed in leaf tissues that showed necrosis and chlorosis after infection with *P.teres*, but low in the uninfected tissues, suggesting that this gene plays an important role during pathogen infection.

2.3 Effects of environment on disease development

The environmental conditions prevailing in the air and soil after contact of pathogen with its host greatly affect the development of the disease. Environmental conditions frequently determine whether a disease will occur or not. Every pathogen has specific environmental conditions that favour its development. The most important environmental conditions during the initiation and development of infectious diseases are temperature and moisture/ relative humidity on / around the plant surface (Agrios, 2005). Soil nutrients are also important in the development of some diseases. Light and wind, however, play smaller roles in disease development but spread. These factors influence the development of diseases due to their contribution towards the growth and susceptibility of the host, on multiplication and activity of the pathogen, or on the host – pathogen interaction in relation to the severity of symptom development. Thus, a change

in any of these factors may favour the pathogen, host or both. In some cases however, one may be favoured over the other, causing a considerable change in disease development. The influence of these factors on disease development is determined by the extent of their deviation from the point at which disease development is optimal.

2.3.1 Effects of temperature and relative humidity/moisture on net blotch development

Different pathogens have different preferences for high or low temperatures. Temperature affect the number of spores formed in a unit plant area and the number of spores released in a given time period (Agrios, 2005).When temperatures remain within a favourable range during sporulation, pathogen growth or reproduction, a polycyclic pathogen such as *Pyrenophora teres* can complete its infection cycle within a very short time. As a result, such pathogens can produce many infection cycles within a cropping season, thus leading to the development of a severe epidemic. Spore production and the infection on growing barley plant by *P.teres* is maximum at temperatures of 25ºC (Kosiada, 2008). Conidial germination require a high relative humidity for 10 – 30 hours or longer, over a wide range of temperatures 8-33°C an optimum between 15-25°C (Mathre, 1982). During epidemic stage, incubation lasts for 4 days at 20°C. Seedling infection is greatest during a period of cool humid weather 10-15°C. Moisture is important in the germination of spores, and penetration of the host by the germ tube. Moisture in forms such as splashing rain and running water also plays an important role in the distribution and spread of some pathogens on the same plant and on their spread from one plant to the other. Moisture increases the succulence of host plants and thus their susceptibility to certain pathogens (Agrios, 2005). In most fungi, moisture increases sporulation, facilitates spore release and spore germination thus, the presence of high

level of moisture enables all these events to take place constantly and repeatedly and leads to epidemics. On the other hand, lack of moisture for even a few days prevents all these events from taking place, so that epidemics are interrupted or stopped completely.

2.3.2 Effects of light to the development of net blotch

Light intensity and duration are known to increase or decrease the susceptibility of plants to infection and also to severity of the disease. The production of weak plants has been attributed to the reduced light intensity. This increases the susceptibility of plants to non-obligate parasites such as *P.teres* but decreases their susceptibility to obligate parasites. An increase in *P.teres* infection has been reported with an increase in duration of darkness than at an alternating light application of 12 hours and 12 hours darkness (Kosiada, 2008). Infection intensity of *P.teres* has been reported to be highest at 10 hour day, this shows that the highest infection occurs at a short day, when the dark hours exceeds the light hours of the day. In some fungi however, abundant sporulation occurs when the fungus is exposed to continuous near ultra violet light and is even more abundant when the exposure is followed with darkness. For instance, abundant sporulation in Ascomycetes and Basidiomycetes have been reported to be induced by exposing the colonies to near ultra violet light (Guo *et al.*, 2003; Wang *et al*., 2005). This also may also apply to *P.teres* since it is an Ascomycetes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field screening for the response of barley genotypes to *Pyrenophora teres*.

3.1.1 Sources and selection of genotypes

Twenty barley lines were obtained from the Moi University - East Africa Breweries Limited Collaborative Research Project. Two genotypes with known resistance to net blotch, two with known susceptibility and others with unknown responses were used (Table 1). These lines were evaluated for their response to *P. teres* under different field environmental conditions.

3.1.2 Experimental sites

Field experiments were carried out at Chepkoilel University College, School of Agriculture Farm and in Purko sheep ranch Mau- Narok near Tipis centre. The sites were selected to represent mid-attitude and high-altitude production areas, respectively. Mau-Narok is located at an altitude of 2900 m above the sea level and lies between latitudes 0°36'S and longitude 36°0'E. The area receives an average annual rainfall of 1200-1400mm. The minimum temperatures of 6-14°C and maximum of 22-26°C (Wanyera *et al*., 2010) have been reported. Chepkoilel is located in Uasin-Gishu district. It is located between longitude 35°18' E and latitude 0° 30'N and at an altitude of 2140 m above the sea level. Rainfall ranges from 900 to 1300 mm with an annual average of 1124 mm. The average annual temperature is 23°C with a minimum of 10°C (Okalebo *et al*., 1999).

Table 1: Characteristics of Barley genotypes and groups screened *P.teres*

GENOTYPE GROUPING	GENOTYPE	SPECIAL ATTRIBUTES
SYNGENTA VARIETIES	Quench	High yielding Resistant to lodging Good malting quality
	Publican	Resistant to lodging Good malting quality High yielding
KARI/KML INTRODUCTION	Cerise Laurel	High yielding Plump grains
SIX ROW BARLEY	NBD Petunia	High yielding Resistant to lodging
	77 Tecoma	Resistant to lodging High yielding
	54 CAN	Resistant to lodging High yielding
	Gloria bar	Resistant to lodging High yielding
HKBL VARIETIES	HKBL 1385-12	Resistant to BYDV Medium yielding
	HKBL 1386-2	High yielding Good malting quality
	HKBL 1512-5	Plump grains Resistant to lodging Good malting quality &high yielding
	HKBL 1539-4	High yielding Good malting quality
	HKBL 1591-3	High yielding
	HKBL 1629-19	High yielding Resistant to lodging
	HKBL 1674-4	High yielding
	HKBL 1719-1	High yielding
	HKBL 1719-18	High yielding
RESISTANT CHECKS	HKBL 1386-13	Resistant to net blotch,scald &BYDV Good malting quality
	Nguzo	Moderate yield .Good malting qualities Resistant to net blotch & BYDV
SUSCEPTIBLE CHECKS	Karne	Moderate yield .Resistant to BYDV,Scald&RWA Susceptible to net blotch .Good malting qualities
	Selian	.Susceptible to net blotch and BYDV .Good malting qualities .Moderate yields

Source: MU/EABL collaborative research project, 2011

3.1.3 Experimental design and layout

Both experimental sites were prepared in July 2011. Mau – Narok was planted in mid-August 2011 and Chepkoilel planted in early September 2011. The 20 barley lines were planted in a Randomized Complete Block Design (RCBD) with four replications. A plot size of 3m by 1.5 m was used. Block spacing of 1.5 m and between plot spacing of 0.5 m was used. All blocks were surrounded by guard rows of a known susceptible line (Sabini). Seed rate of 80 kg ha^{-1} was used. Pre-emergence and post- emergence herbicides were used as recommended.

3.1.4 Inoculation and disease assessment

Due to the continuous presence of the disease in the experimental areas the barley lines were screened under natural infection. A known susceptible variety (Sabini) was planted around the experimental field to act as a spreader. Disease assessment was done visually for the percentage leaf area affected by disease on six occasions at both sites at an interval of two weeks. This was done on a scale of 0 – 9 (0-no disease, 9-all leaves of the plant severely affected (Couture, 1980; Xue *et al*., 1994). During the assessment seedling response data was scored at 21 days while adult plant response data was scored at 63 days and 91 days respectively on a 0-9 scale as follows: 0 = 1 – 10 % of the leaves are affected; 1 = 11 – 20 % of the leaves are affected; 2 = 21 – 30 % of the leaves are affected; 3 = 31 – 40 % of the leaves are affected; 4 = 41 – 50% of the leaves are affected; 5 = 51 – 60 % of the leaves are affected; 6 = 61 – 70% of the leaves are affected; 7 = 71 – 80 % of the leaves are affected; 8 = 81 – 90 % of the leaves are affected; 9 = 91 – 100 %. Based on field reactions, the genotypes were grouped as follows: **0-3** - Resistant, **4-5** moderately resistant, **6-7** moderately susceptible, **8-9** Susceptible. During assessment, barley tissues showing symptoms of net blotch (both

types) were collected for laboratory isolation. These were used to study morphological diversity of *P. teres* and assessment of the virulence and pathogenicity on the barley genotypes that had been evaluated for net blotch disease in the field.

3.1.5 Disease assessment on ears

Due to severe ear infection in some genotypes ear infection was also assessed at dough stage on a scale of 0-9 scale where **0-3** - Resistant, **4-5** moderately resistant, **6-7** moderately susceptible, **8-9** Susceptible. The pathogen confirmation was done according to (McLean, 2011). The infected ears and healthy ears were sampled separately from each plot and thousand kernel weight determined for each sample.

3.1.6 Statistical analysis

The data collected was subjected to analysis of variance (ANOVA), using Genstat software Version 12, to find out whether there were significant differences in the response of the barley genotypes to net blotch. Significant differences were tested at 5% level of significance and mean reaction separated by contrast comparison to help in answering specific questions relating the different groups of genotypes.

3.2 Morphological diversity of *P. teres*

3.2.1 Collection of net blotch infected leaf tissues

During field evaluation, infected leaf tissues were collected. This was done for experimental fields in Mau Narok, Njoro and Chepkoilel. Biased sampling was used where most samples were collected from the most susceptible varieties to increase the pathogen diversity. The infected tissues were air dried and placed in paper bags and kept at room temperature.

3.2.2 Isolation of *P. teres* species

Isolation was done according to McLean, (2011), where infected leaf samples were cut into 1 cm^2 pieces and surface-sterilized in 70 % ethanol for 10 sec, 1% sodium hypochlorite solution for 30 sec, then double rinsed in sterile distilled water for 10 sec. Fragments were blotted dry and aseptically transferred to freshly prepared potato dextrose agar (PDA Oxoid, 3.9 %) containing streptomycin sulphate (0.39 %) in petri dishes. This was then incubated inside a Gallenkamp incubator at 15-20ºC and 12 hour under white fluorescent light and 12 hour dark cycle to induce sporulation. After three to five days, a single conidium representing each collection was transferred, using the needle to potato dextrose agar medium petri dishes and incubated as indicated above for 2 weeks to induce sporulation.

3.2.3 Assessment of morphological diversity

Data on morphological characteristics including conidia shape, mycelium colour (upper surface), colour of substrate (under surface), texture, septation of mycelium and growth of aerial mycelium (luxuriant or scanty) was recorded and used to morphologically assess the diversity of *P. species* infecting barley. The conidia were observed under a light microscope at eyepiece lens magnification of ×10 and objective lense magnification of ×400 (mg ×40). Sporulation potential (spore/ml) for each isolate was scored by counting using the Buker- Turk haemocytometer (Mathur *et al*., 1989).

3.3 Screen house evaluation of *P.teres*

3.3.1 Experimental design

The 20 barley genotypes were planted in split – plot experiment in completely randomized design with two replicates. Identified isolates were assigned to whole plots

with barley genotypes as sub – plots. Each genotype was randomized within whole-plots and replicated twice. At planting, a table-spoonful of DAP fertilizer was applied to every sub-plot and mixed thoroughly with the soil that was previously solarized in black polythene to sterilize the soil before planting.

3.3.2 Inoculum preparation and inoculation

The isolates were prepared using the procedures stated by McLean, (2011) as described in section 3.2.2 above. The mycelia and spores were harvested by flooding the petri dishes with 4 ml of sterile distilled water and scrapping with a spatula. Spores and mycelia suspension were filtered through double layer cheesecloth to retain any fragments of culture media. The density of the conidia was assessed on a Buker-Turk haemocytometer and the concentrations adjusted to 1 x 10^4 per ml. The seedling of the barley genotype were placed in a plastic container for inoculation and transferred to room temperature. Leaves of 21 – days old seedlings were pre-moistened using sterile distilled water and inoculated immediately. About 0.5 ml of 1×10^4 spore/ml concentration was sprayed on seedlings in each container (two replications) using a hand sprayer and covered immediately to maintain high relative humidity. The inoculated plants were maintained under high relative humidity (>95%) by misting after every 12 hours using sterile water and left at room temperature for three days to ensure successful infection . After three days, the plants were acclimatized and then transferred to the screen house for symptom development. Two weeks after inoculation the plants were assessed for percentage leaf area affected by net blotch on a 0-9 scale (Gupta *et al*., 2003.) where; **0-3** - Resistant, **4-5** moderately resistant, **6-7** moderately susceptible, **8-9** Susceptible.

3.3.3 Statistical data analysis

The data were subjected to analysis of variance (ANOVA) using GENSTAT statistical software version 12. The mean reactions to net blotch were separated using contrast comparison and difference between treatments determined at 5% significance level.

CHAPTER FOUR

RESULTS

4.1 Field response of advanced barley genotypes to net blotch

4.1.1 Response of barley genotypes to *P. teres* under field conditions

The results of the response of barley genotypes under field conditions during field screening are as provided in table 2. Site and variety showed significant differences ($p < 0.05$) in terms of response to net blotch by advanced barley genotypes under field conditions. Mau Narok had the highest disease level (6.3) while Chepkoilel score was 5.5 on a 0-9 severity scale (Table 2, Appendix I).

Table 2: Response of grouped barley genotypes to *P.teres* in the field at Chepkoilel and Mau Narok sites.

		SITES		SEVERITY AVERAGES		
VARIETY GROUP	VARIETY	CHEPKOILEL	MAU NAROK	MEAN		GROUP MEAN
SIX ROW BARLY	54 CAN	4.3	5.7	5.0		5.4
	NBD PETUNIA	4.7	5.3	5.0		
	77 ALOE	6.3	6.0	6.2		
	GLORIA BAR	4.7	6.0	5.3		
HKBL VARIETIES	HKBL 1385-12	4.7	6.3	5.5	6.3	5.9
	HKBL 1386-2	7.3	7.0	7.2		
	HKBL 1512-5	5.3	6.7	6.0	5.8	
	HKBL 1539-4	4.3	5.3	4.8		
	HKBL 1591-3	6.3	7.0	6.7		
	HKBL 1629-19	5.0	6.3	5.7	5.2	
	HKBL 1674-4	4.3	5.3	4.8		
	HKBL 1719-1	5.3	6.0	5.7	6.3	
	HKBL 1719-18	6.7	7.0	6.8		
KML/KARI INTRODUCTION	CERISE LAUREL	4.3	4.7	4.5		4.5
SYNGENTA VARIETIES	PUBLICAN	4.7	6.0	5.3		5.3
	QUENCH	5.0	5.7	5.3		
SUSCEPTIBLE CHECKS	SABINI	8.3	8.3	8.3		8.0
	KARNE	7.7	7.7	7.7		
RESISTANT CHECKS	HKBL 1385-13	3.7	4.7	4.2		6.1
	NGUZO	8.0	8.0	8.0		
MEAN		5.5	6.3	5.9		5.9

	SITE	VARIETY	SITE x VARIETY
S.E	0.0726	0.2297	0.3248
S.E.D	0.1027	0.3248	0.4593
F.probability	< 0.001	< 0.001	0.042
% C.V	9.5		

Irrespective of site and group effects, genotypes HKBL 1385-13, CERISE LAUREL, HKBL 1674-4, and HKBL 1539-4, NBD PETUNIA and 54 CAN were moderately resistant to net blotch under field conditions. These genotypes scored 4.2, 4.5, 4.8, 4.8, 5.0 and 5.0 respectively on a 0-9 severity scale. On the other hand, majority of the genotypes were moderately susceptible to *P.teres*. In the moderate susceptible category of genotypes, Quench, Publican and Gloria Bar were the best (5.3) while HKBL 1719-18 and HKBL 1591-3 recorded the highest disease severity. The third category of screened barley genotypes gave susceptible reactions which included Nguzo, Sabini and Karne varieties that scored 8.0, 8.3 and 7.7 respectively. Both Sabini and Karne were used as susceptible checks and their reaction to the disease is evident that the disease pressure was adequate for screening. However, despite being known to be resistant to net blotch, Nguzo variety was susceptible to the disease in both sites thus contradicting the previous findings and indicating that its resistance has been broken (Table2).

4.1.2 Group response of barley genotypes to *P. teres* under field conditions

In terms of groups, Cerise Laurel genotype, an introduction by the KARI/EAML recorded the least level of disease (4.5). The six row barley types (54 CAN, NBD Petunia, 77 Aloe, Gloria Bar) and Syngenta varieties (Publican, Quench) ranked second best in terms of disease resistance after Cerise. Each group scored 5.3 and 5.4 for Syngenta and six row groups respectively.

Susceptible checks (Sabini, Karne) exhibited the highest net blotch severity at both Chepkoilel and Mau Narok sites at 8.0. However, the resistant check group gave a moderate susceptible response, this was due to the response of Nguzo variety which gave a high susceptible response (8.0) within the group at both sites despite being known as a resistant genotype.

The HKBL group of genotypes showed moderate susceptible response. HKBL 15 and 16 series gave almost the same response, however, HKBL 1539-4 and HKBL 1674-4 gave moderate resistant response (4.8) within the groups Table 2. Further grouping of HKBLs into 13 and 17 series (i.e. HKBL 1385-12, HKBL 1386-2; and HKBL 1719-1, HKBL 1719-18) expressed the high level of disease (6.3) and were more susceptible within the HKBL group (Table 2).

4.1.3 Barley genotypes response to ear infections under field conditions and effects on yield

The results of genotypes response to ear infection under field conditions are as presented in table 3. Site and variety showed significant differences ($p < 0.05$) in terms of ear response to net blotch by advanced barley genotypes (Table 3, Appendix II).

Table 3: Ear infection by *P. teres* on advanced barley genotypes at Chepkoilel and Mau Narok sites

		SITES		SEVERITY AVERAGES		
VARIETY GROUP	VARIETY	CHEPKOILEL	MAU NAROK	MEAN		GROUP MEAN
SIX ROW BARLY	54 CAN	7.7	6.3	**7.0**		**6.4**
	77 ALOE	6.7	5.7	**6.2**		
	GLORIA BAR	5.7	4.7	**5.2**		
	NBD PETUNIA	7.7	6.7	**7.2**		
HKBL VARIETIES	HKBL 1385-12	3.3	2.3	**2.8**	**1.9**	**2.9**
	HKBL 1386-2	1.3	0.7	**1.0**		
	HKBL 1512-5	4.7	3.7	**4.2**	**3.6**	
	HKBL 1539-4	3.3	2.3	**2.8**		
	HKBL 1591-3	4.3	3.3	**3.8**		
	HKBL 1629-19	3.7	2.7	**3.2**	**4.0**	
	HKBL 1674-4	5.3	4.3	**4.8**		
	HKBL 1719-1	2.7	1.7	**2.2**	**2.0**	
	HKBL 1719-18	2.3	1.3	**1.8**		
KML/KARI INTRODUCTION	CERISE	1.0	0.3	**0.7**		**0.7**
SYNGENTA VARIETIES	PUBLICAN	1.3	0.7	**1.0**		**0.8**
	QUENCH	0.7	0.3	**0.5**		
SUSCEPTIBLE CHECKS	KARNE	1.3	0.7	**1.0**		**0.9**
	SABINI	1.0	0.7	**0.8**		
RESISTANT CHECKS	HKBL 1385-13	0.7	0.0	**0.3**		**0.5**
	NGUZO	1.0	0.3	**0.7**		
MEAN		**3.3**	**2.4**	**2.9**		**2.9**

	SITE	VARIETY	SITE x VARIETY
S.E	0.0784	0.2478	0.3504
S.E.D	0.1108	0.3504	0.4956
F.probability	< 0.001	< 0.001	0.999
% C.V	9.5		

Six-row barley were the most susceptible to ear infection to *P. teres* scoring 6.4. However, HKBL lines showed resistant reaction to ear infection, giving a mean severity of 2.9. Cerise, resistant checks and Syngenta varieties were almost immune to this disease although even susceptible checks behaved the same.

Mean separation for ear infection among the groups indicated significant differences ($p < 0.05$) between six row and HKBLs; resistant checks and HKBLs; susceptible checks and HKBLs; susceptible checks and six row; resistant checks and six row; HKBL 13 series and HKBL 15 series; HKBL 16 series and HKBL 17 series; HKBL 13 series and HKBL 17 series and six row barley and Syngenta varieties comparisons. However, susceptible and resistant checks; susceptible checks and Syngenta varieties; resistant checks and Syngenta varieties and HKBL 15 series and HKBL 16 series did not differ significantly in terms of ear infection (Table 3, Appendix II).

Ear infection by *P. teres* plays a very significant role in the final yield potential of each barley variety. In terms of a thousand kernels weight (TKW), all genotypes screened for ear infection gave lower weight for the infected kernels compared with the healthy kernels (Figure 1).

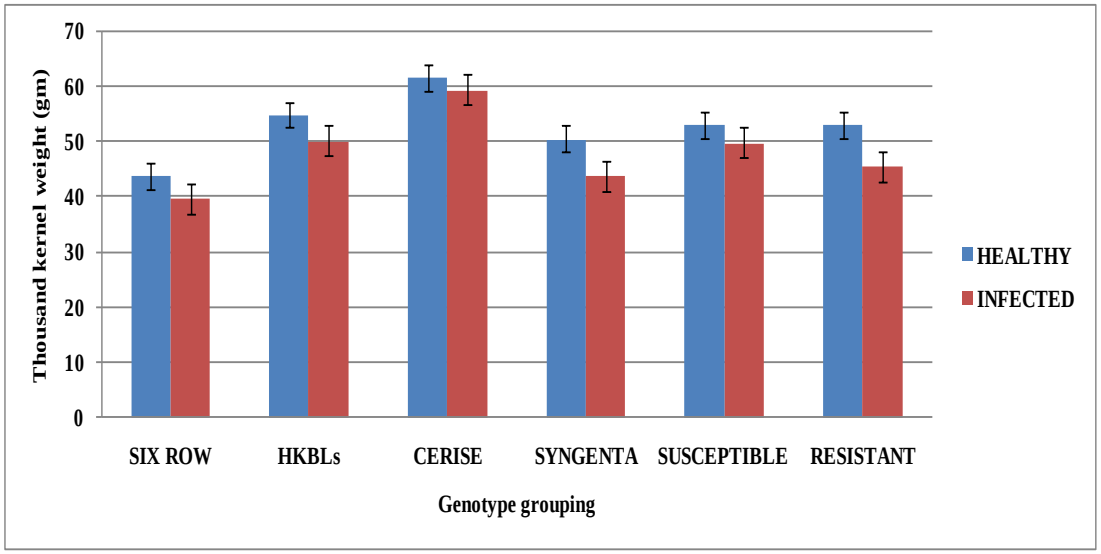

Figure 1 Thousand kernel weights for infected and healthy kernels of advanced barley genotypes. Error bars represent the significant differences.

Despite ear infection, Cerise Laurel had the highest healthy kernel weight of above 60gms while the six row group exhibited the least kernel weight with all genotypes weighing less than 50 g within the group. The HKBL group weighed above 50 g, however, only genotype 1674-4 weighed below 50 g within the HKBL group. Additionally, all varieties had kernel weights ranging between 40 g to 62 g. Though a high foliar infection response was observed in susceptible group of genotypes, they gave a relatively high kernel weight compared to the six row and Syngenta group of genotypes.

4.1.4 Site x Variety interaction effect on severity of net blotch and ear infections

The site x variety interaction effect showed significant differences ($p < 0.05$) in foliar infection by *P. teres* under field conditions in Mau Narok and Chepkoilel sites (Appendix I). However, this interaction did not show significant difference ($p > 0.05$) on ear infection (Appendix II). For example, some individual genotypes from different groups showed lower levels of foliar infection by net blotch fungus at Chepkoilel site compared with Mau Narok site. These included 54 CAN, GLORIA BAR and PUBLICAN. However, certain genotypes gave similar response in both sites and they include Sabini, Karne and Nguzo. Similar observations were made for ear infections by the fungus except that the ear infection levels were higher in Chepkoilel than Mau Narok while leaf infections were higher in Mau Narok than Chepkoilel site.

4.1.5 Adult and seedling resistance mechanisms

During this study a variation in resistance at different stages of barley plant growth and environments was noticed. For instance, in Syngenta group and six row group of genotypes, there was seedling immunity at 21 days after sowing at Chepkoilel site. However, the same genotype groups recorded a higher disease level at 21 days after

sowing in Mau Narok (Figure 2). However, 77 Aloe gave a susceptible reaction within the six row group at Chepkoilel site. Contrary to this, CERISE genotype showed seedling resistance to net blotch in Mau Narok site both at seedling and adult plant stage (Plate 1.) but the same genotype gave a susceptible response at seedling stage at Chepkoilel site with a resistant response at adult plant stage. Susceptible group of genotypes gave a susceptible response at seedling stage, however, within the group Sabini gave a resistant seedling response at both sites. Similarly, not all genotypes with seedling susceptibility recorded high severity of net blotch and instead, they were tolerant to the disease at adult stage of growth for example . HKBL 1539-4, HKBL 1674-4, HKBL 1719-1 within the HKBL group. On the other hand, genotypes such as Nguzo gave a susceptible seedling response and corresponding adult plant susceptibility (Plate 1).

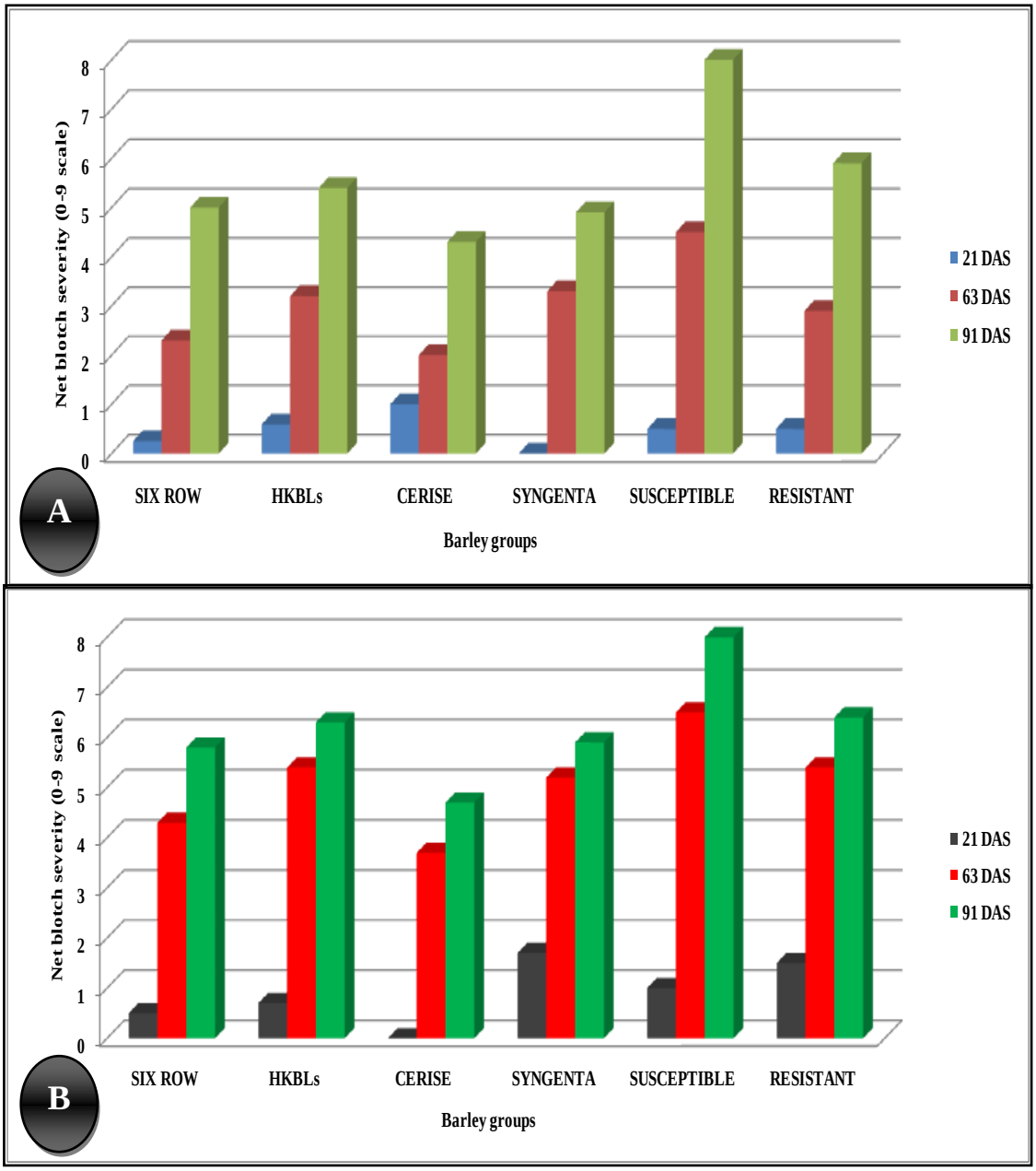

Figure 2: A- Seedling and adult plant response of grouped barley genotypes in Chepkoilel, B -Seedling and adult plant response of grouped barley genotypes to *P. teres* in Mau

Plate 1: a- Nguzo, b- Cerise Laurel showing reaction to disease at seedling stage (21days), c-Nguzo, d - Cerise Laurel showing reaction to disease at adult plant stage (63days) at Mau Narok site. At 21 days Nguzo (a) genotype had dot like lesions on leaves while Cerise Laurel (b) had no symptoms of the disease.

(Source: Author, 2013)

4.2 Morphological diversity of *P. teres* in barley zones

Morphological study indicated that there are five morphologically diverse strains of *P. teres* in Chepkoilel, Mau Narok and Njoro areas where barley is grown. The first group of isolates consisted of dark grey colony colour with cream concentric rings and a grey center. All isolates in this group had floccose colony texture with majority exhibiting sparse sporulation potential. However, all group one isolates had cylindrical conidia shape with inconspicuous scar at the tip except for one isolate from Njoro that produced abundant spores with conspicuous scar at the tip (Table 4, Plate 3)

Group two of the isolates had light grey mycelia with brown concentric ring and a grey center. Unlike group one, this group exhibited granular colony texture and all produced abundant spores. Also, most of the group two isolates had conspicuous scar but with cylindrical conidia just like group one. Only two isolates, one from Chepkoilel and the other from Njoro had inconspicuous scar (Table 4, plate 3).

Group three of the isolates had light grey colony colour but unlike group two, they exhibited brick red concentric ring and light grey centers. Similar to most of the group one, group three isolates had floccose colony texture and low sporulation potential. Majority of the isolates had conspicuous scar at the tip of the conidia and all had cylindrical conidia just like other groups (Table 4, Plate 3).

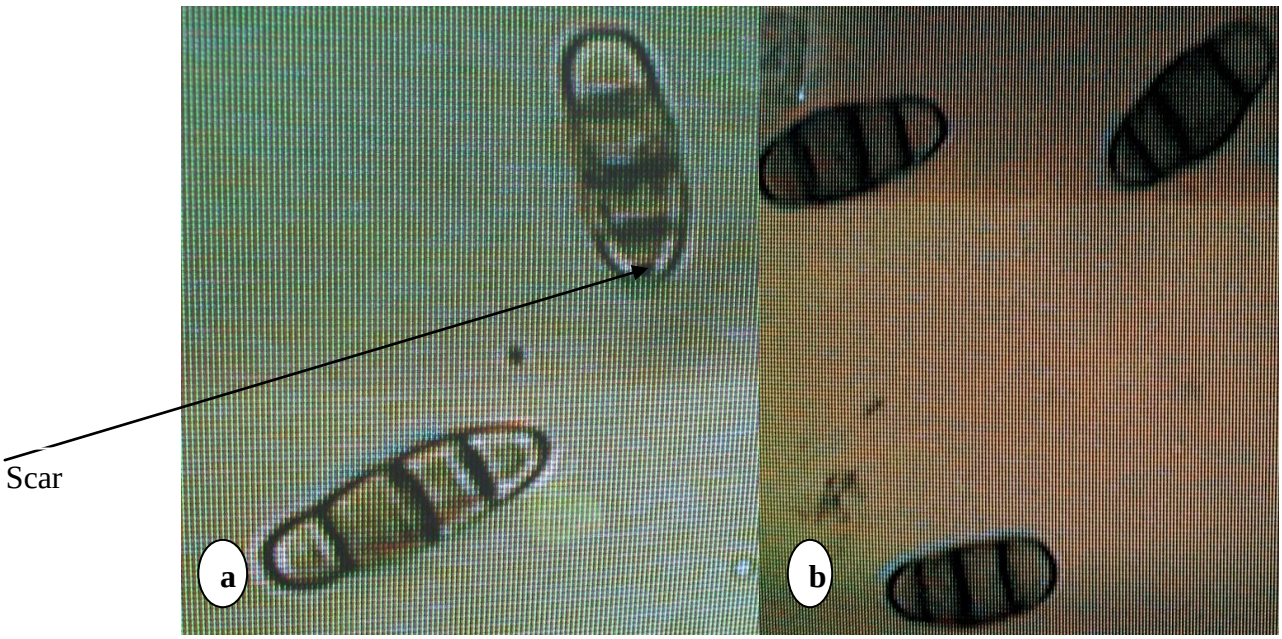

Plate 2. a- conidia with a conspicuous scar at one end and the other end rounded, b- Conidia with inconspicuous scar, both ends rounded.

(Source: Author, 2013)

Table 4: Description of *P.teres* isolates from infected barley tissue from Chepkoilel, Mau Narok and Njoro sites.

MORPHOLOGICAL GROUP	SOURCE	MYCELIA COLOUR/ RING	COLONY TEXTURE	SPORULATION	CONIDIA SHAPE
ONE	Chepkoilel	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	Chepkoilel	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	chepkoilel	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	Mau Narok	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	Mau Narok	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	Njoro	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
	Njoro	Dark grey ,cream concentric rings,grey center	Floccose	Abundant	cylindrical,conspicous scar
	Njoro	Dark grey ,cream concentric rings,grey center	Floccose	Sparse	cylindrical,inconspicous scar
TWO	Chepkoilel	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Chepkoilel	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,conspicous scar
	Mau Narok	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,conspicous scar
	Mau Narok	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,conspicous scar
	Njoro	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,conspicous scar
	Njoro	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Light grey,brown concentric center,grey center	Granular	Abundant	cylindrical,conspicous scar
THREE	Chepkoilel	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Chepkoilel	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Chepkoilel	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Mau Narok	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Njoro	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Njoro	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,conspicous scar
	Njoro	Light grey,brick red concentric ring, light grey center	Floccose	Sparse	cylindrical,inconspicous scar
FOUR	Chepkoilel	Black,no concentric ring, black center	Floccose	Abundant	cylindrical,inconspicous scar
	Chepkoilel	Black,no concentric ring, black center	Floccose	Abundant	cylindrical,conspicous scar
	Mau Narok	Black,no concentric ring, black center	Floccose	Abundant	cylindrical,conspicous scar
	Mau Narok	Black,no concentric ring, black center	Floccose	Abundant	cylindrical, inconspicous scar
	Mau Narok	Black,no concentric ring, black center	Granular	Abundant	cylindrical,conspicous scar
	Mau Narok	Black,no concentric ring, black center	Floccose	Abundant	cylindrical, inconspicous scar
	Mau Narok	Black,no concentric ring, black center	Granular	Abundant	cylindrical,conspicous scar
	Mau Narok	Black,no concentric ring, black center	Granular	Abundant	cylindrical,conspicous scar
	Mau Narok	Black,no concentric ring, black center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Black,no concentric ring, black center	Floccose	Sparse	cylindrical,inconspicous scar
	Njoro	Black,no concentric ring, black center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Black,no concentric ring, black center	Floccose	Sparse	cylindrical,inconspicous scar
FIVE	Chepkoilel	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Chepkoilel	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,conspicous scar
	Chepkoilel	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Mau Narok	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Mau Narok	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Mau Narok	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar
	Njoro	Grey,dark brown concentric ring,grey center	Granular	Abundant	cylindrical,inconspicous scar

The fourth group of *P. teres* isolated from different sites exhibited black mycelia mass with no concentric ring at all. Additionally, this group had of isolates had mixed colony texture from floccose to granular but most of the isolates produced abundant spores except for two Njoro isolates that produced sparse spores. All of the morphologies in this group had cylindrical conidia shape but majority produced inconspicuous scar (Table4, Plate 3). Contrary to the four other groups, group four of the isolates exhibited the slowest growth rate on culture media despite the fact that they were all subjected to the same growth conditions of temperature, media strength and light intensity (Plate 3).

Group five is the last diverse group of isolates which had grey mycelia colour with dark brown concentric ring and a grey center. However, this group resembled group two in terms of granular colony texture and production of abundant spores on growth media. The group produced cylindrical conidia with majority exhibiting inconspicuous scar (Table 4).

Apart from diversity in terms of upper surface of the colony, the five major groups of *P. teres* isolates from different sites varied in their substrate (under surface) colour and conidia sizes (Plate 3). For instance, group one had the smallest spore size while group three had the largest spores. However, all conidia from different groups had the same number of septation which gave four segments per conidia (Plate 3.)

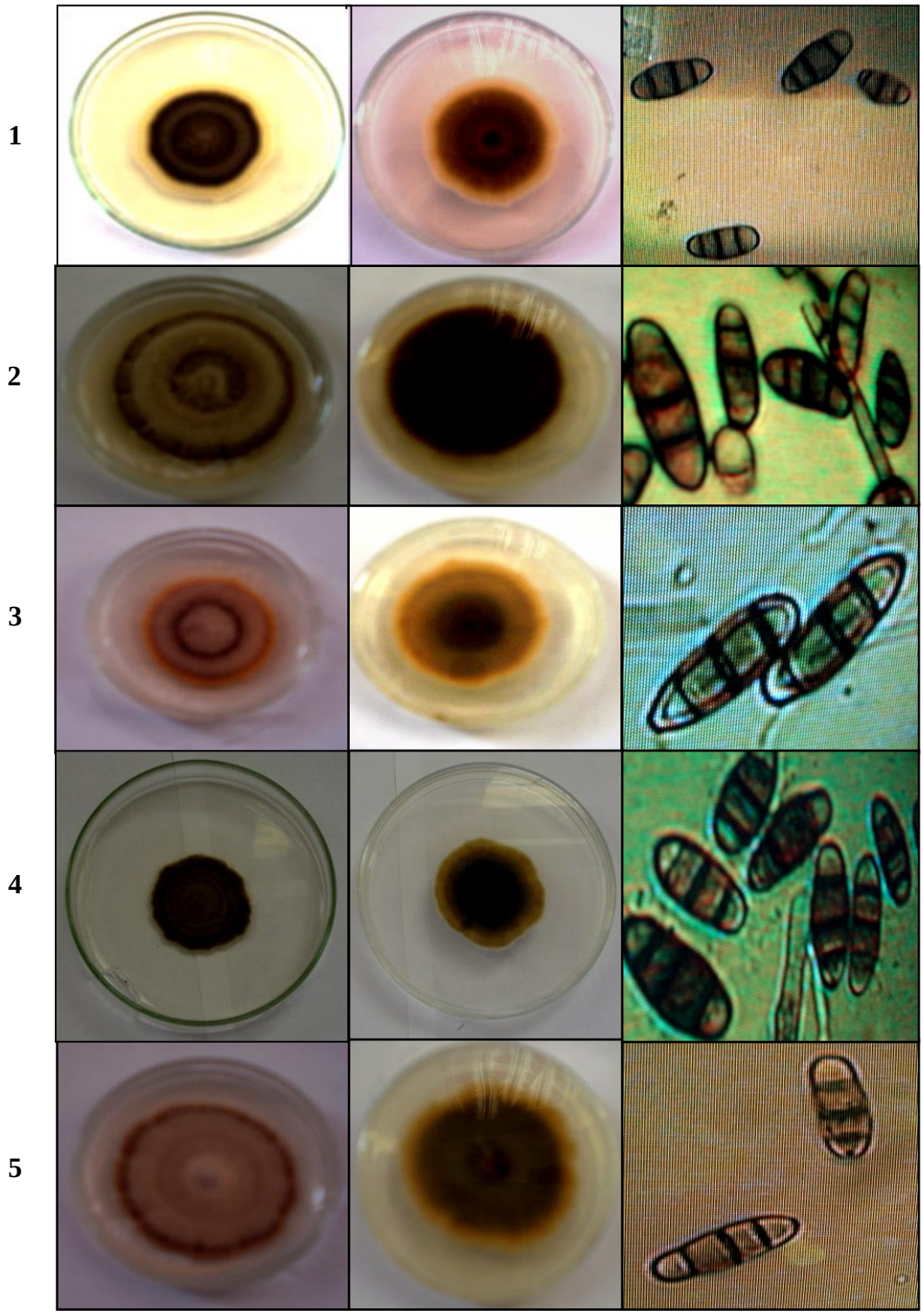

Plate 3: The five different isolates of *P. teres* from different sites; Isolate 2 is the typical *P. teres* which evolved into 4 sub-species

(Source: Author, 2013)

4.3 Virulence of *P. teres* isolates on barley genotypes inoculated in the screen house

4.3.1 Response of barley genotypes to *p. teres* isolates

Advanced barley genotypes showed significant differences ($p < 0.05$) in terms of net blotch reaction. However, these observations did not differ much from those of the field screening (Table 5, Appendix III).

Table 5: Response of advanced barley genotypes to five isolates of *P. teres* under screenhouse conditions

GENOTYPE GROUPING	GENOTYPE	MORPHOLOGICAL GROUPS					Genotype mean	Group mean
		One	Two	Three	Four	Five		
SIX ROW BARLEY	54 CAN	5.1	4.1	4.8	5.6	4.6	4.8	5.2
	77 Aloe	6.2	5.4	5.9	6.4	5.7	5.9	
	NBD Petunia	5.4	4.4	4.9	5.4	4.4	4.9	
	Gloria Bar	5.6	4.6	5.1	5.6	4.6	5.1	
HKBLs	HKBL 1385-12	5.8	5.1	5.3	5.6	4.8	5.3	5.6
	HKBL 1386-2	7.6	6.1	6.8	7.6	6.1	6.8	
	HKBL 1512-5	6.0	5.2	5.7	6.2	5.5	5.7	
	HKBL 1539-4	5.0	3.5	4.5	5.5	4.0	4.5	
	HKBL 1591-3	6.6	5.6	6.1	6.6	5.6	6.1	
	HKBL 1629-19	5.9	5.1	5.6	6.1	5.4	5.6	
	HKBL 1674-4	4.5	4.3	4.5	4.8	4.5	4.5	
	HKBL 1719-1	5.8	5.3	5.5	5.8	5.3	5.5	
	HKBL 1719-18	6.7	6.2	6.4	6.7	6.2	6.4	
SYNGENTA VARIETIES	Publican	5.2	4.7	5.2	5.7	5.2	5.2	5.3
	Quench	5.7	5.4	5.4	5.4	5.2	5.4	
KARI/KML INTRODUCTION	Cerise	4.0	3.2	3.7	4.2	3.5	3.7	3.7
SUSCEPTIBLE CHECK	Sabini	8.7	7.4	7.9	8.4	7.2	7.9	7.5
	Karne	7.4	6.6	7.1	7.6	6.9	7.1	
RESISTANT CHECK	HKBL 1385-13	4.0	3.0	3.5	4.0	3.0	3.5	4.0
	Nguzo	4.1	5.2	4.6	3.9	5.1	4.6	
	MEAN	5.7	5.0	5.4	5.8	5.1	5.4	

	Genotype	Source	Morph grp	Genotype X Source	Genotype X Morph grp
S.E	0.2022	0.1011	0.1011	0.4521	0.4521
S.E.D	0.286	0.1238	0.143	0.5538	0.6394
F. probability	< 0.001	< 0.001	< 0.001	0.995	0.999
% C.V	11.8				

For instance, under controlled environment of screenhouse and inoculation with known isolates of the pathogen, only 54 CAN, NBD Petunia, HKBL 1539-4, HKBL 1674-4,Publican, Cerise, HKBL 1385-13 and Nguzo genotypes expressed moderate resistance to net blotch disease. These reactions are almost similar to the field observations except for Nguzo variety which was very susceptible to *P.teres* under field conditions. The rest of the barley genotypes exhibited responses ranging from moderate susceptible to susceptible reactions with all the susceptible checks (Sabini and Karne) expressing their expected responses (Plate 4). In addition, the resistant checks also gave expected resistant response to the disease under the screenhouse conditions.

4.3.2 Grouped barley response to *P. teres* isolates in the screenhouse

Like the genotype responses to net blotch, group responses to the disease were similar to resembled observed field reactions. For example, among the groups, the six row barley, HKBLs, Syngenta and Cerise gave severity averages of 5.2, 5.6, 5.3 and 3.7 respectively under screenhouse.

In all successful infection by *P. teres*, yellowing of leaves was the first observation on the fourth day after inoculation. These gradually turned into brownish, purplish or black blotches taking the net shapes. The colour of the symptoms produced varied from one genotype to the other even with different morphological groups. For instance, Quench variety produced black coloured symptoms with most lesions taking spot forms. On the other hand, Karne produced brownish and elongated net forms of blotches with chlorotic zones and this was common with all morphologies (Plate 4).

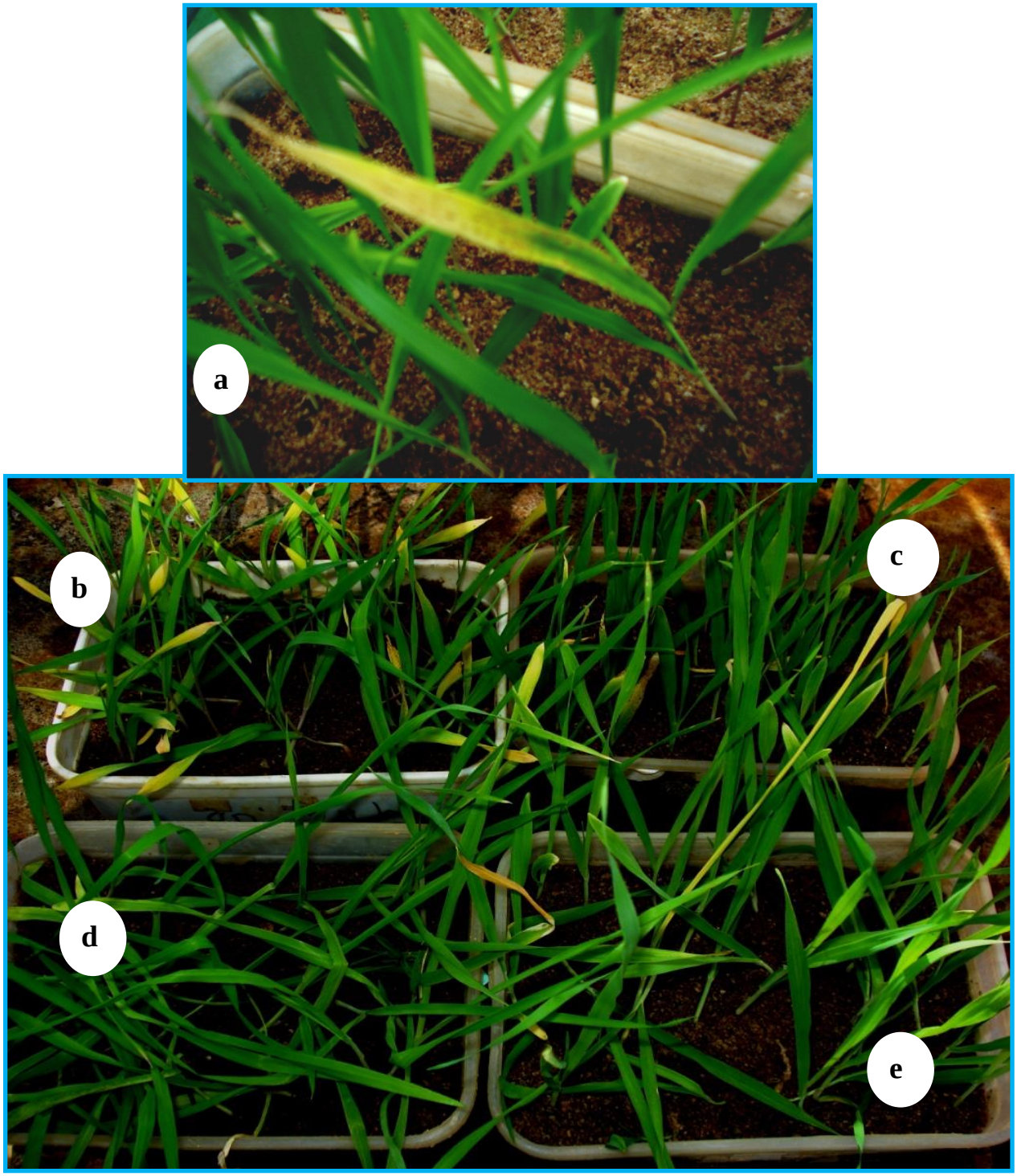

Plate 4. Response of barley lines to infection by *P. teres* in the screenhouse by the 7th day after inoculation. a- Karne(S), b-Sabini (S), c-Publican(MR), d-Cerise (R), e-54 CAN(MR).

4.3.3 Virulence and pathogenicity of morphologically diverse isolates of *P. teres*

The five morphological groups of *P. teres* gave significant differences ($p < 0.05$) in terms of virulence on advanced barley genotypes under the screenhouse conditions. Isolates in morphological group four were the most virulent while group two whose morphology resembles the typical *P.teres* were less virulent on average. However, the virulence data indicates that all the sub-species that evolved from isolate two were generally more virulent than the original species (isolate two) (Table 5, Appendix IV).

Some genotypes exhibited high susceptibility to one morphological group but tolerant response to the other groups. For example, on average, Cerise Laurel genotype was rated 3.5 to group five while the same genotype scored 4.2 to group four. However, some genotypes gave almost the same response across all morphological groups for example Syngenta varieties (Quench and Publican) and HKBL 1674-4 among others (Table 5).

All the isolates were more pathogenic to Sabini with isolate one recording the highest severity of 8.7, causing most of the tissues to dry by the 14^{th} day. Isolate four was the second most pathogenic on Sabini variety after isolate one. However, a contrasting observation was made on Karne variety where isolate four was the most pathogenic followed by isolate one. However, isolates two and five recorded the least pathogenicity on Karne variety (Table 5).

4.3.4 Effect of genotype x isolate source interactions on virulence of *P. teres*

Other than diverse responses to the five morphological groups, grouped barley genotypes as well as the individual genotypes reacted differently to the isolates based on the source/sites from which they were obtained (Table 6).

Table 6: Virulence of *P.teres* isolates from three areas (sources) in Kenya where barley is grown on advanced barley genotypes. Scale 0-9

GROUP	GENOTYPE	VIRULENCE BASED ON ISOLATE SOURCES			GENOTYPE MEAN	GROUP MEAN
		Chepkoilel	Mau Narok	Njoro		
SIX ROW	54 CAN	5.3	4.8	4.0	**4.7**	**5.0**
	77 Aloe	6.0	6.3	5.0	**5.8**	
	NBD Petunia	5.5	5.0	3.5	**4.7**	
	Gloria Bar	5.5	5.0	4.5	**5.0**	
HKBLs	HKBL 1385-12	5.8	5.5	4.0	**5.1**	**5.5**
	HKBL 1386-2	7.3	6.8	6.0	**6.7**	
	HKBL 1512-5	6.0	5.8	5.0	**5.6**	
	HKBL 1539-4	5.0	4.5	3.5	**4.3**	
	HKBL 1591-3	6.5	6.0	5.5	**6.0**	
	HKBL 1629-19	6.0	5.8	4.5	**5.4**	
	HKBL 1674-4	4.8	4.5	4.0	**4.4**	
	HKBL 1719-1	5.8	5.8	4.5	**5.3**	
	HKBL 1719-18	6.8	6.3	6.0	**6.3**	
KARI-KML INTRODUCTION	Cerise	4.0	3.8	3.0	**3.6**	**3.6**
SYNGENTA	Publican	5.5	5.5	4.0	**5.0**	**5.1**
	Quench	6.0	5.3	4.5	**5.3**	
SUSCEPTIBLE CHECKS	Sabini	8.5	7.8	7.0	**7.8**	**7.3**
	Karne	7.5	7.3	6.0	**6.9**	
RESISTANT CHECKS	HKBL 1386-13	4.0	3.5	2.5	**3.3**	**3.8**
	Nguzo	4.9	5.0	3.0	**4.3**	
	SOURCE MEAN	**5.8**	**5.5**	**4.5**	**5.3**	

	Genotype	Source	Morph grps	Genotype x Source	Genotype x Morph grps
S.E	0.2022	0.1011	0.1011	0.4521	0.4521
S.E.D	0.286	0.1238	0.143	0.5538	0.6394
F.probability	< 0.001	< 0.001	< 0.001	< 0.001	0.999
% C.V	11.8				

However, the interactive effect of source of inoculum and genotypes was not significant ($p > 0.05$). For instance, based on sites from which the isolates were obtained (source), Chepkoilel isolates were the most virulent followed by Mau Narok while those from Njoro had less virulent *P. teres* isolates but these differences were significant ($p < 0.05$) (Appendix V).

CHAPTER FIVE

DISCUSSIONS

5.1 Field response of advanced barley genotypes to *P. teres*

Barley genotypes evaluated for their response to net blotch gave varied responses. The genotypes were ranked moderately resistant, moderately susceptible and susceptible based on the intensity of the symptoms on the leaves. The difference in the response for the genotypes towards net blotch in different sites could be due to the differences in environmental conditions during the experimentation period (Appendix VI). Owing to higher temperatures (25°C), coupled with high precipitation, Mau Narok had higher relative humidity, conditions which favour spore production and multiple infection of growing barley plants. Since moisture is also an essential requirement in spore germination during the initial disease infection and the consequent disease progress, higher amounts of rainfall in Mau Narok could have contributed to the higher disease levels in Mau Narok. Temperature affects the number of spores formed in a unit plant area and the number of spores released in a given time period (Agrios, 2005). Maximum spore production has been reported to occur at 25°C and at a high relative humidity (Kosiada, 2008). These conditions may have contributed to the observed significant variation in disease response in the two sites. Other than temperature and relative humidity, light intensity and duration during the incubation period in *P.teres* (Kosiada, 2008) may have contributed to the observed significant variation in disease response by the different genotypes at the two sites.

The observed differences in responses of the various genotypes evaluated also showed that the genotypes were genetically diverse and that their response to net blotch may be under the control of different resistance genes. Resistance to net blotch have been reported to be under

the regulation of more than one gene (Arabi *et al.*, 2003; Liu *et al.*, 2011) which may have conditioned their response to the disease. Besides, the difference in the resistance genes in the genotypes, responses may also have been due to induced defence by biochemical substances such as phytoalexins that are antimicrobial in nature. The phytoalexins are produced by healthy cells adjacent to the point of infection in response to materials diffusing from the necrotic cells (Agrios, 2005). The moderate resistance observed in genotypes such as Cerise Laurel may be due to the production of phytoalexins in concentrations which are sufficient to restrict disease development. However, in susceptible group of genotypes such as Karne and Sabini the production of the phytoalexins may have been prevented by toxin suppressor molecules that are produced by the pathogen during infection. These toxins are reported to be responsible for symptoms observed in susceptible genotypes (Sarpeleh *et al*., 2007). However, the mechanisms by which phytoalexin production, phytoalexin suppressors, gene for resistance or susceptibility and expression of resistance and susceptibility are linked is not well known.

In this study, a moderately susceptible response was observed in the resistant group of genotypes (HKBL 1386-13 and Nguzo). This observation was due to a highly susceptible response observed in Nguzo, contrary to its known trait, that it is resistant to net blotch. This observation could be attributed to the fact that some form of resistance are environment - specific and may only be expressed in one environment and not the other. Cromey and Parks, (2003) in their study showed that a variety with resistance can be effective against virulence of an entire pathogen population in an area, but ineffective against a pathogen population in another area. Thus, the resistance reported in Nguzo might have only been

effective in a pathogen population within the area in which the observations were made. The presence of environment - specific genetic resistance was also observed in another study and was attributed to the existence of multiple resistance loci in different varieties (Grewal *et al.*, 2008). Furthermore, the loss of resistance in this genotype may be due to the increased exposure of the same resistance genes to the present pathogen population. This encourages the pathogen population to overcome the resistance genes by producing pathotypes that are more virulent to an extent that they are no longer effective against the pathogen. Therefore the observation made in this genotype could be due to the existence of different pathogen populations within the fields of study. Sometimes, the same variety can show substantial differences in resistance level when different pathogen populations exist (Jørgensen *et al*., 2000).This indicates that the pathogen may differ in composition and that successful breeding efforts require the use of a pathogen population composed of several or many local subpopulations. *Pyrenophora teres* has the ability to undergo sexual reproduction and this may cause an increase in frequency of pathotypes that have the ability to adapt to the changes in the genetic makeup of the host population (Statkevičiūtė *et al*., 2010). Such pathotypes can also increase in frequency due to the influence of selective pressure from growing resistant varieties (McDonald and Linde, 2002). The breakdown of resistance in variety Nguzo may also have been caused by a heavy spore - load in the sites with increased inoculum potential.

The significant difference in terms site and variety to ear infection indicates that the genotypes have different resistance mechanisms towards foliar and ear infection. It was further noted that a genotype may be resistant to foliage infection but susceptible to ear infection. For instance, six row type genotypes (54 CAN, 77Aloe, NBD Petunia and Gloria

bar) had a moderately resistant response of foliar infection with a moderately resistant response on ear infection. The increased level of ear infection in six row genotypes may be due to the increased sensitivity of the genotypes to environmental changes, observed at early dough stage of the crop growth (Appendix VII). Higher amounts of rainfall observed in both sites at early dough stage period may have caused a rise in moisture levels in the host plants. Since moisture is important in germination of spores and penetration of the host, this may have increased the susceptibility of the host plants, thus, an increased infection by the pathogens at this stage of growth (Agrios, 2005). A low level of ear infection in genotypes that gave a highly susceptible response in the foliage such as susceptible group and Nguzo indicates that genotypes may have had some form of quantitative resistance that was only effective at that point of crop development. Such forms of resistance have also been reported by Steffenson *et al*., (1996) and Robinson and Jalli, (1997).

A reduction in thousand kernel weight observed in infected kernels shows that the disease plays a significant role in the final yield of all barley genotypes. This decrease may have been due to the decrease in the photosynthetic capacity early in the season, leading to a decrease in the carbohydrates level in the kernel. Horsley and Hochhalter (2004) reported a decrease in kernel weight due infection by net blotch disease. Other studies that indicated decrease in thousand kernel weight due to net blotch disease attributed the losses to the percentage leaf area affected by the disease with emphasis on the three top leaves (Jayasena *et al*., 2007; Jebbouj and Youfsi, 2009). However, the overall thousand kernel weight for each genotype seems to vary with the genotype and are not dependant on the disease levels alone.

The significant interaction between genotype and environment (site) may be associated with the different growing conditions of the plants and the variable amounts of inoculum that resulted in different severity levels of net blotch in the two sites. The difference in severity levels in different sites may also have been influenced by the presence of different pathogen isolates in the sites (Jørgensen *et al*., 2000). A significant interaction between genotype and environment has also been explained to affect the level of net blotch epidemics in different experimental locations (Cherif *et al*., 2010). For each genotype, the levels of disease severity may depend on growth stage at infection, the environmental factors and the degree of virulence of the pathogen (inoculum). Environmental effects on the level of disease severity only remain significant until the genotypes reach flowering stage, after which, the environment have no effect on disease severity and disease progress (El Yousfi and Ezzahiri, 2001). Thus, the observed progress after flowering in some genotypes such as Sabini may be due to the virulence of the pathogen and not the effect of the environment. The varied response of different genotypes to infection by *P.teres* may also be due to existence of distinct host-pathogen interaction which is specific and genetically controlled and may render a given pathotype virulent to one genotype and avirulent to the other.

Barley genotypes exploit different mechanisms of resistance to defend themselves against different pathogens, including *P.teres*. Quantitative and qualitative resistance have been reported to be the major forms of resistance in barley (Friesen *et al*., 2006; Robinson and Jalli, 1997). In this study, resistance in the genotypes was observed to vary at different sites and at different crop growth stages. This indicated that resistance in these genotypes is majorly quantitative and is thus affected by changes in environment and may only be effective at a given stage of crop growth and developmental stage (Agrios, 2005). For

instance, six row type (54 CAN and NBD Petunia), Syngenta (Publican and Quench) groups of genotypes exhibited seedling susceptibility at Mau Narok site and some level of resistance at adult stage. However, the same genotypes showed seedling resistance at Chepkoilel site, showing that resistance of genotypes may vary at different growth stages and at different sites. Cultivars that are susceptible to *P.teres* at seedling stage but exhibit resistant reactions at adult stage have also been reported in another study (Scott, 1992). Genotypes such as Sabini showed seedling resistance at both sites but a high susceptibility at adult stage and this suggests that resistance genes in this genotype are only effective at seedling stage of plant growth and not effective at adult plant stage of growth. Williams *et al.* (2003) reported resistance genes that were only effective at seedling stage of growth and such genes may also exist in these genotypes. Some genotypes such as NBD Petunia with seedling resistance had a corresponding lower disease levels at adult plant stage and this may be due to the presence of resistance genes that are effective at both growth stages (Cakir *et al*., 2003; Grewal *et al*., 2008). Resistance to net blotch has been reported to be maintained or improved as the plant matures (Gupta *et al.*, 2003.; Tekauz, 2000). However, Douiyssi *et al.,* (1998) reported that resistance to some isolates decrease with plant age.

5.2 Morphological diversity of *P. teres*

The observed variations in morphology in terms of mycelia colour and colony morphologies in *P.teres* isolates grown on PDA are indications that *P.teres* has several sub species across the barley growing zones – Chepkoilel, Mau Narok and Njoro. Such variations in mycelia colour and colony morphologies have also been observed in other studies with isolates from different geographical regions (Frazzon *et al*., 2002; McDonald, 1967). The existence of

different colony morphologies of isolates from one area was also observed. For instance, all groups had a mixture of isolates from all the three sampling areas making it difficult to group the isolates on the basis of morphologies in terms of area of origin. This could be due to the presence of different pathogen strains in each area. The variation in morphologies within an area has also been reported in other studies (Lehmensiek *et al.*, 2010; McDonald, 1967; Shipton *et al*., 1973) and has been attributed to the fact that the pathogen undergoes evolutionary changes to produce pathotypes that can overcome host resistance. Evolution of pathogens has been reported to majorly occur in cases where the numbers of host species are increased through breeding for resistant varieties of the same host (Cromey and Parks, 2003; Wu *et al*., 2003). Tekauz (1990) also suggested that variability of a population of *P.teres* depend on the number of barley cultivars examined and the differences in their genetic background. The different strains could be due to the ability of *P.teres* pathogen to reproduce sexually thus enabling the pathogen to adapt to changes in genetic makeup of the host plants (Statkevičiūtė *et al*., 2010). This diversity may also explain the variations observed in the response of the different genotypes to the pathogen in the field. This response may be due to the existence of distinct host- pathogen interactions that occur in the presence of different races of a pathogen (Liu *et al*., 2011).

Similarities in *P.teres* strains from the three sites suggest that environmental factors may play little role in the evolutionary processes of this pathogen. The similarities observed among the isolates in the three sampling areas may also be due to the transfer of infected seeds from one area to the other thus transferring the pathogen strains of the same ancestor to the area.

On the other hand, the conidia were straight, cylindrical and round at both ends (Liu *et al*., 2011) in all isolates, an indication that there may be differences in other morphological features but not in the conidial shapes. The number of pseudosepta was four in all isolates while other studies have reported 4 - 6 to be the common number of pseudosepta in isolates from different regions (Liu *et al*., 2011; Smedegard-Petersen, 1971).

During this study, it was also noted that there is a relationship between the colony texture and sporulation potential of the grouped isolates. Granular texture observed in group two, five and some isolates in group four may be associated with abundant sporulation and development of sclerotium – like bodies which project from the surface of the agar (Scott, 1991).

5.3 Virulence and pathogenicity of *P. teres* isolates

In this study, all genotypes developed symptoms following inoculation and so were not resistant to all groups of isolates. However, there were differences in the degree to which the disease developed on the different host genotypes ranging from moderately resistant to susceptible. The *P.teres* isolates evaluated in this study did not show the same reaction pattern across the barley genotypes. This may be due to the diversity of the pathogen or the genetic diversity of the genotypes. Virulence diversity of *P. teres* pathotypes to different genotypes has been reported in different studies (Gupta and Loughman, 2001; Tekauz, 1990; Wu *et al*., 2003). These were attributed to the specificity of host - pathogen interaction.

The significant difference in reactions by different genotypes to the five isolates of *P. teres* may also suggest that the isolates used were genetically different and exhibit different

interaction mechanisms with the genotypes. The major factors that may have contributed to these reactions include the virulence factors such as virulence genes used to induce infection, avirulence genes recognized by the host to induce resistance or a combination of the two mechanisms. The varied virulence levels suggest that the pathogen may exist as specialised pathological races or pathotypes; this implies that such races may exist in pathogen populations in barley production areas in Kenya. However, further studies on more populations containing larger numbers of isolates would be required to confirm the existence of such pathotypes. Diverse pathotype populations have been reported in other studies. For instance, Tekauz, (1990) evaluated a set of Canadian lines and identified 20 different pathotypes based on the reaction of 12 differential lines; 13 pathotypes were identified using 22 differential lines in California (steffenson and Webster, 1992). In Western Australia, Gupta and Loughman, (2001) reported 4 pathotypes using 47 differential lines. Similarly, 4 pathotypes were identified using four differentials in Egypt (El-Fahl *et al*., 1982).

The diversity of pathotypes reported is likely to be the result of sexual reproduction. The relationship between sexual reproduction as it affects the virulence and production of pathotypes has not been reported specifically in *P.teres*. However, pathogens with sexual reproduction as part of their lifecycle combined with asexual reproduction pose greater risk in overcoming resistance genes compared to strictly asexual or strictly sexual pathogens (McDonald and Linde, 2002). *P.teres* meets these criteria. Sexual reproduction impact pathotype generation through mutations that affect virulence and reshuffling of genes during meiosis, which increases the probability of creating new virulence pathotypes (McDonald

and Linde, 2002). This may explain higher virulence observed in isolate four compared to other isolates.

The existence of the different pathogen strains with varied virulence levels also confirms the variation observed in the response of genotypes during field screening. This further explains the existence of specific host - pathogen interaction, where a host response is determined by a successful interaction between the host resistance genes and the corresponding avirulence genes in the pathogen. The specificity of host- pathogen interaction has been reported by (Douiyssi *et al.*, 1998; Gupta and Loughman, 2001; Jalli and Robinson, 2000).

Most of the genotype groups gave nearly corresponding responses to those of the field screening. However, the higher virulence observed in isolate four, may further indicate that this isolate may have undergone changes in its genetic makeup making it more virulent than others. Some genotype groups such as Syngenta group of genotypes, six row group and HKBL group gave almost the same response across all the isolates, indicating that despite the difference in morphological characteristics, some isolates may have some common genetic characteristics. The possible genetic similarity in morphologically different isolates have also been reported and associated to the same descent of the isolates (Frazzon *et al*., 2002; Goodwin *et al*., 1993).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Given the response of screened barley groups, Cerise, Syngenta, HKBLs and Six row types it can be concluded that these groups contain some resistance genes that could be optimized and be incorporated into breeding programmes. However, the resistance genes in most of these genotypes seem to be effective only at certain plant growth stages since some genotypes such as Sabini exhibited resistant response at seedling stage with a highly susceptible response at adult stage. The effects of such genes are usually dependent on environment and the genotypes under the control of such genes are likely to vary in their response in different environments.

- The five morphologically different groups of *P.teres* obtained indicates that there are different strains of the pathogen in the three barley growing zones Chepkoilel, Mau Narok and Njoro and more strains may be found in populations from other barley growing zones since this study only covered a section of the growing areas. These further aids in designing breeding objectives aimed at managing this disease.

- The virulence of *P.teres* is influenced by differences in strains of the pathogen and the environment of the source of inoculum. This is an indication that a genotype may be resistant to one strain of the pathogen in one environment but very susceptible to another strain in another environment or the same environment.

6.2 Recommendations and way forward

- There is need to establish molecular basis of the observed responses both in the field and screenhouse. This will assist in studying the relationship that exists between the observed responses and the genetic constitution of the genotypes. Multiple location studies using the same genotypes is also required to confirm the responses of the genotypes in other environments since environment was found to play a major role in the reaction of some of these genotypes.

- Since the pathogen has been observed to have pathotypes, for successful breeding efforts, there is a need to perform studies on resistance using pathogen populations composed of several or many local subpopulations. This will also assist in generation of genotypes that can overcome the challenges experienced by breeders in cases of new pathotypes.

- Based on the morphological differences and the virulence responses observed in isolates studied, further studies need to be conducted to ascertain the genetic diversity of the isolates and how the genes in these isolates interact with the genes in the host genotypes to give the observed responses.

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APPENDICES

Appendix I*:* Anova table for net blotch severity under field conditions on a scale of 0-9

Variate: Net_Blotch Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.65	0.325	1.03	
Site	1	14.7	14.7	46.45	<.001
Variety	19	160.1333	8.4281	26.63	<.001
Contrast 1: Six row Vs HKBLs	1	2.2012	2.2012	6.96	0.01
Contrast 2: Susceptible checks Vs Resistant checks	1	22.0417	22.0417	69.65	<.001
Contrast 3: Susceptible checks Vs Syngenta varieties	1	42.6667	42.6667	134.83	<.001
Contrast 4: Resistant checks Vs HKBLs	1	0.3039	0.3039	0.96	0.33
Contrast 5: Susceptible checks Vs HKBLs	1	51.3778	51.3778	162.36	<.001
Contrast 6: Susceptible checks Vs Six row barley	1	55.125	55.125	174.2	<.001
Contrast 7: Resistant checks Vs Six row barley	1	4.0139	4.0139	12.68	<.001
Contrast 8: Resistant checks Vs Syngenta varieties	1	3.375	3.375	10.67	0.002
Contrast 9: 13 Series Vs 15 Series	1	0.4444	0.4444	1.4	0.24
Contrast 10: 16 Series Vs 17 Series	1	6	6	18.96	<.001
Contrast 11: 13 Series Vs 17 Series	1	2.9389	2.9389	9.29	0.003
Contrast 12: 15 Series Vs 16 Series	1	2.45	2.45	7.74	0.007
Contrast 13: Six row barley Vs Syngenta varieties	1	0.0139	0.0139	0.04	0.835
Site.Variety	19	10.6333	0.5596	1.77	0.042
Site.Contrast 1	1	0.0583	0.0583	0.18	0.669
Site.Contrast 2	1	0.375	0.375	1.19	0.28
Site.Contrast 3	1	1.5	1.5	4.74	0.032
Site.Contrast 4	1	0.3039	0.3039	0.96	0.33
Site.Contrast 5	1	1.8778	1.8778	5.93	0.017
Site.Contrast 6	1	1.125	1.125	3.56	0.063
Site.Contrast 7	1	0.125	0.125	0.4	0.532
Site.Contrast 8	1	0.375	0.375	1.19	0.28
Site.Contrast 9	1	0.1111	0.1111	0.35	0.555
Site.Contrast 10	1	0.6667	0.6667	2.11	0.151
Site.Contrast 11	1	0.1389	0.1389	0.44	0.51
Site.Contrast 12	1	0.05	0.05	0.16	0.692
Residual	78	24.6833	0.3165		
Total	119	210.8			

Appendix II: Anova table for ear infection under field conditions scored on a scale of 0-9

Variate: Ear infection Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.2667	0.6333	1.72	
Site	1.0	21.7	21.675	58.84	<.001
Variety	19.0	577.1	30.3732	82.45	<.001
Contrast 1: Six row Vs HKBLs	1.0	231.5	231.525	628.5	<.001
Contrast 2: Susceptible checks Vs Resistant checks	1.0	1.0	1.0417	2.83	0.097
Contrast 3: Susceptible checks Vs Syngenta varieties	1.0	0.2	0.1667	0.45	0.503
Contrast 4: Resistant checks Vs HKBLs	1.0	59.6	59.5589	161.68	<.001
Contrast 5: Susceptible checks Vs HKBLs	1.0	31.8	31.8028	86.33	<.001
Contrast 6: Susceptible checks Vs Six row barley	1.0	238.3	238.3472	647.02	<.001
Contrast 7: Resistant checks Vs Six row barley	1.0	276.1	276.125	749.57	<.001
Contrast 8: Resistant checks Vs Syngenta varieties	1.0	0.4	0.375	1.02	0.316
Contrast 9: 13 Series Vs 15 Series	1.0	44.4	44.4444	120.65	<.001
Contrast 10: 16 Series Vs 17 Series	1.0	24.0	24	65.15	<.001
Contrast 11: 13 Series Vs 17 Series	1.0	2.7	2.6889	7.3	0.008
Contrast 12: 15 Series Vs 16 Series	1.0	1.1	1.0889	2.96	0.09
Contrast 13: Six row barley Vs Syngenta varieties	1.0	253.1	253.125	687.14	<.001
Site.Variety	19.0	1.8	0.0961	0.26	0.999
Site.Contrast 1	1.0	0.1	0.0964	0.26	0.61
Site.Contrast 2	1.0	0.0	0.0417	0.11	0.738
Site.Contrast 3	1	0	0	0	1
Site.Contrast 4	1	0.2155	0.2155	0.58	0.447
Site.Contrast 5	1	0.4694	0.4694	1.27	0.262
Site.Contrast 6	1	0.6806	0.6806	1.85	0.178
Site.Contrast 7	1	0.3472	0.3472	0.94	0.335
Site.Contrast 8	1	0.0417	0.0417	0.11	0.738
Site.Contrast 9	1	0.1111	0.1111	0.3	0.584
Site.Contrast 10	1	0	0	0	1
Site.Contrast 11	1	0.0889	0.0889	0.24	0.625
Site.Contrast 12	1	0	0	0	1
Residual	78	28.7333	0.3684		
Total	119	630.5917			

Appendix III: Virulence of *P.teres* isolates to different groups of barley genotypes

Variate: Virulence Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.0012	0.0012	0	
GENOTYPE	19	225.6237	11.8749	29.04	<.001
Contrast 1: Six row Vs HKBLs	1	1.3207	1.3207	3.23	0.077
Contrast 2: Susceptible checks Vs Resistant checks	1	120.7562	120.7562	295.34	<.001
Contrast 3: Susceptible checks Vs Syngenta varieties	1	48.4	48.4	118.37	<.001
Contrast 4: Resistant checks Vs HKBLs	1	40.592	40.592	99.28	<.001
Contrast 5: Susceptible checks Vs HKBLs	1	74.2017	74.2017	181.48	<.001
Contrast 6: Susceptible checks Vs Six row barley	1	72.075	72.075	176.28	<.001
Contrast 7: Resistant checks Vs Six row barley	1	17.6333	17.6333	43.13	<.001
Contrast 8: Resistant checks Vs Syngenta varieties	1	16.2563	16.2563	39.76	<.001
Contrast 9: 13 Series Vs 15 Series	1	0.8167	0.8167	2	0.163
Contrast 10: 16 Series Vs 17 Series	1	8.1	8.1	19.81	<.001
Contrast 11: 13 Series Vs 17 Series	1	6.75	6.75	16.51	<.001
Contrast 12: 15 Series Vs 16 Series	1	1.7633	1.7633	4.31	0.042
Contrast 13: Six row barley Vs Syngenta varieties	1	0.2083	0.2083	0.51	0.478
SOURCE	2	46.9394	23.4697	57.4	<.001
MORPH_GROUPS	4	21.8406	5.4602	13.35	<.001
GENOTYPE.SOURCE	38	7.0481	0.1855	0.45	0.995
Contrast 1.SOURCE	2	0.0596	0.0298	0.07	0.93
Contrast 2.SOURCE	2	0.3844	0.1922	0.47	0.627
Contrast 3.SOURCE	2	0.0375	0.0187	0.05	0.955
Contrast 4.SOURCE	2	0.9083	0.4541	1.11	0.336
Contrast 5.SOURCE	2	0.1817	0.0908	0.22	0.801
Contrast 6.SOURCE	2	0.1125	0.0562	0.14	0.872
Contrast 7.SOURCE	2	0.45	0.225	0.55	0.58
Contrast 8.SOURCE	2	0.1969	0.0984	0.24	0.787
Contrast 9.SOURCE	2	0.2667	0.1333	0.33	0.723
Contrast 10.SOURCE	2	0.025	0.0125	0.03	0.97
Contrast 11.SOURCE	2	0.4	0.2	0.49	0.616
Contrast 12.SOURCE	2	0.07	0.035	0.09	0.918
Contrast 13.SOURCE	2	0.0625	0.0312	0.08	0.927
GENOTYPE.MORPH_GROUPS	76	13.9719	0.1838	0.45	0.999
Contrast 1.MORPH_GROUPS	4	0.0875	0.0219	0.05	0.995
Contrast 2.MORPH_GROUPS	4	2.2656	0.5664	1.39	0.25
Contrast 3.MORPH_GROUPS	4	0.8125	0.2031	0.5	0.738
Contrast 4.MORPH_GROUPS	4	2.7251	0.6813	1.67	0.17
Contrast 5.MORPH_GROUPS	4	0.1083	0.0271	0.07	0.992
Contrast 6.MORPH_GROUPS	4	0.1042	0.026	0.06	0.992
Contrast 7.MORPH_GROUPS	4	2.8333	0.7083	1.73	0.155
Contrast 8.MORPH_GROUPS	4	0.4531	0.1133	0.28	0.892
Contrast 9.MORPH_GROUPS	4	0.3333	0.0833	0.2	0.935
Contrast 10.MORPH_GROUPS	4	0.125	0.0312	0.08	0.989
Contrast 11.MORPH_GROUPS	4	0.8333	0.2083	0.51	0.729
Contrast 12.MORPH_GROUPS	4	0.8167	0.2042	0.5	0.736
Contrast 13.MORPH_GROUPS	4	0.8542	0.2135	0.52	0.72
Residual	59	24.1237	0.4089		
Total	199	339.5488			

Appendix IV: Anova table for morphological group contrast

Variate: VIRULENCE Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.0012	0.0012	0	
GENOTYPE	19	225.6237	11.8749	29.04	<.001
SOURCE	2	46.9394	23.4697	57.4	<.001
MORPH_GROUPS	4	21.8406	5.4602	13.35	<.001
Contrast 1: One Vs Two	1	10.6945	10.6945	26.16	<.001
Contrast 2: Two Vs Three	1	3.507	3.507	8.58	0.005
Contrast 3: Three Vs Four	1	3.507	3.507	8.58	0.005
Contrast 4: Four Vs Five	1	10.6945	10.6945	26.16	<.001
GENOTYPE.SOURCE	38	7.0481	0.1855	0.45	0.995
GENOTYPE.MORPH_GROUPS	76	13.9719	0.1838	0.45	0.999
GENOTYPE.Contrast 1	19	5.8836	0.3097	0.76	0.745
GENOTYPE.Contrast 2	19	2.0086	0.1057	0.26	0.999
GENOTYPE.Contrast 3	19	2.0086	0.1057	0.26	0.999
GENOTYPE.Contrast 4	19	5.8836	0.3097	0.76	0.745
Residual	59	24.1237	0.4089		
Total	199	339.5488			

Appendix V: Anova table for isolate source contrast

Variate: VIRULENCE Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.0012	0.0012	0	
GENOTYPE	19	225.6237	11.8749	29.04	<.001
ISOLATE SOURCE	2	46.9394	23.4697	57.4	<.001
Contrast 1: Chepkoilel Vs Mau Narok	1	4.3891	4.3891	10.73	0.002
Contrast 2: Chepkoilel Vs Njoro	1	46.376	46.376	113.42	<.001
Contrast 3: Mau Narok Vs Njoro	1	26.0042	26.0042	63.6	<.001
MORPH_GROUPS	4	21.8406	5.4602	13.35	<.001
GENOTYPE.SOURCE	38	7.0481	0.1855	0.45	0.995
GENOTYPE.Contrast 1	19	2.7672	0.1456	0.36	0.992
GENOTYPE.Contrast 2	19	3.1448	0.1655	0.4	0.984
GENOTYPE.Contrast 3	19	4.9125	0.2586	0.63	0.866
GENOTYPE.MORPH_GROUPS	76	13.9719	0.1838	0.45	0.999
Residual	59	24.1237	0.4089		
Total	199	339.5488			

Appendix VI: **Weather data of Chepkoilel and Mau Narok sites during field**

TIME (MONTHS)	TEMPERATURE (°C)				PRECIPITATION (mm)	
	Chepkoilel		Mau Narok		Chepkoilel	Mau Narok
	Min	Max	Min	Max		
January	11	24	11	27	35	30
February	10	25	11	28	21	30
March	11	25	12	28	63	62
April	12	24	13	26	105	119
May	11	23	13	25	85	106
June	11	22	12	24	91	73
July	10	22	12	24	147	83
MEAN	**10.9**	**23.6**	**12**	**26**	**78**	**71.9**
August	10	22	12	24	123	97
September	10	23	11	26	51	73
October	11	24	11	25	36	83
November	12	23	12	24	50	100
December	11	23	11	26	20	61
MEAN	**10.8**	**23**	**11.4**	**25**	**56**	**82.8**

Appendix VII: Mean reaction of 20 barley genotypes to 43 isolates of *P.teres* in the screen **house**

ISOLATE	HKBL 1629-19	54 CAN 77 ALOE	CERISE	GLORIA BAR	HKBL 1385-12	HKBL 1386-13	HKBL 1386-2	HKBL 1512-5	HKBL 1539-4	HKBL 1591-3	HKBL 1674-4	HKBL 1719-1	HKBL 1719-18	KARNE	NBD PETUNIA	NGUZO	PUBLICAN	QUENCH	SABINI	Mean
C1G1	5	5	6	3	5	5	3	7	6	5	6	4	5	7	7	5	4	5	5	8
C2G1	6	5	6	4	5	7	3	6	6	5	6	4	5	7	7	6	4	6	5	7
C3G1	6	4	7	4	6	5	3	7	6	5	6	4	6	7	7	5	4	5	6	8
M1G1	5	6	5	4	6	6	4	6	6	4	7	5	6	6	7	6	3	5	6	7
M2G1	5	5	6	5	5	5	4	8	5	5	6	5	6	6	6	5	5	6	6	8
N1G1	6	4	6	4	6	6	4	7	5	6	7	5	6	6	8	4	4	4	5	8
N2G1	6	6	7	4	6	5	4	8	7	5	7	4	6	7	7	6	3	5	5	9
N3G1	5	6	6	3	6	7	4	7	7	5	6	5	5	6	7	4	5	5	6	7
Mean	**5.5**	**5.1**	**6.1**	**3.9**	**5.6**	**5.8**	**3.6**	**7.0**	**6.0**	**5.0**	**6.4**	**4.5**	**5.6**	**6.5**	**7.0**	**5.1**	**4.0**	**5.1**	**5.5**	**7.8**	**5.6**
C4G2	5	4	6	5	5	5	4	6	5	3	5	4	5	6	8	5	4	6	6	8
C5G2	4	4	6	3	5	4	3	7	6	4	6	4	5	6	7	6	5	4	6	7
M3G2	6	4	5	3	5	5	3	6	5	4	6	4	5	6	8	5	5	5	5	8
M4G2	5	4	6	3	5	5	3	6	5	3	6	5	5	6	6	5	6	4	6	8
N4G2	5	5	5	2	4	6	3	7	5	4	5	5	6	7	7	4	5	5	5	8
N5G2	5	4	6	3	4	6	3	6	6	3	5	4	5	6	7	4	6	4	5	7
N6G2	6	3	6	3	4	5	2	5	5	3	5	4	6	7	6	3	5	5	6	8
Mean	**5.1**	**4.0**	**5.7**	**3.1**	**4.6**	**5.1**	**3.0**	**6.1**	**5.3**	**3.4**	**5.4**	**4.3**	**5.3**	**6.3**	**7.0**	**4.6**	**5.1**	**4.7**	**5.6**	**7.7**	**5.1**
C6G3	6	4	6	4	5	5	4	7	6	4	6	4	6	6	7	3	5	6	5	8
C7G3	5	4	6	3	6	6	4	7	6	5	6	5	5	6	7	4	6	4	6	7
C8G3	6	4	6	3	5	6	3	7	6	5	6	5	6	6	7	5	4	5	4	7
M5G3	5	5	5	4	5	4	3	6	6	4	5	4	5	6	6	6	4	6	6	8
N7G3	6	6	6	3	5	5	3	7	5	4	6	4	6	7	6	5	6	6	5	8
N8G3	5	5	6	4	5	6	3	7	5	4	7	4	5	6	8	6	4	4	6	8
N9G3	6	5	6	3	5	5	4	6	6	4	7	5	5	7	7	4	4	5	6	7
Mean	**5.6**	**4.7**	**5.9**	**3.4**	**5.1**	**5.3**	**3.4**	**6.7**	**5.7**	**4.3**	**6.1**	**4.4**	**5.4**	**6.3**	**6.9**	**4.7**	**4.7**	**5.1**	**5.4**	**7.7**	**5.3**
C9G4	6	5	6	4	6	6	3	7	6	5	6	5	6	7	8	4	5	5	6	7
C10G4	6	6	6	5	5	6	4	7	6	5	6	5	5	7	7	6	5	6	7	8
M6G4	5	5	6	5	6	6	5	7	6	6	7	5	5	7	7	6	5	6	5	8
M7G4	7	6	7	5	6	6	3	6	5	5	7	5	6	6	6	4	4	5	6	7
M8G4	7	5	6	4	6	5	4	7	7	4	7	4	6	6	7	5	4	6	5	8
M9G4	6	6	7	4	6	4	3	7	6	6	6	4	6	7	6	6	3	6	5	8
M10G4	7	5	6	4	5	6	4	7	6	5	7	5	5	6	7	5	3	5	5	9
M11G4	6	5	6	5	5	6	5	6	7	4	6	5	6	7	7	4	3	5	5	8
M12G4	6	5	7	5	6	5	4	6	6	6	7	4	6	6	7	6	4	6	6	8
N10G4	5	6	6	5	5	6	4	7	7	5	7	4	6	6	7	5	4	6	4	9
N11G4	6	6	7	5	5	6	5	6	7	6	6	5	5	7	6	6	3	5	5	8
N12G4	6	5	6	4	6	5	4	7	6	5	6	5	6	7	8	6	4	5	6	7
Mean	**6.1**	**5.4**	**6.3**	**4.6**	**5.6**	**5.6**	**4.0**	**6.7**	**6.3**	**5.2**	**6.5**	**4.7**	**5.7**	**6.6**	**6.9**	**5.3**	**3.9**	**5.5**	**5.4**	**7.9**	**5.7**
C11G5	6	5	6	3	4	5	4	6	5	4	5	4	5	5	7	6	5	4	4	7
C12G5	5	5	6	4	4	5	2	6	6	4	5	5	5	6	8	5	4	5	5	8
C13G5	5	5	7	4	4	5	3	6	6	5	5	5	5	6	8	4	6	6	6	7
M13G5	6	5	6	3	5	4	3	6	5	4	5	4	6	7	7	4	6	5	4	7
M14G5	5	5	7	4	5	5	3	6	6	4	6	4	5	6	8	4	4	5	5	8
M15G5	6	4	6	3	4	4	3	6	5	5	6	5	5	6	7	4	5	4	6	7
N13G5	6	5	6	4	6	5	2	7	6	3	6	4	6	7	7	4	5	6	5	7
N14G5	5	4	6	4	5	5	4	6	5	3	6	5	6	5	6	4	5	6	6	7
N15G5	5	4	6	3	4	5	3	6	6	4	5	5	5	6	7	4	4	5	4	8
Mean	**5.4**	**4.7**	**6.2**	**3.6**	**4.6**	**4.8**	**3.0**	**6.1**	**5.6**	**4.0**	**5.4**	**4.6**	**5.3**	**6.0**	**7.2**	**4.3**	**4.9**	**5.1**	**5.0**	**7.3**	**5.2**