

**HOST PLANT RESISTANCE AND CHARACTERIZATION OF BLAST DISEASE
(*Pyricularia grisea*) IN SELECTED FINGER MILLET (*Eleusine
coracana* L.) GENOTYPES IN KENYA**

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**A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements
for the Master of Science Degree in Crop Protection of Egerton University**

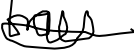
EGERTON UNIVERSITY

OCTOBER, 2021

DECLARATION AND RECOMMENDATION

Declaration


This thesis is my original work and has not been submitted for the award of degree to any other University.

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DEDICATION

This thesis is dedicated to Almighty God, my beloved parents Mr. Joseph Onjiri Aketch and Pamela Vugusta Aketch, my siblings Emma Mmboga, Cindy Moige, Bruce Joel Aketch, Belinda Maloba and my niece Jessy Wiraka.

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ABSTRACT

Host plant resistance (HPR) mechanism is the most economical, safe and convenient method in the management of blast disease (*Pyricularia grisea*), one of the most serious diseases affecting finger millet. Four studies were conducted in Agricultural Training Centres (ATCs) in Bomet, Koibatek and Nakuru, and Egerton University research stations to determine resistance to blast and yield performance of finger millet. Twenty-five finger millet genotypes with diverse genetic backgrounds were planted in a 5 × 5 *alpha* lattice design in 2 seasons 2019/2020. Genotypes; NKRFM1, U-15 and P224 were used as resistant checks while KNE 741 was susceptible. Twelve genotypes were selected from the field experiment and evaluated for both leaf and neck blast severity in RCBD design in the greenhouse at Egerton University. *Pyricularia grisea* strains from Alupe (0.4861° N, 34.1365° E), Koibatek (1.0445° N, 35.5822° E), Busia (0.4608° N, 34.1115° E), Makueni (2.2559° S, 37.8937° E) and Nakuru (0.3031° S, 36.0800° E) were isolated from diseased plants and characterized using microscopy and SSR markers, respectively. Results from the field trials showed that genotypes GBK-127189A, Snapping-Purple, KNE 1124 x KNE 796, Gulu E, SDFM-1702, IE-2183 and Kat Fm1xU15 1.6.6.3.1.1 were significantly resistant to both leaf and neck blast across all seasons and environments ($P < 0.05$). Genotypes Kat Fm1xu15 1.6.6.3.1.1, KNE 1034, SDFM-1702, IE2183 and Snapping Purple showed higher response with <13% DSI on both leaf and neck blast when compared to susceptible check KNE 741 in the greenhouse. Kat FM1xU15 1.6.6.3.1.1, Kal 2 pader, Ikhulule, P-224, Gulu-E and Snapping Green were the preferred varieties by farmers from Nakuru while KatFM1, KNE 741 and KNE 629 were the most preferred varieties for Bomet. *Pyricularia grisea* showed variation in morphology, virulence level and gene diversity. Gene diversity and Shannon's Information index had a mean of 0.4 and 0.58, respectively. There was no geographical grouping but *P. grisea* diversity occurred within populations (87%) as opposed to among populations (13%). The resistant genotypes would be useful in breeding programs in accordance with the farmer's preferred traits and durable resistance achieved from understanding the pathogen.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
ATC	Agricultural Training Centre
AUDPC	Area under Disease Progress Curve
CABI	Centre for Agricultural and Bioscience International
CAN	Calcium Ammonium Nitrate
CIA	Chloroform Isoamyl Alcohol
CRD	Completely Randomized Design
CTAB	Cetyl Trimethyl Ammonium Bromide
DAP	Diammonium Phosphate
DMRT	Duncan Multiple Range Test
DSI	Disease severity index
DSR	Disease severity rating
FAO	Food and Agriculture Organization of the United Nations
FPVS	Farmer Participatory Varietal Selection
HPR	Host Plant Resistance
ICRISAT	International Crops Research Institute for semi-arid Tropics
KALRO	Kenya Agricultural and Livestock Research Organization
MLND	Maize Lethal Necrotic Disease
MSE	Mean Square Error
PCA	Principal Co-ordinate Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PIC	Polymorphic Information Content
PIC	Polymorphism Information Content
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis software
SDS	Sodium Dodecyl Sulphate
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic mean
UV	Ultra-violet

CHAPTER ONE

INTRODUCTION

1.1 Background information

Finger millet blast caused by a fungus *Magnaporthe grisea* (anamorph *Pyricularia grisea*), is a major disease affecting the growth and production of finger millet (*Eleusine coracana* L.) in many parts of the world. This crop is vulnerable to blast disease which occurs mainly during rainy and winter seasons. Leaf blast occurs at early crop stage and is highly damaging. In India, an increase in 1% infection in the neck and finger resulted to corresponding yield losses of 6.75% to 87.5%, respectively (Rao, 1990). In East Africa, Pagliaccia *et al.* (2018) reported yield losses that exceeds 80%. The disease affects a wide range of grasses and sedge species, in rice (*Oryza sativa*) it has been reported to cause yield loss of 60-100% (Kihoro *et al.*, 2013). The disease affects *E. coracana* at all phenological stages from seed to grain formation (Takan *et al.*, 2012).

Millet production in the world is estimated to be 26.7 million tonnes ha⁻¹ (FAO, 2015). India is the leading producer worldwide (8.8 million tonnes) with yield of 4789 which is approximately 33% of world production (FAO, 2015), Eurasia and central Asia (14%), Africa (16%) and the rest of the world (37%). In East Africa Uganda produces 841,000 tonnes with an area of 412,000 Ha. In Kenya finger millet grain production is estimated at 260,000 tonnes with an area of 185,000 ha, with corresponding yield of 750 kg ha⁻¹ (FAO, 2019). Finger millet is mostly grown by smallholder farmers and the main production areas in Kenya are Western 77,000 ha (29%), Nyanza 57,000 ha (15%) and Rift Valley 65,000 ha (13%) (FAO, 2015). However, finger millet has witnessed an expansion in the last five years to >200,000 ha (Upadhyaya *et al.*, 2016) due to combined research and promotion efforts that have provided new varieties, improved agronomy, and adoption of the crop by farmers. In regions such as Bomet County, finger millet acts as an alternative adopted crop to maize (*Zea mays*) which is often affected by Maize Lethal Necrosis Disease (MLND) disease (Wekesa *et al.*, 2019).

Finger millet is the most important small millet grown for subsistence in Eastern Africa and Asia. In East Africa, it is majorly used for food in form of thin porridges, malting and brewing. In India, it is mainly used for brewing (Mitaru *et al.*, 1993). It is highly nutritious food for the vulnerable and people with low immunity (Takan *et al.*, 2012). It contains nutritional elements which are easy to digest thus a major source of food for pregnant women, the sick, lactating mothers and children, and diabetics (Gupta *et al.*, 2017; Singh & Raghuvanshi, 2012). Being resilient, *E. coracana* can tolerate high levels of drought

because of its nature of having small grains which allows it to escape most grain pest and allow the crop to have a good shelf life. Therefore, it offers food security for the rural communities who are smallholder farmers (Gupta *et al.*, 2017; Sivakumar, 2006).

Despite all its importance, finger millet is affected by various biotic and abiotic stresses such as blast, foot rot, smut, leaf blight, shoot fly, pink stem borer, streak mottling virus, drought and soil infertility among others. Blast is the most devastating disease affecting different aerial parts of the plant at all stages of its growth. (Mgonja *et al.*, 2007; Srivastaya *et al.*, 2009). Average yield loss due to blast is estimated to be about 28-36% and is usually associated with Kernel abortions and shriveled grains caused by damage of panicle during reproductive stages (Nagararaja *et al.*, 2007; Sreenivasaprasad *et al.*, 2004). There is limited knowledge of host-pathogen relationship most importantly host plant resistance, characterization of blast and farmer preferred genotypes and their traits. The identification of varieties that are high yielding with a durable mechanism of blast resistance is key. The race components of *P. grisea* in major finger millet growing areas of Western, Nyanza and Rift Valley provinces is not well documented. Recent studies by Wekesa *et al.* (2019) showed that in Western Kenya, disease severity was highest in early maturing varieties as compared to the late maturing varieties. The report further identified genotypes GBK 000702, GBK 000513, GBK 029869, Gulu-E, GBK 000752 and Busibwabo as resistant to blast. Other constraints to finger millet production and utilization that need attention include ineffective weed management, poor kernel quality, inefficient seed systems and production-supply chain problems, notably through 'spill-in' and adaptation of relevant technologies developed elsewhere. This will make finger millet sector likely to make a significant contribution to fighting malnutrition and poverty in Kenya and East Africa. Kimurto and Obare (2012), Ogundi *et al.* (2018) noted that there is need for promoting bio-fortified pearl millet (*Pennisetum glaucum*) products through market segmentation with more attention given to high-income households with good knowledge of millet nutritional benefits for increased commercialization.

Furthermore, lack of suitable variety tolerant to disease stresses during the crop growing period has encouraged the integration of scientists, researchers, resources and dedicated hard working farmers through Farmer Participatory Varietal Selection (FPVS) through which there is access and improvement of finger millet genotypes (Christinck Weltzien, 2017). Breeding for adoption to farmers in marginal sets of environments require selection of high yielding and blast resistant varieties (Wang *et al.*, 2015). In most cases, subsistence farmers growing millets and other minor crops in unfavorable environments use

low levels of inputs and have not benefited by high yielding varieties. Identification of most suitable variety through FVPS for growing in a specific niche is important as it offers a second crop which otherwise would have been difficult to adopt under the variety evaluation system confining to research stations. In Kenya, there is evidence and information that finger millet production has reduced drastically over the last decade due to constraints such as blast disease, use of low yielding varieties, limited mechanization and low demonstration of improved varieties through appropriate evaluation system like FVPS among other constraints (Handschuh & Wollni, 2016). Therefore, there is need to address the production challenges and help to boost food security through identification of cultivars with partial or durable tolerance to blast and high yielding with wide adaptability in target regions.

1.2 Statement of the problem

Blast caused by *Pyricularia grisea* is an economically important disease of finger millet worldwide. In Kenya, decline in finger millet production is exponentially related to blast disease attack which highly threatens crop and causes exceeding yield losses of over 50% under favorable conditions. Most finger millet landraces and commercial cultivars are susceptible to blast and current methods of control like use of fungicides, cultural or biological control are limited due to their ineffectiveness, high cost to small-holder farmers or not suitable to current finger millet production systems. Although a lot of efforts have been geared towards breeding for resistance to blast by National Research Systems (NARS) (KALRO and Universities) and regional; Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) and International Crops Research Institute for semi-arid Tropics (ICRISAT) millet breeding programs, resistant/tolerant genotypes have low resistance production cycles within short cultivation time, there is resistance /tolerant breakdown due to the high rate of mutation of blast isolates . There is also limited success in identifying cultivars with partial or durable resistance that are high yielding with wide adaptability and stability across major varied agro-ecological zones where finger millets are produced especially in Western, Nyanza, Rift Valley and Coastal areas. The current local landraces and released varieties like P-224 and EUFM 401 (KNE 741) and NKRFM-1 are susceptible to blast disease although the use of host plant resistance (HPRs) is recommended as the most cost effective and environmentally acceptable option. Furthermore, there is limited information reported on identification of finger millet genotypes that are widely adapted to varied agro-ecological zones and resistant to blast in major growing areas of Kenya. Through FVPS approach, farmers' selection of preferred varieties that are high

yielding with wide adaptability coupled with blast tolerance can easily be adopted in target regions. As key contribution to low yield, majority of farmers grow local genotypes that are late maturing and highly mixed with a limited adoption with less use of the improved genotypes such as U-15, Gulu E and EUFM 502 (KNE 629). Knowing the pathogen diversity and its virulence is key to designing breeding program through selection of appropriate parents and gene recombination. Furthermore, characterization of the pathogen from diverse agro-ecologies has remained a challenge because of the pathogen diversity, genetic variability and frequent genetic mutations which mainly occur due to sexual variation in *Ascomycete* fungi and leads to emergence of new strains, making breeding for resistance difficult. The study therefore aimed at determining levels of host resistant/tolerance genotypes, characterization of *P. grisea* and assessing adoption of blast tolerant and high yielding varieties among farmers in Central-Rift Kenya.

1.3 Objectives

1.3.1 General objective

To contribute to enhanced food and nutritional security through increased finger millet production by effective management of blast disease using host plant resistance and identification of farmer preferred high yielding varieties through FVPS approach in Central Rift, Kenya.

1.3.2 Specific objectives

- i. To determine levels of resistance/tolerance to blast disease in selected finger millet genotypes
- ii. To assess farmers' preferred finger millet genotypes and traits through FVPS approach in major growing areas of Kenya.
- iii. To characterize morphological and genetic diversity of *Pyricularia grisea* isolates in major growing areas of Kenya.

1.4 Hypotheses

- i. There is no significant difference in levels of resistance among selected finger millet genotypes against blast disease under varied growing conditions.
- ii. There is no significant difference in the preference of finger millet and their traits in the growing areas of Kenya.

- iii. The *Pyricularia grisea* strains from major growing areas do not show any morphological and genotypic difference associated with virulence levels.

1.5 Justification

Finger millet is grown in most parts of the world. The adoption of finger millet by farmers continually increases because of its resilience and high nutritional ability. According to UNDP (2013), 0.9 billion people in the world have been reported to be malnourished while 1.2 billion continue to live in poverty despite the world's effort to reduce hunger and poverty. The current increase in population with less food provision will lead to a high demand of crops like finger millet because it survives under harsh climatic conditions. Finger millet is a resilient crop to climate change, soil depletion and water scarcity. The utilization of plants own defense mechanism is a highly economical, cost-effective, safe and convenient method of blast control in resource-poor and marginal farmers who reside and cultivate finger millet in major growing area of Kenya such as Bomet, Nakuru and Baringo. The use of low unimproved genotypes has led to the development of improved genotypes by the local and regional companies with an aim of boosting productivity. Farmer participatory varietal selection (FVPS) creates a bridge between scientist and farmer interests. FPVS have been used in development and dissemination of improved crop varieties making it easier for farmers' adoption. FVPS is an important approach for identifying cultivars for harsh environments and acceptable to resource poor farmers (Gowda *et al.*, 2000). Various blast fungal variability exist in a population of *Ascomycota* fungi and various isolates have been identified due to continual introduction of modern cultivars. A rapid and accurate detection of the evolved pathotype and their virulence has been shown to undoubtedly formulate strategies of coming up with resistant cultivars that are high yielding for these regions for adoption by farmers. Finger millet is important nutritionally and economically for subsistence to local farmers. In addition, it is a crop that can be used to improve food security of the country. The rapid population growth in Kenya has led to the increase in demand of food from marginal areas makes finger millet essential to be grown and utilized for food sufficiency (Macauley, 2015). Despite all these, *E. coracana* is regarded among 'Orphan Crops' since they have low production levels compared to other popular food crops (Belton & Taylor, 2004). The identification of high yielding varieties of finger millet and the race components of *P. grisea* in major finger millet growing areas of Western, Nyanza and Rift Valley provinces is not well documented. Therefore, there is need to address this by identifying blast resistant

genotypes, evaluate pathogen strains affecting finger millet and assessing preference of farmers for adoption.

CHAPTER TWO

LITERATURE REVIEW

2.1 Finger millet botany and morphology

Finger millet (*Eleusine coracana* L. Gaertn) is an annual crop cereal in the family *Poacea*. *E. coracana* is an allotetraploid ($2n\ 4x = 36$) annual cereal crop that includes two distinct subspecies subsp. *Africana* (wild finger millet) and cultivated finger millet subsp. *coracana*. (Chandrashekar, 2010; Hilu, 1994). It is a strong tillering grass grown for its grain which is mostly consumed as food or brewing by resource poor farmers in Africa and Asia (Pradhan *et al.*, 2019). Animal feed can also be obtained from its straw. It has erect, light green stems that grows to a height of up to 170 cm, leaves are dark green, smooth with hair along the edges. The flower has a cluster of 4-19 finger-like spikes that resemble a hand clenched inward when mature, the spikes resemble fingers, hence its name finger millet (Heuzé, 2015). The spikes bear up to 70 alternate spikelet which carry 4-7 small seeds (Devos & Dida, 2006). The seed contains a seed coat which can easily be removed since the seed pericarp is independent from the kernel (Heuzé, 2015).

2.2 Origin, ecology and cultivation of finger millet

Eleusine coracana oldest archeological sample originated from Axum, Ethiopia in 3000 B.C (Hilu *et al.*, 1979). *Eleusine coracana* was first domesticated 5000 years ago in the highlands of Ethiopia and Uganda (Hilu *et al.*, 1976). From a series of spontaneous hybridization of the diploid *E. indica* (AA genome) and unknown B-genome donor, *E. coracana* was developed from the wild type *E. coracana* subsp. *Africana* ($2n=4x=36$). Finger millet was then domesticated and moved to lowlands of Africa in 1000B.C. Due to international trade *E. coracana* was introduced to India which is now the centre of diversity of finger millet and Africa (Neves *et al.*, 2005).

Eleusine coracana is adapted to a wide range of environmental and climatic conditions and thrives at higher elevations than most other tropical cereals and tolerates salinity better than most cereals. It grows on lateritic or black heavy vertisols and a fertile well-draining sandy loam soil with a pH 5-8 (Green life Crop Protection Africa, 2018). It has some tolerance for alkaline and moderately saline soils. In terms of altitude *Eleusine coracana* is mainly found between 1000-2000 meters above sea level in eastern and southern Africa and up to 2500-3000 meters above sea level in the Himalayas.

It is mainly grown for subsistence and performs well in relatively dry areas of western and Lake Basin regions of Kenya (FAO, 2012). Finger millet sowing is done by broadcasting

or by use of furrows at a depth of 2.5 cm, 10-12 cm between plants or use of a row and a drill and a row-to-row distance of 25 cm. The seed are so small and therefore land preparation should be to a fine tilth. The temperature range for optimal growth is 15-28°C. Application of Di-ammonium phosphate during planting is necessary. (Green life Crop Protection Africa, 2018). The seeds bed should be prepared to fine tilth and drills made 30cm apart. Planting is then done early on the onset of rains by broad casting along the drills.

2.3 Finger millet production and distribution in Kenya

Kenya largely produces finger millet (called *wimbi* in Swahili) has been grown by farmers especially in marginal areas since the historical times for subsistence (Onyango, 2016). In 1978 to 1981 the yield of finger millet in Kenya drastically reduced from a production of 1.6 tonnes per hectare ($t\ ha^{-1}$) to $0.7\ t\ ha^{-1}$ due to government sensitization on maize (*Zea mays*) which led to displacement of finger millet, poor growing conditions and insufficient breeding programs (Dida *et al.*, 2008). In 2015, 2016 and 2017 there has been decline in production from 99,000 tonnes to 54,000 tonnes. The yield per farm also reduced from $10.968\ tonnes\ ha^{-1}$ with an area of 90263 ha in 2015 to $6.111\ t\ ha^{-1}$ with an area of 88368 in 2016 and $6.136\ t\ ha^{-1}$ with an area of 88000 ha (FAO, 2018). According to Chrispus Oduori 2016, Kenya Agricultural and Livestock Research Organization (KALRO) principal researcher based at Kisii, more than 30,000 ha in Western region of the country is under finger millet and more than 65,000 ha are under the crop nationally. The data form the Ministry of Agriculture 2015, reveal that the area under finger millet has increased significantly from 53,155 ha in 2008 to 104, 576 ha in 2009 yielding 626,856 bags up from 426,928 bags the previous year. In 2005 to 2006 production periods recorded an increase in acreage and yield while the 2006-2007 production showed reduction in acreage while yield increased. The major producers of finger millet include, China (1,225,579 tonnes), Ethiopia (260,030 tonnes), Niger (2,677,860 tonnes), Nigeria (4,884,890 tonnes), Mali (1,390,410 tonnes), Burkina Faso (970,927 tonnes), (FAO, 2009). Locally, finger millet is majorly grown in Kisii, Migori, Busia, Homabay, Kisumu, Siaya, Machakos, and Kericho counties by local farmers for its ability to grow in adverse agro- climatic conditions (Onyango, 2016). *E. coracana* accounts for 11 % of production in the world as compared to 50% pearl millet, 30% Proso millet (Dida *et al.*, 2008; FAO, 2005).

2.4 Finger millet varieties grown in Kenya

Farmers in Kenya are known to continually use the local varieties of *Eleusine coracana*. In Busia County, the local landraces grown by farmers include *Ikhulule*, *Khayoni*, *Agriculture* and *Madarekasabale*. In Teso, *Obokoro*, *Aran*, *Ebonit*, *Eleurot*, *Eblue* and *Emumware* and in Kisii *Enyakundi*, *Enyandabu*, *Endere*, *Enaikuru*, *Morogi*, *Amatugi*, *Omokoni* and *Matege* are commonly planted. Good genetic resources such as resistance to diseases and high yielding traits can be sourced from these local land races. For instance, *Ikhulule*, *Emumware*, *Enaikuru*, *Enyandabu* and *Emumware* are high yielding *Enyakundi* while *Matege* are resistant to blast disease. Improvement of finger millet varieties in Kenya has continually grown over the past years. The development and release of these varieties has led to improvement of finger millet production in the country. However, the challenge of adoption is still a challenge to the farmers in the country and therefore the use of FVPS which conducted in this study brought knowledge of farmers of genotypes to farmers therefore improving the chances of adoption.

2.5 Diseases infecting *Eleusine coracana*

The major disease affecting finger millet is blast disease (*Magnaporthe grisea* (anamorph *Pyricularia grisea*). Mold infestation has also been a challenge; however, it has been reduced due to the hardness and small grain of the seed (Audilakshmi *et al.*, 1999). Other diseases include downy mildew *Sclerospora graminicola*, rust *Puccinia substriata* and seedling leaf blight *Helminthosporium nodulosum* (CABI, 2008). The chemical and physical composition of finger millet grain determines the resistance mechanisms to pest and pathogens. The physical appearance of the grain is regarded as the first line defense against infection and infestation.

2.6 Blast disease

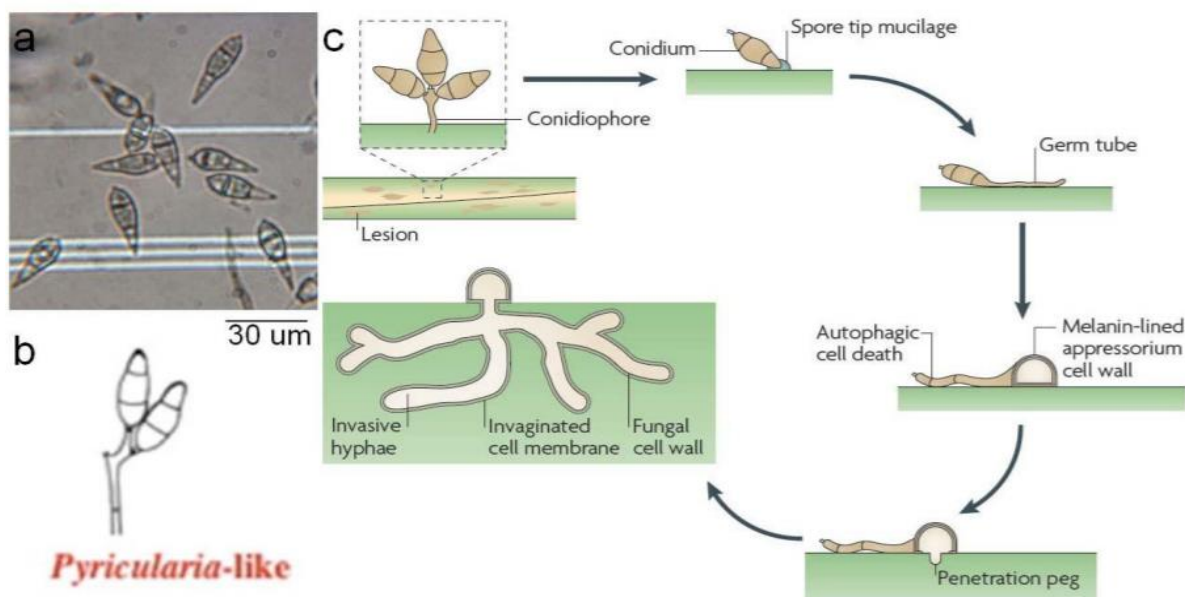
2.6.1 Biology and structure of *Pyricularia grisea*

Pyricularia grisea is a filamentous *Ascomycete* fungus. It produces ascospores which are septate and contained in structures called asci. The asci are also found in special structures called perithecia contained in the stroma covered by a loose hyphal mat. It has haploid spores with nucleus contained in the stroma. It belongs to a family *Magnaporthaceae*, order *Diaporthales*, class *Ascomycetes* and phylum *Ascomycota* (Murata *et al.*, 2014). The fungus produces both sexual (teleomorph) and asexual spores (anamorph). Sexual production of spores occurs in ascus while the asexual reproduction occurs on fruiting bodies called

pycnidia and the hyphae. Both of these reproduction mechanisms lead to single or groups of conidia with a mass of mycelia. *Pyricularia grisea* is identified by its shape, pattern and spore shape. Under favorable conditions such as humidity of >89% and temperature of 25-28 °C, *P. grisea* produces millions of conidia within 1-2 days (Ruiz, 2003).

2.6.2 Epidemiology and life cycle of *Pyricularia grisea*

Under conditions of high humidity >89% and temperature of 25-28°C, the asexual spores (conidia) infect the plants. *Pyricularia grisea* has a hemibiotrophic lifestyle which has two stages biotrophic which hinders the plant immune system and necrotrophic where cell death occurs after infection. The air borne spores land on leaves and produce spore-tip mucilage from the tip of its apex which the attaches on the hydrophobic leaf surface. An *appressorium* then forms from the tip of the elongating germ tube. Due to turgor pressure that builds on the *appressorium*, a penetration peg is formed which then penetrates the epidermal cells (Fernandez & Kim, 2017; Hayden, 1999). The appressorium then germinates into a germ tube by rapid growth of the hyphae also called invasive hyphae. Once penetration has occurred, the invasive hyphae swell and fills the cell, penetration occurs rapidly to form a colony. High relative humidity leads to sporulation which occurs in the grey area of the lesion (Figure 2.1) (Takan *et al.*, 2004). Conidiophores produce conidia which then project through the stomata and extrusion through the epidermal cell. Three to eight days after appearance of the lesion, conidia formation is usually at peak. The spores are carried from one plant to another by dew or rain and the distribution is relative to the amount of disease propagule in seed lots. Production of the pathogen takes place 4-5 days later. New conidia are produced the 6th day after sporulation (Udagawa & Yaegashi, 1978). The disease affects the crop at all stages of growth from seeding to grain formation. *Pyricularia grisea* is favored by cloudy skies, frequent rain and drizzles which accumulate dew on leaves for a long time and the rate of sporulation increases with increasing relative humidity by 90% or higher. The appearance of lesions on the leaves, nodes and the heads which are spindle shaped wide at the center and a brown margin to older lesion indicates the symptoms of infections (Dida *et al.*, 2008). The symptoms appear similar to rice infections however different pathotypes have been identified with respect to genetic diversity, reproductive potential and pathogenicity that are specifically linked to the host (Takan *et al.*, 2011)



Figures 2.1: Disease cycle of *Pyricularia grisea* from infection stage to production of symptoms. Source: Talbot and Wilson (2009)

2.6.3 Symptoms of *Pyricularia grisea*

Blast affects leaf, neck and head region leading to leaf, neck and head blast. On the leaves, spindle shaped spots with grey or white center. The margin of the wound appears to be reddish brown that will start as small and continue to enlarge to give blast appearance (Manyasa *et al.*, 2019; Wekesa *et al.*, 2019). On the neck region, brown colour which finally changes to black is observed two to four inches. This can result to breaking of the neck. The infection spread out to the head region where the florets will appear brown and dry with poor grain development also the severely affected florets will result in total loss of yield (Anjum *et al.*, 2016).

In older plants, the disease appears on leaf lades with typical spindle-shaped spots. Under congenial conditions, such spots enlarge, coalesce and form bigger lesions. The lesions are like hose on seedling and are about 0.3-0.5cm in breadth and 1-2 cm in length. The apices of infected leaves beyond the lesions hang down and sometimes break. Stem infection causes blackening of nodal region, penetrating into the tissues (Dida *et al.*, 2008). Maximum damage is caused by infection of the neck which then turns black and shrinks with olive grey fungal growth seen on the affected area. Severe infection causes ears to hang down from the stalk and sometimes break away. In later stages infection of the fingers occurs which usually begins from the apical portions and extend towards the base. The infected ears become chaffy

and only a few shriveled grains may be found the affected ear head turns black. The extent of grain losses depends upon the time of infection (Tamilnadhu Agritech Portal, 2017).

2.6.4 Economic importance and distribution of blast

Pyricularia grisea is a disease of economic importance disease with related yield losses of 50-80%. *Pyricularia grisea* infects finger millet at any growth stage from the seedling to grain formation stage leading to shriveling of seed causing lower market value of the grains hence losses (Ekwamu, 1991). Alternatively, a total loss can occur in severe infections leading to no seed formation hence leading to losses to the farmer. The average loss due to the disease in endemic areas was reported to be 80-90% (Singh *et al.*, 2012). The affection on the leaves causes reduced photosynthetic area leading to accumulation of less assimilates hence lower yield of the crop (Babu *et al.*, 2013). All parts above the neck may die when the infection occurs at the neck. An increase in 1% infection in the neck and finger resulted in a corresponding increase of 0.32 and 0.084% in yield losses and grain yield losses ranged from 6.75 to 87.5%. In India and in East Africa the yield losses exceeding 80% have been reported in rice while in finger millet recent reports point out a loss of 37-45% in India (Prajapati, 2018; Rao, 1990).

2.7 Management of blast

There are several ways of managing blast which includes host plant resistance, cultural, chemical and biological methods.

2.7.1 Host-Plant Resistance

Use of resistant varieties is the most effective, economical and environmentally friendly for disease control (Jacob, 2019). Development and introduction of resistant finger millet combined with other disease control practices is the most practical approach of disease control at field level (Jacob, 2019). Resistance genes offer a durable resistance mechanism against the pathogen. This includes partial resistance which offers a stable form of resistance and is controlled by multiple genes. Usually, it is expressed in form of reduced infectivity, prolonged latent periods lead to less lesion development after infection (Perlevliet, 1988). Pyramiding resistant genes after proper identification of the genotypes can be done leading to genotypes with partial resistance. However, these cultivars can differ from resistant to extremely susceptible and to moderately resistant (Perlevliet, 1988; Sridhar *et al.*, 2007). Four partial-resistance genes in rice have been identified and have been described as specific: *Pif*,

Pi21, *Pb1*, and *Pi34* (Elsa *et al.*, 2008). Most of the resistance genes are concentrated in certain genomic regions, particularly on chromosomes 6, 11, and 12 in rice (Monosi *et al.*, 2004). *Rwt4* gene in wheat is reported resistant to *Pyricularia grisea* in Japan. Muyonga *et al.* (2000) after screening five finger millet varieties for blast disease tolerance, Sirare had more tolerance to blast than P224 and Nyakairo, Gulu-E and Ikhulule were moderately tolerant. Various plants react differently when exposed to different environments and this is also exhibited in cases of tolerance of some plants against certain diseases and yield variations. Gbadeyan *et al.* (2018) reported that under different ecological conditions, there is occurrence of resistance break down which is also variable among the varieties. For instance, varieties such as IE-1010, EKR-227 and P283 recommended for low altitude areas have also been released. Varieties like KNE-1034 and KNE-479 were developed by ICRISAT specifically for areas with harsh climatic conditions such as Machakos County. Okhale-1 and U-15 are also among the released varieties developed by KALRO-Kibos.

2.7.2 Chemical control

Fungicides have been reported to be widely used by farmers and several have been shown to give good control of blast leading to increase in yield (Rajashekar *et al.*, 2006). Benlate reduces leaf blast by 28% on young millet and 38% on neck blast. Pyroquilon and Tricyclazole are also systemic fungicide used to control neck blast (Mgonja *et al.*, 2011). The use of Mancozeb has also been effective against blast. It is a protective fungicide that inhibits spore germination and interferes with the biochemical processes within fungal pathogen cell. Carbendazim is an effective, broad spectrum, systemic and curative drug that inhibits the development of fungi interfering with spindle formation during cell division by mitosis (Khanzada & Shah, 2012).

2.7.3 Biological control

Isolates from Phyllosphere is reported to reduce growth of the pathogen in culture and suppress spore germination (Sreenivasaprasad *et al.*, 2004). Spray of inoculum on culture is effective in reducing the number of lesions on leaves. Use of actinomycetes and botanical like Sanna extracts will be of great importance if it proves to reduce or suppress the growth of the pathogen. The naturally occurring antagonistic microorganisms have been the focus of intense research throughout the world for the control of fungal diseases (Wolie *et al.*, 2013). The use of bio controls such as *Pseudomonas fluorescens*, *Trichoderma harzadium* or a combination of the two on both seed treatment and field trials have been shown to control

Pyricularia grisea (Kumar, 2011). In Kenya the control of finger millet blast by use of *Streptomyces* was done in Maseno and the results showed the effectiveness of this organism against the disease (George *et al.*, 2013).

2.7.4 Cultural control

Intercropping with crops like pigeon pea (*Cajanus cajan*), peanut (*Arachis hypogaea*) or cow pea (*Vigna unguiculata*) reduces intensity of the pathogen (Elsa *et al.*, 2008). Diseased plants can be uprooted and burned or composted to prevent the spread of the disease to the next crop season. For a healthy crop, healthy seeds are necessary and many precautions are to be taken. The excessive use of nitrogen fertilizer promotes luxuriant crop growth, which increases the relative humidity and leaf wetness of the crop canopy, and so favors blast (Nguetti *et al.*, 2018). The application of silicon fertilizers (e.g., calcium silicate) to soils that are deficient in this element has reduced blast but because of its high cost, it should be applied efficiently (Abed-Ashtiani *et al.*, 2012). Cheap sources of silicon, for example, selecting the straw of rice genotypes with high silicon content, can be considered to make this approach economically viable (Jena & Mackill, 2008). However, the effectiveness of these control strategies is limited due to ability of pathogen to survive in plant debris for a long time, land unavailability to practice crop rotation and small-scale farmers use their own seeds from previous season. Mulching could reduce infections caused by splashing.

2.8 Breeding for resistance to *Pyricularia grisea* and characterization of finger millet blast

Breeding programs for mitigation of finger millet blast have been put in place to find a suitable source of resistance among genotypes. Manyasa *et al.* (2019) in research done in Busia, Kenya found that finger millet genotypes G18, G43, G39, G16, G60 and G67 were all resistant to both leaf neck and head blast hence they had higher yields compared to the susceptible types. The gene action on finger millet blast resistance is not well documented with little or less information on the resistance of finger millet to blast on varied ecological conditions (Shailaja & Thirumeni, 2010). Wekesa *et al.* (2019) identified genotypes GBK 000702, GBK 000513, GBK 029869, Gulu-E, GBK 000752 and Busibwabo as resistant to blast in Western, Kenya. Other varieties include Kahulunge and Ekama in Tanzania and Seremi 2, Etiyo brown and Ngome in Uganda (Manyasa *et al.*, 2019). Host plant resistance has been the key to identify potential parents that can further be used to obtain resistant genes and be helpful to the breeders.

2.8.1 Morphological characterization of finger millet blast disease

Various studies have been done on the morphological diversity of rice and finger millet blast morphology, however, studies done Africa are limited. Shanmugapackiam *et al.*, 2019 reported that there are significant variations of finger millet blast isolates collected from various regions. The variations were linked to colour, margin, mycelial growth and sporulation of the isolates. The same findings were found to be true by Babu *et al.* (2015), Meena *et al.* (2005) and Shahriar *et al.* (2020) who described *Magnaporthe* fungi having varied structures, growth mechanism and colour due to the sexual hybridization that occurs at different stages of the fungi. Also, it was concluded by Meena *et al.* (2005) that it is important to describe the structure of the pathogen using distinct features such as conidia, conidiophore morphology and colour. In Kenya, there has been limited research on the finger millet morphology and cultural diversity of the pathogen which is the main aim of the study.

2.8.2 Genetic characterization of *Pyricularia grisea* pathogen

Genetic characterization of *P. grisea* is important for host plant resistance studies as it helps scientist avoid cryptic error. *Pyricularia grisea* diversity has widely been studied in rice especially in Kenya. Kariaga *et al.* (2017) characterized variation in *Pyricularia oryzae* of rice and revealed variation in colony colour, diameter and morphology of the pathogen. Further, molecular diversity of the eight isolates clustered into four haplo-groups indicating variation in make-up of the pathogen. Random Amplified Polymorphic DNA (RAPD) has also been used in characterization of *Magnaporthe grisea* and 52 pathotypes were found with high genetic variability (Sharma *et al.*, 2002). A study by Anjum *et al.* (2016) showed a high genetic variability among the *Pyricularia grisea* isolates using Simple Sequence Repeats (SSR) and shift in genetic make-up of races of *Pyricularia grisea* pose a great challenge in the resistant lines hence understanding the variability can lead to strong stability of a genotype against attack. In this study SSR markers were used to show the variability of *P. grisea* selected from different growing areas of Kenya for breeding purposes. Genetic research on the *P. grisea* collected from different areas of Kenya are limited. The main aim of the study is to determine if there is genetic diversity in isolates collected from various growing areas of Kenya.

2.9 Farmer Participatory Varietal Selection (FPVS)

Farmers' living conditions, different attitudes and socio-economic challenges pose a challenge in dissemination of innovations and technologies among farmers (CIMMYT, 1988;

Ojulong *et al.*, 2016). On-farm research and involvement of farmers in evaluation of new and released varieties plays a major role in adoption of these varieties and dissemination of technologies (Lancon *et al.*, 1989). In development of new varieties little is known to breeders as most farmers not only look at yield, they select more other traits during the farmers' varietal evaluation (Angarawai *et al.*, 2016). Socio-economic improvement of small-scale farmers has been witnessed due to increased participation of farmers through FVPS which has led to successful technology transfer and creation of new innovations (Dargo & Shiferaw, 2017). In many countries, high yielding varieties have been developed and released for general cultivation through breeding of exotic and indigenous lines (Singh *et al.*, 2016). However, farmers have an uneven adoption of these varieties due to the neglect of their interests.

The Farmer participatory Variety Selection (FPVS) has been an effective way of bridging the gap. It involves a series of steps which include, rural appraisal survey, selection of varieties or lines, conduction of farmer participatory trials and dissemination (Witcombe *et al.*, 2002). Various mixed varieties including released cultivars, cultivars at advanced stage of testing, and non-segregating lines from plant breeding programs are selected and planted. FVPS conducted where farmers get to assess the crop varieties and score the varieties depending on the agronomic traits and performance (Vom *et al.*, 2010). Farmers give opinions on the pre and post-harvest qualities which include the grain quality, plant height, days to maturity, tillering ability, tolerance to biotic and abiotic stresses and post-harvest qualities such as taste, cooking ability of the crops and other traits that are important to the farmer (Atlin, 2004). FPVS offers a new way in the field of centralized plant breeding to combat the problems faced by the farmers in marginal environments regarding adoption of varieties developed finger millet, enhances access to crop diversity, increases productivity and ensures food security (Gowda *et al.*, 2000).

FVPS has been successfully done in many crops including rice (Orlando *et al.*, 2020; Panwar *et al.*, 2019; Paris, 2011), beans (*Phaseolus vulgaris*) (Tamene, 2016; Yadavendra *et al.*, 2017), barley (*Hordeum vulgare*) (Demsie & Ferede, 2020), wheat (*Triticum sp.*) (Van *et al.*, 2020), sorghum (*Sorghum bicolor*) (Sissoko *et al.*, 2019; Vom *et al.*, 2020). Banbara nuts (*Vigna subterranean*) in Malawi (Pungulani *et al.*, 2012) and finger millet (Ojulong *et al.*, 2017; Tarekegne *et al.*, 2019). Various varieties of finger millet have been up-scaled, released, adopted and disseminated to farmers in countries such as Tadesse, Wama, Degu and ACC#213572 for Delgi and Chilga in Ethiopia (Fentie, 2012), and U-15 and P-224 in Tanzania and Kenya (Ojulong *et al.*, 2017). Farmer ratings, comments and yield reports have

been used and shown to be a reliable practice to the researchers (Paris, 2011). In this study FVPS was conducted to assess the selected 25 varieties in order to know the preferred traits and varieties selected by farmers in Nakuru and Bomet Counties in Central Rift Kenya.

CHAPTER THREE

SCREENING OF FINGER MILLET GENOTYPES FOR RESISTANCE TO BLAST (*Pyricularia grisea*) UNDER VARIED GROWING CONDITIONS

Abstract

Pyricularia grisea is the most destructive fungal pathogen causing blast disease in finger millet hence screening for resistance across multi-locations for identification of resistant and high yielding genotypes is the safest way of controlling the disease. Twenty-five 25 selected genotypes were screened in a 5×5 *alpha* lattice design at ATC- Koibatek (1.0445° N, 35.5822° E), Bomet (0.8015° S, 35.3027° E) and Nakuru (0.3031° S, 36.0800° E) and 2 seasons 2019/2020. To determine resistance to blast and yield performance, four checks were used NKRFM1, U-15 and P-224 as resistant while KNE 741 was susceptible. A second trial involved evaluation of 12 genotypes selected from field trial (based on morphology, maturity and disease resistance/tolerance) for resistance to blast (both leaf and neck blast) in the greenhouse. Results showed that seasons, environments, environment and their interactions were significant ($P < 0.05$) in the expression of neck blast, however, the environment was not significant in the expression of leaf blast recurrence ($P > 0.05$) indicating the prevalence of the disease in the regions. In both seasons and environments, genotypes resistant to both leaf and neck blast included; Snapping-Purple, IE-2183, GBK-127189A, Gulu-E, KatFm1xU15-1.6.6.3.1.1, KNE-1124xKNE-796. These genotypes had a higher grain yield of 1.50-2.58 t ha⁻¹. IE-2183 and Snapping-Purple variety had 0% Disease Severity Index (DSI) in the greenhouse confirming high levels of resistance. Commercial checks NKRFM-1, and U-15 had average yields ranging from 2.05 and 1.83 t ha⁻¹ while P224 was among the highest yielding variety with 2.68 t ha⁻¹ as compared to susceptible check KNE-741 had a lower yield of 0.55 t ha⁻¹. Correlation analysis showed that an increase in leaf blast by 0.51 units significantly increases the chances of neck blast infections by 37% in the field conditions. The study showed a considerable variation in response to blast disease indicating that the resistant varieties could be used for further improvement through breeding. Genotypes Snapping-Purple, IE-2183, GBK-127189A, KatFm1xU15-1.6.6.3.1.1 and KNE-1124xKNE-796 could further be evaluated in multi-location for possible release as commercial varieties.

3.2 Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) is a staple food crop in South Asia and Africa. It is among the oldest domesticated cereal grain in Africa. The crop ranks third amongst the dryland cereal in importance to the world that is; sorghum (*Sorghum bicolor*)

and pearl millet (*Pennisetum glaucum*) (Onyango, 2016). The annual world production of finger millet is at 4.5 million tons. India is the largest producer of finger millet with 8.8 million tons. Africa produces 2 million of the production (FAOSTAT, 2015). East Africa production is about 800,000 ha with 470,000 ha in Uganda, 350,000 ha in Tanzania and 77,880 ha in Kenya (Onyango, 2016). It is highly resilient with a special ability to survive under extreme weather conditions of altitudes between 1000-2000 in East and Southern Africa and 2500-3000 in Himalayas. It has wide adaptability of soils of pH ranging from 5-7, well-drained sandy-loam soils to black heavy vertisols with adaptability to survive under saline conditions. Rainfall requirements can also vary from 500-1000 mm well distributed throughout the growing season (Green life Crop Protection Africa, 2018). According to FAO (2019), although finger millet production has been increasing by 4.5 million tons in 2018 the increase is not concomitant with the demand for finger millet because of the rapidly increasing human population and industrialization. To overcome the challenge, finger millet production has to be increased by at least 40% like other major cereals (Kumar *et al.*, 2018). Since to date, there is no published data on economic loss due to blast disease in finger millet (Masaki & Mbinda, 2020)

Broadly, finger millet is an important cereal crop in southern Asia and Eastern Africa with excellent nutraceutical properties, a long storage period and a unique ability to grow under arid and semi-arid environmental conditions (Masaki & Mbinda, 2020). The crop is also one of the important millet crops and has greater nutritional value due to its methionine-containing protein which is not found in rice, maize and sorghum. It is one of the novel crops containing a wide range of nutrients from calcium 358 mg/kg, iron 46 mg/kg, proteins 7.4%, zinc 0.22% and fiber. It is considered a stable food for pregnant and lactating mothers, children, diabetics and the sick (Lansakara *et al.*, 2016; Takan *et al.*, 2012).

Despite the mentioned benefits, *E. coracana* production and productivity is declining due to several biotic stress such as blast disease (*Magnaporthe grisea* (anamorph *Pyricularia grisea*), downy mildew *Sclerospora graminicola*, rust *Puccinia substriata* and seedling leaf blight *Helminthosporium nodulosum*. Insect pests include shoot flies (*Atherigona* sp. Muscidae: Diptera), spotted stem borer (*Chilo partellus*; Pyralidae; Lepidoptera), shoot bug (*Perigrinus maidis*; Delphacida; Homoptera) and caterpillars (Lepidoptera) (Bhagwat & Shyam, 2015). Avenues for improvement of crops to combat individual and multiple stresses are a major problem in Kenya causing substantial yield losses. Blast disease infects the crop from the seedling stage until the harvest period (Mgonja *et al.*, 2007; Srivastaya *et al.*, 2009). Small, spindle-shaped white spots appear at the initial stage of infection which later turns to

grey or white with a brown or black margin at the point of infection. Infection of the panicle is the most severe with yield losses of above 80% reported. It leads to no seed formation or formation of shriveled seeds on the panicle leading to a total yield loss. The infection starts from the tips downwards.

Due to the importance of finger millet blast disease, which may reduce the crop grain yield to levels of up to 100%, effective disease control measures are needed to ensure global food security, especially in arid and semi-arid regions of African and Asia where the crop is majorly cultivated. Over the years, various pursuits have been made to develop new cultivars that are resistant to the disease such as the development of U-15 and P-224 in Uganda (Manyasa *et al.*, 2019). Control and management of diseases largely depend on chemicals which are usually costly to the marginal farmer.

Understanding the weather patterns especially rainfall, temperature and relative humidity and how they play a role in disease infection and development is important (Takan *et al.*, 2013). Yield losses due to blast have recorded a loss of 90% and provision of knowledge on the host-pathogen and environment is important (Babu *et al.*, 2016). Furthermore, the frequent breakdown of blast resistance because of the susceptibility to rapidly evolving virulent genes of the pathogen causes yield instability in all finger millet growing areas (Masaki & Mbinda, 2020). Therefore, the deployment of novel and efficient strategies that provide dynamic and durable resistance against many biotypes of the pathogen and across a wide range of agro-ecological zones guarantees future sustainable production of finger millet. For a long time, the interest and priority in the research of finger millet has increased compared to other cereals like maize and rice. The limited genomic resources of finger millet have greatly hampered studies of the genetics of resistance to the blast disease compared with other major cereals (Masaki & Mbinda, 2020). As a result of these shortcomings, the understanding of broad-spectrum resistance to finger millet blast disease remains a knowledge gap.

On the other hand, HPR is a mechanism triggered upon pathogen attack that prevents the attack of the pathogen to host tissues, therefore, offering resistance. It is an effective method in terms of cost, environmentally friendly for the control of diseases and pests with the inclusion of *P. grisea*. Host plant resistance has been successful in the management of head blight (*Fusarium graminearum*) in wheat (Rudd *et al.*, 2001), rice blast (*Magnaporthe grisea*) in rice (*Oryza sativa*) (Sharma *et al.*, 2012), Zebra chip disease (*Candidatus Liberibacter solanacearum*) in potato. It has been recommended for the management of finger millet blast (*Pyricularia grisea*) since the marginal farmers who cannot

be able to access the expensive chemicals are able to adapt and use new and improved varieties which are resistant to the disease as well as high yielding (Manyasa *et al.*, 2019). Although screening and selection of finger millet cultivars or new advanced lines toward selected strains of blast fungi have been successful in many breeding programs, caution is needed to avoid the risk of limited success since the method relying primarily on the phenotypic features and virulence tests using various hosts which are extremely variable due to the genetic instability of the blast pathogen (Que *et al.*, 2019; Rasool *et al.*, 2020). The main challenge of the breeders is to develop new varieties with a broad spectrum to the environmental impacts and resistance to diseases which takes huge costs and longer periods, additionally enhancing quick adoption. Hence the knowledge of the pathogen and host plant resistance of diverse genotypes of finger millet across different geographical locations is helpful in identification of multi-location and controlled environment offers resistant lines with stable and durable resistance. The objective of the study was to screen for host plant resistance to blast disease of selected finger millet genotypes under different growing conditions.

3.3 Materials and methods

3.3.1 Environment description

This study was conducted in three environments in Kenya selected as major growing areas: at Agricultural Training Centre (ATC)-Koibatek, Baringo County. ATC Longisa, Bomet County, ATC- Nakuru County. These sites were selected because of the high occurrence of blast disease in the areas and farmers' interest in the crop. Koibatek ATC is located at longitude 35° 58' 0.01" E and latitude 0° 28' 0.01" N. It has a low agricultural potential with a low altitude region of 1890 meters above sea level in agro-ecological zone UM4. Mean annual rainfall is 767 mm and mean temperature ranges between 18-24.3°C. Soils are *Vitric Andosols* with moderate to high soil fertility, well-drained deep to the sandy soils with pH of 5.2. ATC Bomet, the experiment was done at ATC Bomet, which lies at longitude 35°20'29.62" E and latitude of 0° 46'52.64" N. It is a medium-altitude zone with an evenly distributed rain throughout the year and a mean rainfall of 1000-1400 mm. The mean monthly temperature is 17.2 °C. ATC Nakuru lies at a longitude of 36°04'0.01" E and a latitude of 0°16'59.99" N with a mean annual rainfall of 1012 mm, well-distributed and temperature ranges of 15°C-20°C, respectively. Soils are *Mollic Andosols*, well-drained dark reddish-brown. (Jaetzold *et al.*, 2012).

The greenhouse experiment was conducted at Egerton University 0.3714°S, 35.94 10°E. The selection was based on the susceptibility and resistance of the genotypes from the

field conditions for further confirmation of the reaction levels under controlled conditions (Iqbal *et al.*, 2010).

3.3.2 Genotypes

Twenty-five finger millet genotypes were screened for resistance to *Pyricularia grisea* under field conditions. The genotypes were sourced from ICRISAT, Egerton Seed Unit, KALRO, Genebank of Kenya and Local varieties. NKRFM1, U15 and P224 are commercial that were used as resistant checks while KNE-741 was used as a susceptible check since they have varied levels of resistance, phenology and maturity depending on the research done on the varieties (Table 3.1).

Table 3.1: List of Finger millet genotypes evaluated ATC Bomet, Koibatek and Nakuru

NO	Genotype	Source	Description
1	P-224	Kenya Seed	Commercial variety
2	U-15	KALRO	Commercial, Red early maturing
3	NKRFM1	Egerton	Commercial Red early maturing
4	KatFm1×U15-1.7.8.2.1	KALRO	Advanced breeding line
5	IE 615	ICRISAT	Advanced breeding line
6	KNE1034	ICRISAT	Advanced breeding line
7	GBK027189A	Gene Bank	Advanced breeding line
8	IE 2183	ICRISAT	Advanced breeding line
9	IE 2872	ICRISAT	Advanced breeding line
10	Kal Dokolo	Local	Local land race
11	Otiyo brown	Local	Local land race
12	Kal 2 Pader	Local	Local land race
13	KNE 628	ICRISAT	Advanced breeding line
14	Kal pader	Local	Local land race
15	Snapping purple	Egerton	Commercial variety
16	KAT FM1	KALRO	Commercial variety
17	EUFM 2 (KNE 741)	Egerton	Early maturing and escapes drought
18	Ikhulule	Local	Moderately tolerant to blast
19	Gulu E	ICRISAT	Moderately tolerant to blast
20	EUFM4	Egerton	Newly released commercial for lowlands
21	EUFM1 (KNE 629)	ICRISAT	High tillering and late maturing
22	Snapping green early	Egerton	Early maturing, easy for harvesting
23	KATFM1×U15 1.6.3.3.1	KALRO	Advanced breeding line
24	KNE 1124×KNE 796	ICRISAT	Advanced breeding line
25	KATFM1×U151.6.6.3.1.1.	KALRO	Advanced breeding line

Greenhouse experiment involved a total of 12 selected finger millet genotypes with different diseases reactions from the experiment conducted in the field including susceptibility, moderately susceptible, moderately resistant and resistant. This was a confirmatory test for the genotypes. (Table 3.2).

Table 3.2: List of Finger millet genotypes evaluated in greenhouse

No	Genotype	Source	Description
1	KNE-741	Egerton	Commercial variety
2	U-15	KALRO	Commercial variety
3	KNE-1034	ICRISAT	Advanced breeding line
4	KalPader	Local	Local landrace
5	Katfm1x u151.7.8.2.1	KALRO	Advanced breeding line
6	KNE-629	ICRISAT	Commercial variety
7	KNE1124XKNE796	ICRISAT	Advanced breeding line
8	GULU-E	ICRISAT	Commercial variety
9	SDFM-1702	Egerton	Advanced breeding line
10	Katfm1xu151.6.6.3.1.1	KALRO	Advanced breeding line
11	Snapping-Purple	Egerton	Commercial variety
12	IE-2183	ICRISAT	Advanced breeding line

3.3.3 Experimental procedure

a) Experiment layout

Land preparation was done as recommended. In order to achieve good soil tilth for finger millet planting and for fast germination of small-seeded grain, a fine tilth was obtained through harrow. The experiment was laid in an alpha lattice design (5 × 5) design with three replications. A plot of 2 m × 2 m with 4 rows spaced at 0.3 m apart with a plant-to-plant distance of 0.15 m was used. The experiment was conducted in 2 seasons. June-December 2019 and March-September 2020. Susceptible genotype (KNE 741) was planted in all three environments around each plot as a disease spreader to enhance disease development and spread to the test genotypes. Planting was done using recommended 50 kg/ha nitrogenous fertilizers NO₃⁻ of 19.36 kg/ha and NH₄⁺ of 5.62 kg/ha with DAP of 18.46.0 as a source and a rate of 25 kg/ha with 26% N CAN as a source which was the top-dresser. Weed was controlled manually by the use of hand hoe. Data were taken on 5 plants randomly selected

and tagged in all the plots at the two center rows to avoid the border effect at all environments.

b) Greenhouse experiment

Five seeds were disinfected and planted 2 cm below the soil surface in medium-size pots of 30 cm diameter and 30 cm in depth filled with a sterile mixture of black soils (Vertisols) and forest soil autoclaved at 121°C a 103.42 kPa in a Randomized Complete Block Design (RCBD) with three replicates. Soils for sowing were prepared using, a mixture of sand and topsoil, mixed in a ratio of 3:1 and poured into pots to about $\frac{3}{4}$ pot full. Three liters of water were then mixed with an equivalent of 50 Kg ha⁻¹ of Diammonium phosphate during planting and 50 Kg ha⁻¹ of Calcium Ammonium nitrate was applied at head emergence in each pot.

c) Isolation of *Pyricularia grisea* from diseased tissue and inoculation of test genotypes in the greenhouse

Isolation of the fungus was done from diseased tissue picked from a susceptible variety KNE 741 ported at Egerton University Biological Science Laboratory. Isolation was done using Tuite (1969) standard tissue isolation procedure (Tuite, 1969). The margin of the infected tissues was cut in triangular shapes of 5-10 mm with the inclusion of both diseased and living tissue. The tissues with lesions were surface sterilized with 0.5% sodium hypochlorite solution to remove contaminants, then dipped in sterile distilled water for two seconds to saturate the specimen and dealcoholize (Aneja, 2005). The dipped tissues were then laid in glass plates with filter paper to dry the excess water. They were then placed on growth media Oat Meal Agar (OMA) which contains; 60 g Oat Meal, 12.5 g/L Agar with a pH of 7.2. OMA was amended with 60 mg/l Neomycine sulfate to avoid bacterial contamination (Tredway *et al.*, 2002). The solution containing Oat Meal and the amendments were autoclaved at 121°C at 15 pounds pressure and left to cool for 20 minutes. The tissues were then incubated at 25 ± 1°C in artificial light on a 12 h light/dark photoperiod for 15–25 days for sporulation and growth of the fungi (Aneja, 2005). Pure colonies were obtained from each region through five subsequent sub-culturing.

The spore suspension was prepared by flooding the plates with distilled water and scrapping the growth of the fungi (Gashaw *et al.*, 2014). Extraction of spores was done by pipetting 10 ul of the spore solution and sieved using a muslin sieve to remove the media. After preparation of the spore solution, 10 ul of the spore solution was placed on a

hemocytometer and the number of spores was counted on chambers A, B, C, D and E. The spores were then adjusted to determine the number of spores per ml by multiplying the value by 2000. The spore suspension was then adjusted to desired concentration (1×10^5 spores/cm³) with the help of a hemocytometer using the C1V1 formula and 0.01 % Tween 20 a surfactant which amends the properties of the carrier to ensure it dissolves added. The suspension was then sieved through a double layer of muslin sleeve then poured into a calibrated hand sprayer.

After 10 days of emergence, thinning was done and three plants were retained. Inoculation was done at two-week intervals from the seedling stage to the boot stage with 5-7 ml of the spore suspension from the most virulent pathogen isolated from diseased plant. This was done after sunset to benefit from darkness and higher humidity during the night. A conducive environment for the disease was provided in the greenhouse through frequent mist sprays of the plants and the surrounding environment with sterile water to maintain high humidity level i.e., >80% after inoculation and temperature of 30°C (Nagaraja *et al.*, 2010). The plants were then covered with white parchment bags for 24 hours to allow the spore attachment to the host. The inoculated plants were monitored daily for blast development and disease evaluations 21 days after inoculation and scores on leaf and neck severity plus the latent period of infection was measured.

3.3.4 Data collection

a) Disease data collection

Disease severity rating (DSR) (% damage) was done on the first four leaves (flag) where five plants were randomly tagged per plot. Disease severity on tagged plants was recorded at tillering, flowering, and physiological maturity stages on five randomly selected and tagged individual plants. The selected plants were from each plot in the field and the greenhouse respectively using modified Cobb scale (Kiran *et al.*, 2017). Leaf blast was scored based on the percent surface area of the infected leaves and evaluated as *P. grisea* severity (Table 3.3). Neck blast severity was based on the relative lesion size on the neck a 1 to 5 progressive rating scale was used where 1 = no lesions to pinhead size of lesions on the neck region, 2 = 0.1 to 2.0 cm size of typical blast lesion on the neck region, 3 = 2.1 to 4.0 cm, 4 = 4.1 to 6.0 cm, and 5 = >6.0 cm size of typical blast lesion on the neck region (Figure 3.1). The data was then subjected to AUDPC and terminal severity data was used to compare cultivars as resistant, moderately resistant, susceptible and moderately susceptible.

Table 3.3: A quantitative severity scale for foliar blast disease on finger millet

Scores	Reaction category	Appearance of genotypes
1	Highly resistant	Less than 10% of the leaves damaged
2	Resistant	11-20% of the leaves damaged
3	Intermediate	31 to 40% of the leaves damaged
4	Susceptible	51 to 70% of the leaves damaged
5	Highly susceptible	71 to 100% of the leaves damaged

Source; (Babu *et al.*, 2015)



Figure 3.1: Panicle showing peduncle damage rating where 1- no symptom and 5- severe symptom. Source: Babu *et al.* (2015)

b) Agronomic data collection

Data on yield and yield parameters taken in all 3 sites (Bomet, Nakuru and Koibatek) included measurements of five randomly selected plants from the five tagged plants.

The number of tillers which were counted on the five plants while plant height was measured using 100 cm ruler from the base to the tip of the panicle on the five tagged plants selected randomly in the middle row. Plants were evaluated flowering dates when plots had attained 50% flowering. When the crop had 50% DTM (Days to Maturity), the number of fingers per panicle was counted and the length of the panicles was measured using a 30 cm ruler for the straight type of fingers and a string was used to measure the folded fingers and the string placed on a ruler.

Grain yield was measured by harvesting the heads from the net plot area (1m²) (2 middle rows) manually using a knife. They were then sun-dried for 7 days, threshed using sticks and cleaned by winnowing. After obtaining the seeds it was further sun-dried to 15% moisture content for storage. Each plot was weighed separately and the plot yield converted

to (t /ha). The 1000 KW and 100 KW seed weight was measured using an electronic seed counter Contador.

Weather data was recorded periodically on all the environments during the first and second seasons in all the sites. In order to measure weather parameters, rainfall was measured using a rain gauge, maximum and minimum temperatures measured using a thermometer and humidity using a hygrometer (Table 3.4).

Table 3.4: Temperature, rainfall and relative humidity recorded in ATC- Nakuru, Baringo and Bomet counties during the short rain season (2019) and the long rain season (2020)

NAKURU 2019						NAKURU 2020					
Month	Temperature		Rainfall (mm)	Relative humidity		Month	Temp		Rainfall (mm)	Relative humidity	
	Max (°C)	Min (°C)		Max (%)	Min (%)		Max (°C)	Min (°C)		Max (%)	Min (%)
JUN	24.23	14.06	104.5	65	35	MAR	27.87	14.19	120.4	70	55
JULY	24.22	12.71	98.05	66	29	APR	25.76	14.5	228.9	79	75
AUG	24.45	11.29	44.06	63	25	MAY	25.70	14.12	89.4	75	69
SEPT	26.00	12.23	62.06	60	25	JUNE	24.7	13.6	113.5	72	65
OCT	24.16	13.19	197.66	60	28	JULY	23.61	13.35	137.3	76	68
NOV	24.90	13.60	86.27	62	39	AUG	24.87	13	105.1	57	23
DEC	24.22	13.45	236.45	58	10	SEPT	26	12.48	86.8	60	21

BARINGO 2019						BARINGO 2020					
Month	Temperature		Rainfall (mm)	Relative humidity		Month	Temp		Rainfall (mm)	Relative humidity	
	Max (°C)	Min (°C)		Max (%)	Min (%)		Max (°C)	Min (°C)		Max (%)	Min (%)
JUN	27.3	19.00	101.1	93	48	MAR	28.96	18.51	235.4	45	0
JUL	27.8	17.74	145	90	45	APR	28.93	19.1	215.2	75	9
AUG	28.96	17.64	39	93	45	MAY	28.09	18.96	90.0	89	15
SEPT	29.13	17.76	326	81	30	JUN	27.3	18	77.1	79	10
OCT	28.51	18.16	299.9	83	35	JUL	27.25	17.80	200.3	98	20
NOV	28.46	18.56	230.2	87	39	AUG	28.38	17.25	87.9	45	0
DEC	27.45	18.25	181.9	65	29	SEPT	28.55	17.52	80.9	45	5

BOMET 2019						BOMET 2020					
Month	Temperature		Rainfall (mm)	Relative humidity		Month	Temp		Rainf all (mm)	Relative humidity	
	Max (°C)	Min (°C)		Max (%)	Min (%)		Max (°C)	Min (°C)		Max (%)	Min (%)
JUN	24.20	14.03	231.73	59	30	JAN	25.8	13	36.3	39	31
JULY	24.19	12.67	126.40	80	21	FEB	27.65	13	41.3	32	30
AUG	24.39	11.29	87.17	74	20	MAR	27.87	14	120.4	60	35
SEPT	25.93	12.36	72.64	77	20	APR	25.77	15	228.9	82	60
OCT	24.16	14.22	229.19	80	25	MAY	26	14	113.5	83	58
NOV	24.90	13.60	99.52	67	39	JUN	24.7	14	137.3	55	30
DEC	24.19	13.77	248.81	40	25	JULY	23.6	13	105.1	30	21

3.3.5 Data analyses

Area Under Disease Progress Curve (AUDPC) was used to estimate the severity of leaf and head blast disease in finger millet. It was computed using Wilcoxson *et al.* (1975) formula (Equation 1).

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

second variable xy = product of the two paired scores (Herrero *et al.*, 2011).

Analysis of variance for green house experiment was computed using PROC GLM in Statistical Analysis Software (SAS institute Inc; Cary, 2002) using the following model;

$$Y_{ij} = \mu + B_i + R_j + \varepsilon_{ij} \dots \dots \dots \text{Equation 5}$$

Where, Y_{ij} is the overall observation, μ is the overall mean, B_i is the i^{th} effect due to blocks, R_j is the j^{th} observation due to variety and ε_{ij} is the random error term. Leaf and neck blast severity was utilized in Area Under Disease Progress Curve (AUDPC) using the Wilcoxon *et al.*, 1975 formula above (Equation

Mean separation was done using Tukey Honest Significant Difference (HSD) using the formula;

$$R = q[\alpha, p, fe] \times \sqrt{\frac{MSE}{r}} \dots \dots \dots \text{Equation 6}$$

Where R . Tukey's at 5% level of significance, q = Number of treatments mean, fe =Degree freedom of error, α = Level of significance, MSE = mean square error and r =Number of replicates.

Correlation was done to determine the effect of leaf blast severity on neck blast and agronomic traits (yield, panicle weight and 1000 KW) using equation 4 on 3.3.5.1 above.

3.4 Results

3.4.1 Host plant resistance to leaf blast severity of finger millet genotypes in varied agro-ecologies (Nakuru, Bomet and Koibatek)

The combined ANOVA showed that there were significant ($P < 0.05$) mean differences in environment (E), season (S), and genotype (G). There were effects due to interactions; $E \times S$, $R \times E$, $S \times R$, $E \times S \times R$, $G \times E$, $G \times S$ and $G \times E \times S$ which showed effects on the severity of both foliar and neck blast test genotypes as shown in Table 3.5. The significant difference ($P < 0.05$) in seasons showed that the long rain season (2020) had a significantly ($P < 0.05$) higher leaf severity (72.99) compared to the short rain season (2019) (39.42) (Appendix 2; Figure 3.2).

Overall combined means of leaf severity value (AUDPC) was 56.2 with Koibatek having the highest mean of 59.6 (35.36%) while Nakuru and Bomet did not differ significantly ($P < 0.05$) with a mean value of 55.2 (31.75%) and 53.76 (31.88%), respectively

(Appendix 2). The results further indicated that during short rain season one (2019), the interaction between each environment and variety had a strong influence on leaf blast severity ($P \leq 0.001$), however, all environments were not significantly different ($P > 0.05$) on the occurrence of leaf severity (Appendix 2). Bomet had a mean of 39.89 (33.71%) followed by Nakuru 39.77 (33.60%) and Koibatek 38.67 (32.67%) (Appendix 2).

In general, during season one, mean separation of varieties from the combined analysis revealed that KNE-741, P-224, KNE-1034, Kat-Fm1 were susceptible to leaf blast with mean severity values of ≥ 64 in all the environments in contrast to GBK-127189A, Snapping-Purple, KNE-1124 x KNE-796, Gulu-E and U-15 were resistant to leaf blast with mean severity values of ≤ 49 (Table 3.6).

In season two, long rain season (2020), the interaction G x S had a strong influence on leaf blast severity. Comparison of environments indicated that Koibatek had a significantly ($P < 0.05$) higher mean severity 82.73 while Nakuru and Bomet did not differ significantly ($P < 0.05$) with 70.39 and 68.94 respectively (Appendix 2). Mean separation of varieties in season two revealed genotypes resistant to leaf blast during this season included GBK-127189A, Kal Pader, Kal Dokolo, IE-615, Kat Fm1xU15 1.6.6.3.1.1 and KNE1124 x KNE796 had severity values ≤ 66 and therefore rated as resistant. In contrast, genotypes P224, Kal 2 Pader, Snapping green early, KatFm1x U15 1.6.3.3.1, KNE 1034 and SDFM-1702 had severity values of ≥ 83 and were susceptible to leaf blast (Table 3.6).

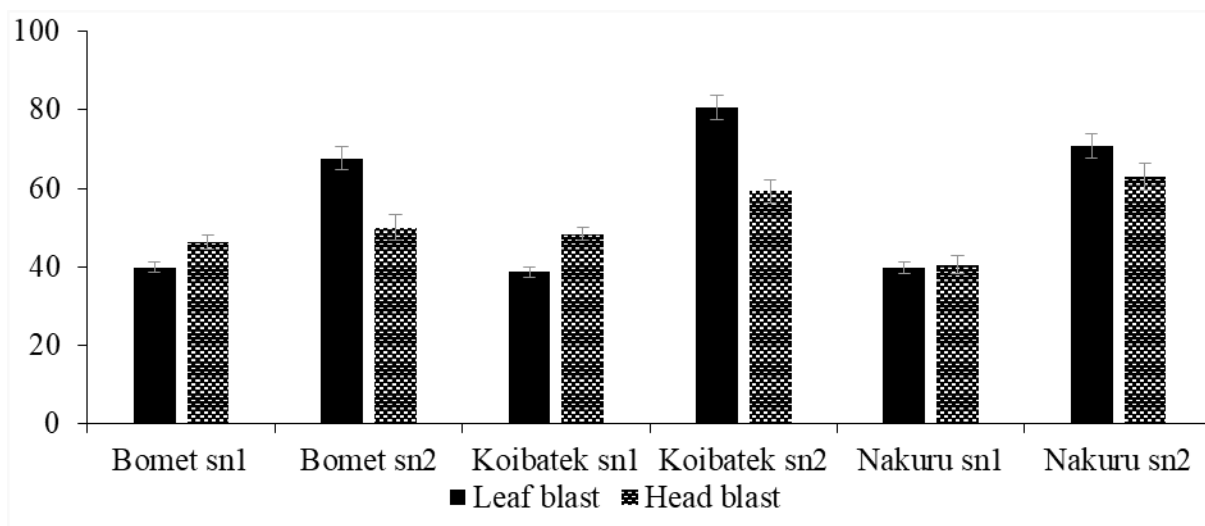


Figure 3.2: Leaf and neck blast severity during the first and second seasons of the three environments. * sn1=Season 1, sn2= Season 2

The combination of both seasons showed that genotypes including Snapping-Purple, IE-2183, GBK-127189A, Gulu E and Kal 2 Pader were resistant to leaf blast with AUDPC values of ≤ 33 in Nakuru. P-224, IE-2183, Snapping green, GBK-127189A and SDFM-1702 were resistant in Koibatek with ≤ 31 . Snapping-Purple, IE2183, GBK127189A, GULUE, IE2872 and Kal Pader were resistant in Bomet with ≤ 33 . Resistant check P224 had 36, 24.5 and 44 AUDPC mean values in Nakuru, Koibatek and Bomet respectively (Table 3.6).

Overall, genotypes resistant to leaf blast in all environments combined included GBK-127189A (40.88), Snapping-Purple (45.13), KNE 1124 x KNE796 (42.27), Gulu E (48.93) and U15 (49.93) while varieties KNE 741 (70.11), P224 (66.72), KNE 1034 (65.14), KatFm1 (64.23) were the susceptible to leaf blast in all the environments and seasons (Appendix 1).

Table 3.5: Combined ANOVA table for finger number, finger length, height, tillers, days to maturity, kernel weight, Biomass, yield, harvest index, leaf and neck blast AUDPC of 25 finger millet genotypes for the short rain season (2019) and long rain season (2020)

Source of variation	df	Finger number	Finger length	Height	Tillers	Days to maturity	Kernel weight
Environment (Env)	2	75.29***	85.16***	38561.72***	130.12***	651.68*	0.59*
Season	1	215.75***	110.60***	2797.61***	2337.86***	6483.84***	55.74***
Environment × Season	2	127.69***	123.84***	11906.51***	144.49***	729.75*	22.95***
Replicates (Rep)	2	1.41	2.03	50.57	12.50**	307.01	0.02
Environment × Replicates	4	9.78***	1.41	224.72	27.18***	565.52*	0.04
Season × Replicates	2	12.62***	0.32	947.82**	3.90	37.04	0.09
Environment × Season xRep	4	1.25	3.75**	122.71	9.83*	88.91	0.17
Block (Replicates)	12	1.64	3.49***	286.20*	9.69***	183.93	0.09
Genotype	24	4.59***	8.55***	227.77*	15.93***	233.67***	0.65***
Environment × Genotype	48	4.28***	5.56***	193.05*	10.89***	380.44	0.32***
Season × Genotype	24	5.53***	5.14***	227.52*	17.17***	207.12	0.59***
Env × Season × Genotype	48	5.26***	5.60***	181.59*	9.00***	164.35	0.29***
Error		1.29	1.04	123.63	2.44	161.39	0.12
CV		18.73	15.62	17.36	21.96	13.47	12.61
R ²		80.72	83.56	80.93	88.34	55.50	83.02

*Significant at P<0.05, **P<0.01 and ***P<0.001 respectively

Table 3.5. Continued...

Source	df	LAUDPC	NAUDPC	Biomass	Yield t/ha	Harvest index
Env	2	1405.84**	1242.94**	11.46***	263.17***	62.20***
Season	1	126711.14***	17020.60***	0.65	583.55***	175.02***
Env × Season	2	2073.50***	3383.76***	3.51*	275.55***	65.44***
Rep	2	3094.95***	416.72	0.70	0.77	0.25
Env × Rep	4	506.67*	367.71	1.15	1.60	0.73
Season × Rep	2	1906.56***	530.87	1.29	0.86	0.24
Env × Season × Rep	4	206.09	191.57	0.58	3.26***	0.86
Blocks (Rep)	12	427.35**	1011.24***	0.92	2.18***	0.39
Genotype	24	655.25***	1410.00***	2.82***	4.24***	0.74
Env × Genotype	48	571.04***	1152.79***	1.56**	3.81***	1.06***
Season × Genotype	24	877.52***	1106.46***	2.49***	4.29***	1.19**
Env × Season × Genotype	48	813.10***	996.81***	0.70	4.01***	0.08***
Error		174.82	183.80	0.88	0.49	0.47
CV		23.52	26.46	39.06	33.64	66.93
R ²		84.55	80.66	56.22	94.52	82.06

*LAUDPC = Leaf blast severity expressed as AUDPC, NAUDPC= Neck blast severity expressed as AUDPC and AUDPC= Area under disease progress curve. *** = p<0.001, **=p<0.01 and *=p<0.05 respectively.

3.4.2 Host plant resistance to neck blast severity of selected genotypes in varied agro ecologies (Nakuru, Bomet and Koibatek)

Results from combined ANOVA indicated that E, S, E x S, Replications (R) and the interactions E x S, R x S, R x E, E x S x R, E x G, S x G and E x V x S had a significant effect ($P \leq 0.01$) on the severity of neck blast on the selected finger millet genotypes (Table 3.5). The long rain season (2020) had a significantly ($P < 0.05$) high neck blast severity compared to the short rain season (2019) (Appendix 1). When the environments were compared, all were significantly different, Nakuru had a high neck blast severity differ significantly ($P < 0.05$) with AUDPC mean severity in the two environments with 53.81 and 51.73 AUDPC values, respectively (Appendix 1).

In both seasons, when compared to the resistant and commercial check P224, genotypes Snapping-Purple, IE-2183, GBK-127189A, Gulu E, Kal 2 Pader and IE-2183 were resistant to neck blast with AUDPC mean severity values of ≤ 33 in Nakuru KATFM1 x U15 1.6.6.3.1.1, IE-2872, Snapping-Purple and KATFM1x U15 1.7.8.2.1 were resistant in Koibatek with mean severity of ≤ 36 . Genotypes Snapping-Purple, IE2183, GBK127189A, GULUE and IE-615 were resistant in Bomet with AUPDC mean severity of ≤ 30 (Table 3.6; Appendix 2).

During the short rain season (2019), the ANOVA table indicated that environment, variety, the interaction between environment and variety had a strong influence on neck blast severity ($P \leq 0.01$) (Appendix 3) 64.77 in contrast to Koibatek and Bomet with AUDPC mean severity of 59.27 and 48.94 respectively 46.52 respectively while Nakuru had the lowest mean of 41.89 (Appendix 5).

In season two, long rain season (2020), ANOVA indicated that variety, the interaction of variety and environment had a strong influence on neck blast severity ($P < 0.001$) (Appendix 4). One environment was significantly different ($P < 0.01$) with lower head infections expressed in Bomet as compared to Nakuru and Koibatek and AUDPC mean values of 48.71, 51.73 and 53.81 respectively (Appendix 5).

Table 3.6: Leaf and neck AUDPC of 25 varieties planted in varied agro-ecologies (Nakuru, Koibatek and Bomet ATC regions)

	Nakuru ATC				Koibatek ATC				Bomet ATC			
	LAUDPC		HAUDPC		LAUDPC		HAUDPC		LAUDPC		HAUDPC	
	SN1	SN2	SN1	SN2	SN1	SN2	SN1	SN2	SN1	SN2	SN1	SN2
GBK	30.3	31.4	22.1	29.8	28.0	33.8	33.8	30.8	31.5	30.3	29.1	22.1
127189A												
GULU E	31.5	31.5	21.0	29.5	36.1	56.0	56.0	44.6	31.5	31.0	35.0	21.0
IE 2183	31.5	29.1	21.0	29.1	22.1	33.8	33.8	35.6	29.7	31.5	29.7	21.0
IE 2872	33.8	38.7	40.8	38.5	37.3	39.6	39.6	42.8	33.8	32.6	38.5	47.5
IE 615	31.5	35.0	26.8	35.0	36.1	39.6	39.6	34.5	35.0	36.5	35.0	25.4
IKHULUL	33.8	42.0	43.1	40.8	43.1	54.8	54.8	68.1	42.0	39.6	40.3	54.8
E												
KAL 2	35.0	31.4	31.5	35.0	37.3	46.6	46.6	42.8	29.1	38.5	35.0	31.5
PADER												
KAL	42.0	46.6	43.1	57.1	37.3	43.1	43.1	72.3	46.6	42.0	57.1	34.8
DOKOLO												
KAL	35.0	35.7	36.1	44.3	56.0	69.1	67.6	36.5	38.5	47.1	44.3	42.8
PADER												
KATFM1	47.8	54.1	52.5	70.0	49.0	66.5	66.5	62.5	54.8	57.8	70.0	52.5
KATFM1×	38.5	30.3	54.8	29.1	40.8	32.6	66.5	44.6	42.0	40.8	46.6	42.0
U15.1.6.3.												
3.1												
KATFM1×	40.8	39.9	42.0	46.6	29.1	66.5	32.6	36.5	30.3	38.5	29.1	54.8
U15												
1.6.6.3.1.1												
KATFM1	43.1	43.1	47.8	53.6	32.6	44.3	44.3	38.3	43.1	43.1	53.6	47.8
× U15												
1.7.8.2.1												
KNE 1034	38.5	51.3	29.1	65.4	40.8	63.0	63.0	58.6	51.3	38.5	63.0	29.1
KNE 1124	31.5	37.3	33.8	47.8	31.5	39.6	39.6	40.9	38.5	30.3	47.8	33.8
× KNE 796												
KNE 628	51.3	41.9	71.1	43.1	43.1	60.6	60.6	77.1	45.5	51.3	43.1	71.1

KNE 629	36.1	41.1	33.8	49.0	38.5	47.8	47.8	44.6	39.6	36.1	49.0	33.8
KNE 741	68.8	60.7	78.1	77.0	66.5	77.0	77.0	78.6	60.6	65.4	77.0	78.1
NKR FM1	38.5	54.6	59.5	65.3	49.0	59.5	59.5	36.5	56.0	38.5	65.3	59.5
OTIYO	49.0	37.3	52.5	49.0	43.1	45.0	46.6	53.5	37.3	49.0	49.0	52.5
BROWN												
P-224	44.3	29.1	40.8	29.1	24.5	19.8	19.8	27.4	29.1	44.3	29.1	29.4
SDFM	33.8	34.4	25.6	50.1	26.8	36.1	36.1	46.5	36.1	32.6	50.1	25.6
1702												
Snapping- Green- Early	39.6	37.4	57.1	52.5	29.1	29.1	39.6	40.6	36.1	39.6	52.5	57.1
Snapping- Purple	29.1	24.5	21.0	23.8	35.0	35.0	33.8	39.3	24.5	28.0	31.5	21.0
U-15	32.6	57.6	37.3	66.5	49.0	49.0	60.6	61.8	57.1	28.9	66.5	22.9
Means	39.7	40.5	70.3	66.2	38.6	48.3	82.7	60.8	39.8	46.3	68.9	50.7

In general, genotypes AUDPC values showed that NKRFM1 (71.43), KNE-628 (66.16), Etiyo Brown (64.96), Kal Dokolo (61.10) and KATFM1 (61.10) were the susceptible to neck blast when compared to the susceptible KNE 741 (60.6-78.1) (Figure 3.3) while genotypes resistant to neck blast included, Snapping-Purple (33.19), GBK-127189A (35.37), SDFM-1702 (40.64), P224 (41.86), Kat Fm1xU15 1.6.6.3.1.1 (43.02), IE-2183 (43.94) and GuluE (44.70) in all the environments and seasons (Appendix 2).



Figure 3.3: Genotype KNE 741 showing its susceptibility to both leaf and neck blast

3.4.3 Genotypic variation on yield and yield traits of finger millet varieties

Results from the combined ANOVA across seasons and environments (Table 3.5 and appendices 4 and 5) indicated that environment, season, environment x season, variety, environment x variety, season x variety, environment x season x variety had a significant ($P < 0.05$) effect on yield and yield traits of finger millet genotypes. The overall grand mean yield from the two seasons (2019-2020) in all the three environments was 1.68 t ha^{-1} . Season two (2020) had the highest mean yield of 1.99 t ha^{-1} while season one had 1.38 t ha^{-1} (Appendix 1) Bomet had the highest yield across all seasons with a mean of 2.06 t ha^{-1} , followed by Nakuru with 1.53 t ha^{-1} while Koibatek recorded the lowest yield of 1.46 t ha^{-1} (Appendix 1).

When comparing seasons and environments, Bomet season two had high performance compared to season one with 2.72 and 1.39 t ha^{-1} respectively (Appendix 1). Overall, the high yielding genotypes in Bomet included Kal Dokolo, IE-615, KNE 741, Ikhulule, IE-2872, P224, KatFm1 \times U15 1.6.3.3.1 with yields $\geq 2.2 \text{ t ha}^{-1}$ respectively (Appendix 2: Figure 3.4).

In Koibatek, season two performed highly compared to season one with 1.67 and 1.25 t ha^{-1} respectively. Yield was generally lower in season one ranging from 1.12 - 1.48 t ha^{-1} in the first season in contrast to season two which ranged from 1.43 - 1.90 t ha^{-1} . Generally, the high yielding genotypes in Koibatek included KatFm1 \times U151.6.6.3.1.1, KNE 1034, Kal-Dokolo and Kal-Pader with 1.57 , 1.58 , 1.60 and 1.64 t ha^{-1} (Figure 3.5).

In Nakuru, season two performed slightly higher than season one with 1.56 and 1.50 t ha^{-1} respectively while season two had yields ranging from 1.16 - 1.75 t ha^{-1} . Genotypes such as Ikhulule, KAT FM1, GBK 027189A and Gulu E were high yielding genotypes in Nakuru with yields of 1.70 , 1.72 , 1.72 and 1.82 t ha^{-1} respectively (Figure 3.6).

In general, genotypes performed differently across all environments and seasons. Mean separation of varieties across all environments and season showed that Kal Dokolo, Ikhulule, KAT-FM1, P-224, IE- 2872, Gulu E, IE-615 recorded yield values of 1.83 , 1.81 , 1.79 , 1.78 , 1.76 and 1.76 t ha^{-1} respectively (Appendix 2).

The response on yield showed that there was variation amongst the genotypes on yield components which included the number of tillers, number of fingers, length of fingers, days to maturity, 1000KW, biomass and harvest index. The comparison of environments showed that Koibatek, Bomet and Nakuru had various maturity period of 96.69 , 94.06 and 92.23 days after planting respectively. In Bomet, genotype days to maturity ranged from 62 to 106 days while in Koibatek maturity days ranged from 88 to 105 , this was in contrast to Nakuru which varied from 79 to 103 . The susceptible genotypes such as the check KNE-741

had the earliest maturity of 62, 91 and 93 days in Bomet, Koibatek and Nakuru respectively. Genotypes with middle range maturity included the number of tillers differed among the genotypes with a range of 5 - 9 tillers per plant.

The high tillering varieties with >7 tillers included SDFM 1702, Snapping-Purple, IE-615, U-15, IE-2872, Gulu-E, GBK-127189A, KAT-FM1 and Kal-Pader. Finger length varied from 4-7 cm with genotypes Snapping-Purple variety and NKR FM1 having the highest finger length (7 cm) while genotypes KATFM1×U151.6.3.3.1, Kal 2 Pader and KATFM1×U15-1.6.6.3.1.1 had small finger lengths of > 5.5 cm.

Biomass of fingermillet varieties planted in all environments ranged from 1.7 kg to 3.6 kg. The biomass of KNE-628, Kal-2-Pader KNE-1124×KNE-796 and NKR FM1 genotypes were high with >2.8 kg while Snapping-Purple variety had the lowest biomass of 1.72 kg. 1000 kernel weight (KW) had varied mean values ranging from 2.3 g to 3.1 g, genotypes KATFM1×U151.6.3.3.1, U-15, Otiyo-Brown and KAT-FM1 having > 3.0 g weight. The harvest index ratio (HI) of finger millet varieties across regions varied from 0.7 to 1.5. High HI was measured in genotypes P-224, Otiyo Brown, KNE-1034, NKR-FM1, KNE-741, and IE-615, KATFM1×U15-1.6.6.3.1.1, Ikhulule, Gulu E, IE-2872 and Kal Dokolo. These genotypes had >1 total harvest index ratio (Appendix 2). The genotypes with lowest harvest index included Snapping Purple Variety, KAT-FM1, Kal-Pader, IE-2183, GBK-127189A and KATFM1×U15-1.6.6.3.1.1 which had > 0.9 HI ratio.

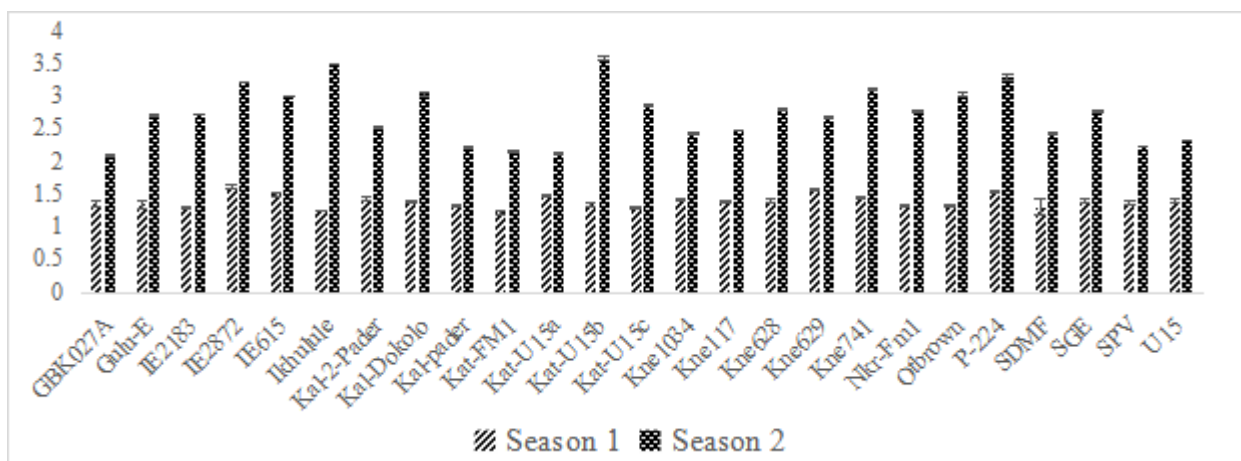


Figure 3.4: Yield performance of selected finger millet genotypes in Bomet season 1 and 2

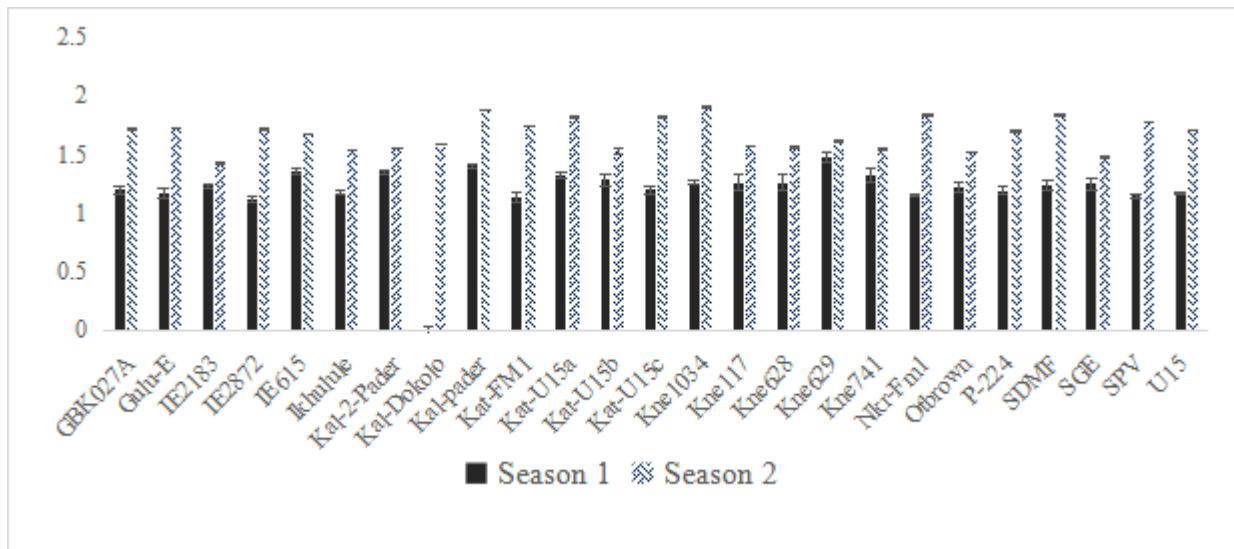


Figure 3.5: Yield performance of selected finger millet genotypes in Koibatek season 1 and 2

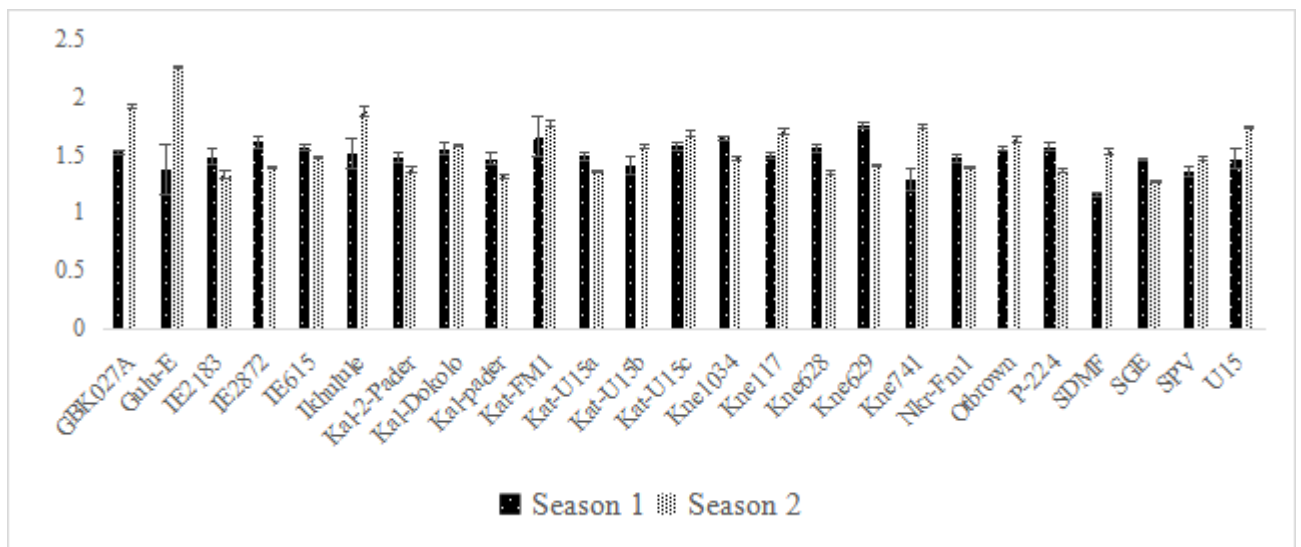


Figure 3.6: Yield performance of selected finger millet genotypes in Nakuru season 1 and 2

3.4.4 Pearson's correlation between yield, disease scores and yield components

The analysis of correlation between yield and yield components showed that disease severity of both leaf and head had negative effect on finger length, finger number, tillers and days to maturity. Leaf blast severity had a significant positive correlation ($P < 0.001$ $r = 0.372^{***}$) with neck blast, however it was negatively correlated to biomass ($r = -0.037$). Neck blast had negative correlation on biomass and yield but only significant in 1000KW ($P < 0.05$ $r = -0.026$, -0.041 and -0.104^* respectively). Maturity had a negative significant ($P < 0.01$) correlation on leaf blast, neck blast, harvest index and yield ($r = -0.168^{**}$, -0.2011^{***} , -0.137^{**} , -0.159^{***}), however, a positive significant ($P < 0.01$ $r = 0.209^{***}$) was noted with 1000 KW.

Plant height had a significant positive correlation with leaf blast ($P < 0.05$ $r = 0.094^*$) but negatively correlated with neck blast ($r = -0.066$) however it was positively correlated to finger length and finger number ($r = 0.167^{***}$, $r = 0.021$). The number of fingers per panicle had a significant ($P < 0.05$) positive correlation with tillers, 1000 KW and biomass while significantly negatively correlated with leaf blast ($P < 0.01$), harvest index and yield (Table 3.7).

Table 3.7: The relationship of leaf and head blast severity expressed in AUDPC, yield and yield traits of selected finger millet genotypes.

	Height	F.L	F.N	Tillers	DTM	AUDPC	NAUDPC	1000KW	Biomass	H.I	Yieldt
Height	1										
F. length	0.16***	1									
F. no	0.02	0.53***	1								
Tillers	0.15**	0.27***	0.44***	1							
Maturity	-0.15**	-0.02	0.04	0.04	1						
Leaf Blast	0.09*	-0.12**	-0.20***	-0.42***	-0.16**	1					
Neck blast	-0.06	-0.03	-0.03	-0.22***	-0.20***	0.37***	1				
Kw	0.15**	0.14**	0.22***	0.39***	0.20***	-0.27***	-0.10*	1			
Biomass	0.22***	0.15**	0.10*	0.07	-0.01	-0.03	-0.02	0.15**	1		
Harvest index	-0.14**	-0.20***	-0.22***	-0.41***	-0.13**	0.29***	0.01	0.21***	-0.24***	1	
Yieldt	-0.11*	-0.17***	-0.21	0.42***	-0.16***	0.30***	-0.04	-0.20***	0.03	0.88***	1

*Significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$, F.L= Finger Length, F.N=Finger Number, DTM=Days to Maturity, LAUDPC=Disease severity on the leaves, HAUDPC=Disease severity on heads, 1000KW= 10000 kernel Weight, H.I=Harvest Index.

3.4.5 Screening for resistance under controlled conditions in the greenhouse conditions

The results of controlled experiment under greenhouse showed that there was significant variation ($P < 0.05$) in neck and leaf blast severity with genotypes ranging from resistant R, MR, MS and S (Figure 3.8: Figure 3.9). Among the 12 finger millet genotypes screened for neck blast resistance, 25% of the genotypes were R and MS, 33% were MR and 16% were S. The R genotypes included SDFM-1702 (Figure 3.7: Table 3.9), IE2183 and Snapping-Purple variety which had a 0% Disease Severity Index (DSI), U15, KNE1034, KNE1124 x 796 and Katfm1 x u151.6.6.3.1.1 MR-13% DSI in contrast to KNE741, Kal pader and Gulu E MS-62.5% DSI and 2 genotypes Katfm1xU15 1.7.8.2.1 and KNE-629 S-87.5% DSI to neck blast (Figure 3.7: Table 3.9). In comparison to field conditions, genotypes SDFM 1702, IE-2183 and Snapping Purple had lower infection rates of < 43 AUDPC mean severity values in all varied agro-ecologies (Appendix 2).

Leaf blast scoring showed that genotypes ranged from R with infection rates of 1-4% on the leaf surface, MS with infections covering 30-50% of leaf surface, and S with above 50% infection rates on the leaf surface measured as disease severity index (DSI) infection on the leaves. Among the 12, 33% were R (Gulu E, Katfm1x U151.6.6.3.1.1, KNE 1034 and SDFM-1702), 16.6% were MS (KNE629 and Kal pader) and 50% of the genotypes were S (Snapping-Purple variety, IE2183, KNE1124x796, Kat Fm1 1.7.8.2.1, U15 and KNE741) (Figure 3.7). In comparison to field conditions, only KNE-1034 was susceptible to leaf blast > 60 mean AUDPC severity value while genotypes such as Gulu-E, Katfm1xu151.6.6.3.1.1 showed resistance to leaf blast with mean severity values expressed as AUDPC of < 50 . SDFM-1702 was moderately susceptible with < 60 AUDPC mean severity value (Appendix 2)

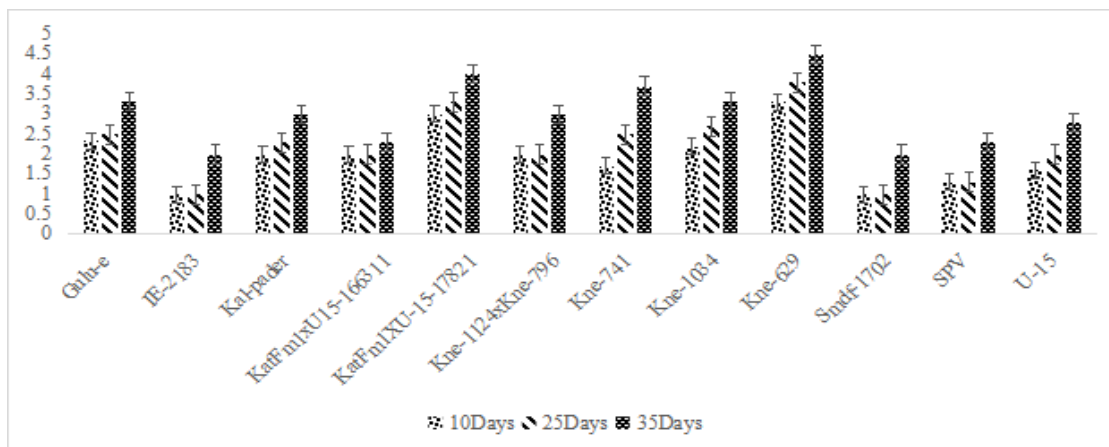


Figure 3.7: Genotype response to neck blast at 10, 25 and 35 days after inoculation

3.4.6 Effect of *Pyricularia grisea* on yield and yield components of selected genotypes under greenhouse

ANOVA revealed that genotypes differed significantly ($P < 0.001$) with respect to days to heading, maturity, height, finger number, panicle weight, 1000 kernel weight, NAUDPC, finger length, yield and biomass. Leaf AUDPC however was not significantly ($P > 0.05$) different among the finger millet genotypes (Table 3.8). Mean separation using Least Significant Difference (LSD) was done and the genotypes ranked on their agronomic performance. Assessment of genotype maturity was based on days to 50% heading which ranged from 58.67-84.66 days and an overall mean of 73.55 days. Genotypes KNE-741 and Katfm1xU15-1.7.8.2.1 were early to head with 58 and 65 days respectively while KNE-629, KatFm1xU15-1.6.6.3.1.1 and Gulu-E headed late with 84, 83 and 81 days respectively.

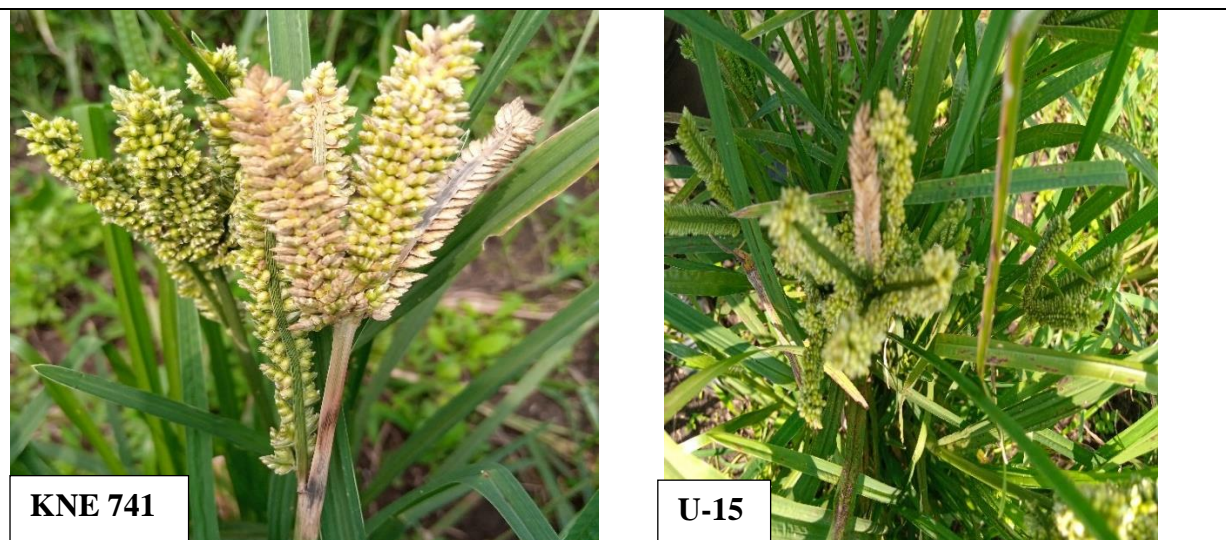


Figure 3.8: Infection response of *Pyricularia grisea* in a susceptible genotype KNE 741 and MR U-15 on the neck region 62 days after planting

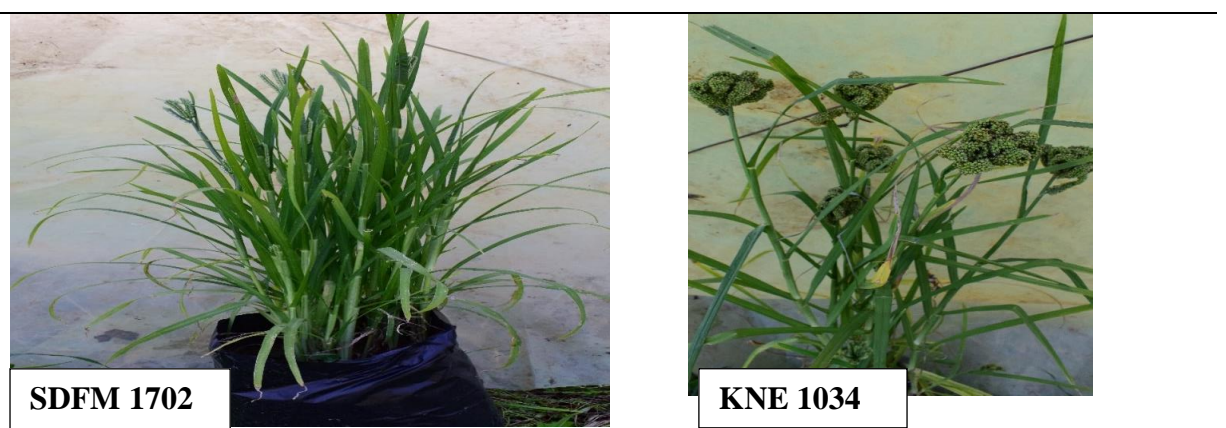


Figure 3.9: SDFM 1702 and KNE 1034 showing resistance to both leaf and neck blast to *Pyricularia grisea* 47 days after planting

Genotypes had different variations in yield and yield components. For instance, days to physiological maturity ranged from 93-72 days with a mean of 85.55. The genotypes that were early in physiological maturity included KNE-741 and IE-2183 matured early with 72 and 76 days respectively while KNE-1034, Kal pader and SDFM-1702 matured late with 93, 91 and 90 days respectively.

Height variations ranged from 83.33-34.67 cm with a mean of 63.24 cm. The tallest varieties included KNE-629 and KNE1124xKNE796 with 83 and 82 cm respectively while the shortest varieties included KatFm1xU15-1.6.6.3.1.1 with 34 cm.

Panicle weight varied from 18.69g- 10.92g with a mean of 13.94 g. The heavy panicle included U-15, Gulu-E and Kal Pader with 18.69 g, 16.12 g and 16.54 g while those with light panicles included KNE741 and Snapping-Purple variety with 10.92 g and 11.05 g respectively. The length of fingers ranged between 9.71-4.37 cm with a mean of 6.89 cm. Genotypes Katfm1xU15-1.6.6.3.1.1, KNE 1124xKNE796, KNE-1034 had the longest fingers of 9.71 cm, 8.15 cm and 8.23 cm respectively while KNE 741 and IE2183 had the shortest fingers of 4.37 cm and 5.27 cm respectively. The number of fingers ranged between 10.55-5.21 and a mean of 6.96. Genotypes Kal pader and IE2183 had the highest number of fingers while KNE 1124xKNE796 and SDFM-1702 had the lowest numbers. The mean 1000 kernel weight ranged from 2.82-1.58 g with a mean of 2.14. Highest weight of 1000 kernels were recorded in KNE 1124 x KNE 796, IE-2183 with 2.82g, 2.64 g respectively while the lowest recorded were Gulu E and KNE 629 1.58g and 1.69g respectively.

Leaf AUDPC severity values ranged from 88.68-47.22 with a mean of 66.67. IE-2183, Snapping-Purple had the highest severity of 88.68 and 78.76 respectively while Gulu E, KNE 1034 and KatFm1xU15-1.6.6.3.1.1 had the lowest severity of 47.22, 50.92 and 53.67 respectively. Neck AUDPC ranged from 94.84-31.50 with a mean of 58.80. Highest mean severity values of 84 and 94 were recorded in genotypes Katfm1xU15 1.7.8.2.1 and KNE 629 respectively while Snapping-Purple, IE2183 and SDFM-1702 had the lowest with 37.17, 33.17 and 31.50 respectively. The mean biomass ranged from 105-46.67 g with a grand mean of 75.41 g. Overall yield of the genotypes ranged from 15.56-4.69 g with a grand mean of 10.47 g. The highest yields were those from genotypes U-15, Katfm1xU15 1.7.8.2.1 and IE-2183 with 15.56 g, 13.16 g and 13.16 g respectively while the lowest yield was that from KNE 629 and KNE1034 genotypes with 5.48g and 4.69g respectively (Table 3.9).

Table 3.8: Analysis of variance table for Leaf, Neck AUDPC, yield and yield components of 12 fingermillet genotypes evaluated in the greenhouse for host plant resistance to *Pyricularia grisea*

Sov	df	DTH	Maturity	Height	Finger No	PW	KW	Biomass	Yield	LAUDPC	NAUDPC	F.L
Genotype	11	170.08***	120.02***	504.10***	6.12***	17.98***	5.09***	791.09***	29.34***	521.64	1116.00***	6.62***
Blocks	2	41.69**	16.36	260.70	1.38**	49.34***	0.74***	18.75	38.69***	74.91	311.41**	2.27
Error	22	6.03	20.81	19.37	0.23	1.64	0.03	46.78	1.41	16.41	51.34	0.75
CV		3.34	5.33	6.95	6.87	9.19	8.39	9.06	11.35	6.08	12.18	12.63
R²		93.64	74.71	93.44	93.30	89.13	89.15	89.46	92.8	94.22	91.94	82.28

*Sov = source of variation, df = Degree of freedom, DTH=days to heading, Mat= maturity, F.N=Finger number, PW=Panicle weight, K.W= Kernel weight, LAUDPC=Leaf severity expressed in AUDPC, NAUDPC= Neck severity expressed as AUDPC, F.L =Finger length. *** = p<0.001, ** =p<0.01 and * =p<0.05 respectively.

Table 3.9: Effect of *Pyricularia grisea* on performance of 12 finger millet genotypes under greenhouse

Genotype	50% DH	DPM (Counts)	Height (cm)	F.L (cm)	F.N (Counts)	P.W (g)	Biomass (g)	1000 KW(g)	Yield (g)	NAUDPC	LAUDPC
KNE 741	58.67 ^f	72.00 ^e	57.52 ^{de}	4.37 ^e	5.21 ^f	10.92 ^e	83.33 ^b	2.26 ^{cde}	10.06 ^{de}	65.21 ^{bc}	71.46 ^c
U-15	72.67 ^{cbd}	84.00 ^{bcd}	60.50 ^{cd}	7.48 ^{bc}	7.22 ^{cd}	18.68 ^a	71.66 ^{cd}	2.42 ^{bcd}	15.56 ^a	52.49 ^d	75.25 ^{cb}
KNE 1034	70.33 ^d	93.00 ^a	67.82 ^{bc}	8.23 ^b	6.01 ^{ef}	12.18 ^{de}	71.67 ^{cd}	1.92 ^{fgh}	4.69 ^f	56.50 ^{cd}	50.92 ^{fg}
Kal Pader	76.67 ^b	91.67 ^{ab}	52.46 ^e	5.32 ^{de}	10.55 ^a	16.54 ^{ab}	66.67 ^d	2.02 ^{ef}	10.90 ^d	71.82 ^b	60.31 ^{de}
KatFm1x u151.7.8.2.1	65.00 ^e	80.33 ^{cd}	62.97 ^{bcd}	6.88 ^{bc}	6.84 ^{cd}	14.42 ^{bc}	88.33 ^b	2.01 ^{efg}	13.29 ^b	84.00 ^a	75.03 ^{bc}
KNE 629	84.67 ^a	87.33 ^{abc}	83.33 ^a	6.42 ^{cd}	6.61 ^{de}	11.65 ^e	53.33 ^e	1.69 ^{hi}	5.48 ^f	94.85 ^a	62.18 ^d
KNE1124XKNE796	71.00 ^{cd}	88.33 ^{ab}	82.21 ^a	8.15 ^b	5.77 ^f	11.88 ^e	88.33 ^b	2.82 ^a	11.99 ^{bcd}	57.44 ^{cd}	79.54 ^b
GULUE	81.67 ^a	88.00 ^{abc}	69.55 ^b	6.14 ^{cd}	6.89 ^{cd}	16.12 ^{bc}	83.33 ^b	1.58 ⁱ	11.25 ^{cd}	64.05 ^{bcd}	47.22 ^g
SDFM1702	74.66 ^{cb}	90.33 ^{ab}	59.15 ^{de}	7.27 ^{bc}	5.55 ^f	15.05 ^{bc}	65.00 ^d	2.47 ^{bc}	11.28 ^{bcd}	31.50 ^e	56.96 ^{def}
KatFm1xu151.6.6.3.1.1	83.66 ^{cb}	88.00 ^{abc}	34.67 ^f	9.71 ^a	7.55 ^{bc}	14.21 ^{cd}	105.00 ^a	2.15 ^{def}	9.98 ^{de}	57.43 ^{cd}	53.67 ^{efg}
Snapping-Purple	73.00 ^{cbd}	87.33 ^{abc}	68.33 ^b	7.41 ^{bc}	7.11 ^{cd}	11.05 ^e	81.67 ^{bc}	1.70 ^{ghi}	8.05 ^e	37.17 ^e	78.76 ^b
IE2183	70.67 ^{cd}	76.33 ^{de}	60.31 ^d	5.27 ^{de}	8.22 ^b	14.56 ^{bc}	46.67 ^e	2.64 ^{ab}	13.16 ^{bc}	33.17 ^e	88.68 ^a
LSD	4.15	7.72	7.45	1.47	0.81	2.17	11.58	0.304	2.01	12.13	6.85

*DTH=days to heading, Mat= days to physiological maturity, F.L=Finger length, F.N=Finger number, P.W=Panicle weight, K.W= Kernel weight, NAUDPC= Neck severity expressed in AUDPC, LAUDPC=Leaf severity expressed in AUDPC and AUDPC- Area Under Disease Progress Curve. Means under the same letter in the same column are not significantly different at P<0.01.

3.4.7 Correlation of leaf blast, neck blast and yield and yield parameters

Pearson's correlation coefficient indicated that neck blast had a negative correlation to leaf blast, panicle weight, yield and was only significant ($P < 0.01$) with 1000 kernel weight (KW). Leaf blast had a significant ($P < 0.01$) positive correlation with 1000 KW and yield, however, a negative correlation with panicle weight was noted. A significant ($P < 0.001$) positive correlation was also noted between 1000 kernel weight and yield. A significant ($P < 0.05$) positive correlation was also noted between panicle weight and yield (Table 3.10). In comparison to the field experiment, neck blast maintained a positive correlation with 1000 kernel weight, leaf blast and also panicle weight. In contrary to the field experiments, leaf blast had negative correlation with 1000KW.

Table 3.10: Correlation table of Neck and leaf blast disease on yield and yield parameters.

	NAUDPC	LAUDPC	KW (g)	P.W (g)	Yield (g)
NAUDPC	1				
LAUDPC	-0.1718	1			
1000KW	-0.3904**	0.4656***	1		
Panicle W	-0.1692	-0.1819	0.1787	1	
Yield	-0.1112	0.4242**	0.5359***	0.3536*	1

*NAUDPC= Neck blast severity expressed as AUDPC, LAUDPC= Leaf blast severity expressed as AUDPC, KW= 1000 Kernel weight.

3.5 Discussion

3.5.1 Response of selected genotypes to blast disease on leaf and panicle

From the results, genotypes respond distinctly when infected with *Pyricularia grisea* under different environments and seasons. In the year 2019 disease severity was low compared to 2020. This is because of the corresponding weather differences in the years. This is similar to Asea and Onega (2016) and Titone *et al.* (2015) who evaluated leaf and neck blast and showed that variability in weather changed the magnitude which therefore increased the severity. The occurrence of blast disease in these areas is highly dependent on weather. *Pyricularia grisea* requires temperatures ranging from 25-30 °C and humidity of above 80% maximum production of spores (Sreenivasaprasad *et al.*, 2004). Koibatek had higher leaf and neck blast occurrence compared to Nakuru and Bomet. These high occurrences of infections could be due to the favorable temperature of 17-29 °C and humidity of 65-93% which is necessitated the multiplication of the spores of *P. grisea*. Bevitori &

Ghini, (2014) observed that the formation of appressorium and multiplication of spores occurs at an average temperature of 24 °C to 27 °C and favorable humidity of above 80%. The variation in severity among the genotypes is dependent on the genotype susceptibility to both leaf and neck blast.

Genotypes such as NKR-FM1, KNE 629, P-224 and Snapping-Purple are resistant to neck blast but are shown to be susceptible to leaf blast in environments like Bomet. The presence of host plant resistance is exhibited in these genotypes because the spread of the pathogen does not affect the productive fingers and this suggests that they could have mechanisms to resist diseases. This may include, shedding off their leaves and hypersensitive reactions to avoid disease spread to other parts of the plant as explained by Preisig and Kuć, (1985). From the comparison, there is stability in the resistance of some genotypes such as SDFM-1702, IE-2183, Snapping Purple variety, Gulu-E, KatFM1x U15 1.6.6.3.1.1 as the results showed that genotypes resistant to the field had the same resistance in the greenhouse on both leaf and neck blast. The high resistance of these genotypes could be due to the genetic makeup of the genotypes. The resistant genes contained in these genotypes may be specific to *Pyricularia grisea* pathogen. The R gene will recognize the host interference through infection by the pathogen which then encodes with protein molecules such as the nucleotide-binding protein and leucine-rich proteins (Marone *et al.*, 2013). The pathogen avirulent gene (Avr) will match specifically with the resistant gene to confer resistance. Specific recognition leads to an immediate response of the plant against the pathogen such includes the hypersensitive reaction (HR) (Balint-Kurti, 2019).

3.5.2 Variations in yield and yield components among finger millet genotypes

Higher yields were achieved in the year 2020 as compared to 2019. This is because of the favourable weather conditions experienced during the long rain season in contrast to the short rain season. The ideal rainfall received during the long rainy season which runs from March to August facilitates the uptake of nutrients and healthy growth of the plants in most parts of the country explains the reason for high yields (Guan *et al.*, 2015). Wekesa *et al.* (2019) also pointed out that under good rain-fed conditions such as the long rain season there is high grain filling due to adequate moisture provided while during the short rains, grain filling is minimal and evident due to moisture stress. Early maturity achieved during the short season could be explained by the short rains received during the season. Plants mature faster when exposed under low moisture content due to high metabolic processes, less

photosynthetic assimilates and availability of nutrients leading to a substantial loss in grain production hence less yield (Osakabe *et al.*, 2014). The maturity of the genotypes had an influence on the severity of both leaf and neck blasts. Genotypes such as Snapping green early and KNE 741 recorded early maturity, therefore exposing them to attacks from diseases such as blast disease leading to low yields. Among the genotypes, Snapping-Purple, IE-615, U-15, IE-2872, Gulu E, KAT FM1 and Kal Pader had a higher number of tillers, this led to a corresponding higher yield of > 90% that is > 2 t ha⁻¹. This is because a high number of tillers leads to more headcount and therefore more yield. Furthermore, when there is a blast attack on the mother plant the surviving tillers would still compensate for the loss. Varieties such as P-224 and Gulu-E had high levels of neck blast resistance as well as high yielding. Neck blast is the most damaging type leading to over 80% losses. When genotypes such as P-224 and Gulu-E resist the attack of the blast, their yields will be higher since the formation of seed will be to the maximum with no disease interference.

3.5.3 Correlation between disease severity, yield and yield components

From the Pearson's correlation analysis, an increase in leaf blast by 0.51 significantly increases the chances of neck blast infections by 37%. Similar to these findings, Owere *et al.* (2015) and Nagaraja *et al.* (2007) reported a positive correlation of leaf blast to neck to be 27.1% with some genotypes resisting blast infection in rice. Babu *et al.* (2013) found a correlation of 92% of leaf and neck blast. This meant that the genotypes that were most susceptible to leaf blast could also be infected by neck blast. However, this is contrary to the resistant checks U-15 and NKRFM1 were both susceptible to leaf blast but resistant to neck blast. This is because the genotypes have resistant genes which confer further spread of blast to the neck region. The relationship of height and blast disease is evident both to the leaf and neck blast severity. Height had a positive correlation with the occurrence of leaf blast. Leaf blast seemed to occur more irrespective of other agronomic aspects. This is because of the favourable climatic conditions that promoted the occurrence of the disease and spread. However, neck blast had a negative correlation with height meaning shorter genotypes were more susceptible to neck blast than the taller genotypes. Wekesa *et al.* (2019) from a study done on 100 genotypes in Busia and Kakamega revealed that greater plant height showed less neck blast severity with lower AUDPC values. Genotypes such as GBK-127189A, Kal Pader and KNE 629 were found to be tall and hence resistant to neck blast. These genotypes can be selected as parent material for the provision of genes through crossing to boost the resistance

mechanism of genotypes such as KNE 741. Similar to these findings is Bregaglio *et al.* (2017) showed that variation in seasons and genotypes are important factors in blast disease development and therefore growing of finger millet genotypes under natural conditions is an efficient way of assessing the resistance of genotypes.

Maturity was significantly negatively correlated to both neck and leaf blast, harvest index and yield. This is because the early maturing genotypes such as KNE 741 (88 days) were most susceptible to both leaf and neck blast because of the favourable microclimate while the late-maturing genotypes KATFM1xU15-1.6.6.3.1.1 (100 days) escaped the attack of the disease because of the weather conditions prevailing at harvest period. These attacks of the diseases had a negative impact on both the yield and harvest index of the crop leading to a corresponding decrease. A decrease in harvest index due to the occurrence of leaf and neck blast is a clear indication of less assimilates accumulation to the productive parts hence a decrease in yield (Unkovich *et al.*, 2010). Similar to these findings, Babu *et al.* (2013); Nagaraja *et al.* (2007) also pointed out that the genotypes that mature early are easily affected by both leaf and neck blast disease. There was a significant positive correlation of height, finger length and finger number. Upadhyaya *et al.* (2006) evaluated 610 genotypes of finger millet and found similar results and further suggested that it is important to select such traits for improvement of finger millet genotypes. Finger number did not seem to have an influence on leaf and neck blast severity however it was positively correlated to the number of tillers, 1000KW and biomass. An increase in the number of tillers would possibly increase the biomass of the plant. Upon increase of the biomass, the weight of the seeds also increases. This is because of the build-up of sufficient photosynthetic assimilates for the growth and development of the plant itself (Smidansky *et al.*, 2003). The negative correlation of finger number with leaf and blast could be explained by the genotype response to attack by the blast as opposed to the plant characteristics. An increase in the number of tillers increased the yield of finger millet. Nandini *et al.* (2010) found a positive correlation of tillers and 1000KW with yield. Contrary to studies such as Nandini *et al.* (2010) and Grando *et al.* (2001) 1000 KW, finger length and finger number were negatively correlated to yield. This could be due to the huge variability shown within the genotypes across various varied environmental conditions. Neck blast results from the field and greenhouse both showed a positive correlation with 1000 KW, leaf blast and panicle weight. This is because the attack of finger millet on the neck region by *Pyricularia grisea* leads to the formation of shrivelled grains which are light in weight, some will not form grains on the panicle hence leading to lower weight of panicle.

3.6 Conclusion and recommendation

The aim of this experiment was to determine host plant resistance in selected finger millet genotypes across three major growing areas of Kenya in different growing conditions. The variation in expression of the disease yearly with an increase in disease progressively from 2019 to 2020 was an indication that weather has a major role on the occurrence of the disease. *Pyricularia grisea* growth, development and expression is highly dependent on weather conditions. Genotypes variability in resistance against attack is an indication of different genetic make-up of different genotypes. Genotypes GBK-127189A, Snapping-Purple, KNE1124xKNE796, Gulu E and U15 were resistant to leaf blast across all environments, while Snapping-Purple, GBK-127189A, SDFM-1702, SDFM-1702, P224, KatFm1 x U15 1.6.6.3.1.1, IE-2183 and Gulu E were resistant to neck blast across all seasons and environment. These genotypes can easily be up-scaled and used as parent sources for resistance of blast disease. Confirmatory test under greenhouse showed that genotypes U-15, Katfm1 x U15 1.7.8.2.1 and IE 2183 were indeed resistant. Despite the severity of KatFm1 x U15 1.7.8.2.1 it was able to be among the high yielding varieties, indicating that it has some level of tolerance to blast. Resistance to leaf blast was depicted in Gulu-E, KNE 1034 and they could be used as sources for improvement of other genotypes. Neck blast resistance was observed in Snapping-Purple variety, IE 2183 and SDFM-1702. All these varieties with good resistance also had high yields. This shows that they can be used by breeders for the improvement other genotypes to enhance both blast resistance and high yield. However, further research should be carried out on more genotypes of finger millet in the above areas.

CHAPTER FOUR

FARMER PREFERENCE FOR SELECTED FINGER MILLET (*Eleusine coracana*) VARIETIES IN RIFT VALLEY KENYA

Abstract

Farmer participatory and varietal selection (FVPS) is an efficient method of achieving productivity. It enhances adoption of improved high yielding finger millet varieties. Farmer participatory and varietal selection was conducted at Agro-ecological zone III (Agricultural Training Centers- Nakuru and Bomet) an aim of finding the most preferred trait and variety of finger millet. One hundred farmers assessed and scored their preferred traits and varieties in each environment. The scores were ranked on a scale of 1-5 in Focused Group Discussions (FDGs) and analyzed using *Kruskal Wallis H*-test of non-parametric data. . Farmers preferred high yielding varieties with qualities such as uniformity (3.91), folded fingers (3.81), snapping ability (3.79), drought tolerance (3.76) and tillering ability (3.66) of genotypes. KatFM1xU151.6.6.3.1.1, Kal 2 pader, Ikhulule, P-224, Gulu-E, Snapping green early and GBK 027189A were the preferred varieties by farmers from Nakuru while KatFM1, KNE 741, KNE629, KatFM1 x U151.6.6.3.1, U-15 and Kal Dokolo were the most preferred varieties for Bomet. FPVS provides a platform for identification of most preferred traits of finger millet and knowledge dissemination of improved varieties to farmers.

4.2 Introduction

Finger millet (*Eleusine coracana*) is a highly nutritious cereal food for the weak and people with low immunity (Takan *et al.*, 2012). It contains nutritional elements which are easy to digest thus a major source of food for pregnant women, the sick, lactating mothers, children and diabetics (Singh & Raghuvanshi, 2012). *Eleusine coracana* is the most important small millet grown for subsistence in Eastern Africa and Asia. In East Africa, it is majorly used for food in form of thin porridge, malting and brewing (Mitaru *et al.*, 1993). In Kenya, finger millet also commonly called ‘*wimbi*’ is used for making porridge, thick porridge ‘*ugali*’ and for brewing. Finger millet production has declined over the years from 99000 tons in 2015 to 54000 tons in 2016 and 2017. It is commonly planted in Western Kenya, around Lake Victoria and in Eastern Kenya.

Finger millet production is still low due to continuous use of poor unimproved landraces that are most susceptible to blast and low yielding, insufficient information on improved varieties poor dissemination of seeds, post-harvest handling of finger millet and poor attitude linked to the crop (Degu *et al.*, 2009; Molla *et al.*, 2020). This has been a major challenge for the adoption of finger millet to farmers in Kenya. High-yielding varieties have been developed and released for general cultivation through the breeding of exotic and indigenous lines by researchers but adoption is still a challenge (Singh *et al.*, 2016).

Farmer Participatory Varietal Selection (FPVS) is an approach that involves farmers in the evaluation of experiments done within their area or in their fields (Jackson and Kassam, 1998). This approach ensures the transfer of knowledge, adoption of new technologies and spread on information to other farmers (Bugeza *et al.*, 2017). This approach has resulted in the adoption of technology and improvement of livelihood to both farmers and researchers (Witcombe *et al.*, 2005). It has been successfully done in many crops including rice (Orlando *et al.*, 2020; Panwar *et al.*, 2019; Paris, 2011), beans (Tamene, 2016; Yadavendra *et al.*, 2017), barley (Ferede & Demsie, 2020), wheat (Van *et al.*, 2020), sorghum (Sissoko *et al.*, 2019; Vom *et al.*, 2020). Banbara nuts in Malawi (Pungulani *et al.*, 2012) and finger millet (Ojulong *et al.*, 2017; Tarekegne *et al.*, 2019). Various varieties of finger millet have been up-scaled, released, adopted and disseminated to farmers in countries such as Tadesse, Wama, Degu and ACC#213572 for Delgi and Chilga in Ethiopia (Fentie, 2012), and U-15 and P-224 in Tanzania (Ojulong *et al.*, 2017) through FVPS. Different environmental conditions, traits of interest, cultural and religious beliefs, gender, marketability and value addition among others influences the choice made by farmers during evaluation (Cleveland *et*

al., 2000) Recent research incorporates farmers for improved uptake, knowledge dissemination and promotion of innovations which can easily be found during FVPS. This study aimed at identifying the most preferred traits and varieties of finger millet identified in Agricultural Ecological Zone (AEZ) III, Agricultural Training Centres (ATC) Nakuru and Bomet for future breeding, up-scaling and release of the farmer-chosen varieties.

4.3 Materials and methods

4.3.1 Environment description

The experiment was conducted during the long rain season 2020, January- June and short rain season of June-December 2019, in ATC-Bomet and ATC-Nakuru, respectively. The experimental layout, design weather and soil description are as described in chapter three, section 3.3.1.

4.3.2 Data collection and analyses

Data was collected from center two rows to reduce the border effect. All data collected was subjected to Statistical Analysis Software (SAS) and SPSS. Pair-wise comparison, mean tables were then constructed to give the best varieties and traits in both environments. SAS was used to compute univariate procedure to give standard error of the means.

4.3.3 Plant genotypes

Twenty-five finger millet genotypes were planted in Agri-Ecological Zone (AEZ) III in both ATC Nakuru and ATC Bomet as described in 3.2.2.

4.4 Farmer Participatory Variety Selection

4.4.1 Target population and sampling procedure

The target groups of study were smallholder millet farmers in Nakuru and Bomet counties. The groups were composed of people of varying gender and age, income groups, lifestyles and education levels. The areas had a high concentration of people whose project targets and deliverables were directly impacted on their livelihoods. A sample size was generated using Yamane formula (Yamane, 1973);

$$n = N / (1 + Ne^2)$$

Where n= corrected sample size, N= Population size, e= (0.08)² the margin of error

$$30 = 300/1 + 300(0.08)^2$$

=102 farmers

4.4.2 Ranking finger millet basing on traits by farmers

Ranking and selection of best performing varieties and varietal differences were conducted when the crop was at physiological maturity. At this stage, farmers were able to note the agronomic traits of finger millet such as flower type (folded or straight), number of tillers, grain colour, uniformity among other traits scored by farmers. Farmers were grouped into Focused Group Discussions (FGDs) consisting of 10 farmers and one extension officer assigned to each group. They were then provided with scoring templates that contained the variety numbers (1-25) arranged vertically on the left of the sheet and the scoring traits arranged horizontally. Each of the varieties was scored on a scale of 1 to 5 (very poor to excellent respectively) (ICRISAT, 2011). The scores were then utilized for the identification of the most preferred traits and varieties. Traits included; high yielding based on agronomic traits of the plant, size of the fingers, high tillering ability, resistance to bird infestation and lodging among others. Early maturity was based on early days to heading, anthesis and physiological maturity. Marketability- based on the colour of the grain and ability to be availed to the market on time depending on maturity. Drought tolerance based on the ability to mature early and escape drought and a high number of tillers.

Blast tolerance was based on ability to resist and tolerate blast disease both on finger and leaf while big fingers was based on the finger length and finger number. Lodging was based on the ability to resist lodging by having strong stems and medium height. Grain colour was based on red, black-brown and white. Tillering was based on the number of tillers per plant and uniformity based on the ability to have a uniform height and mature uniformly. Varieties were ranked based on the scores received per trait and afterward, the scoring sheet was collected and separated according to gender. Both qualitative and quantitative data was then used as an indication of final scoring and selection of traits and varieties chosen by farmers. This data did not only give the best traits and varieties chosen by farmers in both regions but also the overall comments and opinions of farmers on finger millet in both regions. Survey data collected (Tables 6.5 and 6.6) was subjected to the *Kruskal-Wallis* test of non-parametric data and the highest means and ranking was used to obtain the best varieties selected by farmers using Statistical Package for Social Sciences version 20. A pairwise ranking matrix was also done to obtain the best traits selected by farmers.

Statistical Analysis Software (SAS) was used in univariate analysis to find the standard error of the means and to check the normality of the data.

4.5 Results

4.5.1 Farmer preference of finger millet traits

Results of the study showed that farmers had high preference to varieties with higher uniformity, drought tolerance, snapping ability, tillering ability, folded/straight fingers and high yielding and resistance to lodging in both environments (Table 3). In Nakuru county, farmers recognized good taste, grain colour and folded or straight fingers as least important while in Bomet marketability, early maturity and resistance to diseases received the lowest score (Table 4.1). A pair-wise comparison was done to determine the most preferred trait of finger millet as scored by farmers and the best traits selected and ranked (Table 4.4). The presence of various varieties ranging from commercial varieties, advanced breeding lines and local varieties, there were different opinions of varieties basing on their performance.

Selection was based on yield and yield component traits including; uniformity, drought tolerance, tillering ability, big finger, lodging and folded or straight fingers. Resistance to challenges such as; bird infestation and resistance to diseases. Good marketing ability such as; good taste, grain colour. Farmers selected the best traits of finger millet based on the yield traits and yield performance of the varieties including high yielding, tolerance to diseases and birds, high tillering ability, plant height and early maturity.

Table 4.1: Results of Focused Group Discussions (FDGs) on preferred finger millet traits done in both environments Nakuru and Bomet

Finger millet traits	Scores per environment		
	Nakuru	Bomet	Mean scores
Uniformity	3.59	4.23	3.91
Folded fingers	3.96	3.65	3.81
Snapping ability	3.71	3.88	3.79
Drought tolerance	3.36	4.15	3.76
Tillering	3.61	3.71	3.66
Lodging	3.68	3.33	3.51
High yielding	3.63	3.37	3.50
Bird resistance	3.37		3.37
Marketability	3.53	3.13	3.33
Early maturity	3.32	3.03	3.18
Resistance to diseases	3.42	2.38	2.90
Good taste	2.76		2.76

*Blanks indicate that the parameters bird resistance and good taste were not assessed in Bomet.

The farmers evaluated and ranked the best traits of finger millet through Focused Group Discussions (FDG) and using a pairwise ranking matrix (Table 3), the best traits were selected for future breeding purposes. Pair-wise ranking and farmers' preference linked to high yielding, high tillering, resistance to diseases, grain colour and good threshability (Owere *et al.*, 2014; Sibiya *et al.*, 2013; Watson 2019).

4.5.2 Farmer preference on the varieties

The findings revealed that there was variation in the scoring of the varieties in both environments. In Nakuru ATC, majority of farmers participated in the evaluation process leading to a higher scoring compared to ATC-Bomet. Scores ranged from a mean of 3.9 to 2.9 and 4.3 to 2.2 highest to lowest in Nakuru and Bomet respectively (Table 4). In Nakuru ATC, Kal 2 Pader (3.9), P-224 (3.93), KatFM1xU151.6.6.3.1.1 (3.9), GBK 027189A (2.8), Snapping green early (3.7) and KatFM1xU151.7.8.2.1 (3.7) were the most preferred varieties (Figure 4.1) while in Bomet ATC, KatFM1 (4.3), KNE 741 (4.3), KNE629 (4.2), KatFM1xU151.6.6.3.1 (4.1) and Gulu E (3.9), GBK 027189A (3.8) and Kal 2 pader (3.8)

were the most preferred varieties (Figure 4.2). In both environments KatFM1xU151.6.6.3.1.1 (4.0), Kal 2 pader (3.85) and GBK 027189A (3.8), Gulu E (3.75) and P-224 (3.75) ranked the best. On the selection of varieties, the best selected varieties in both areas had a high yielding, high tillering ability, resistance to diseases and pest, high number of tillers and uniformity (Table 4.2).

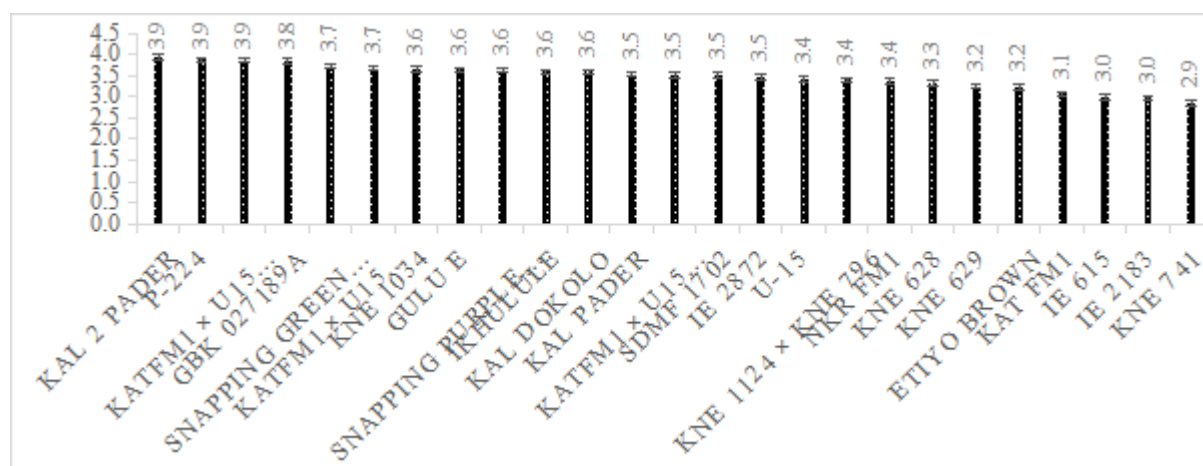


Figure 4.1: Mean variety scores on a scale of 1-5 of 25 varieties in ATC Nakuru

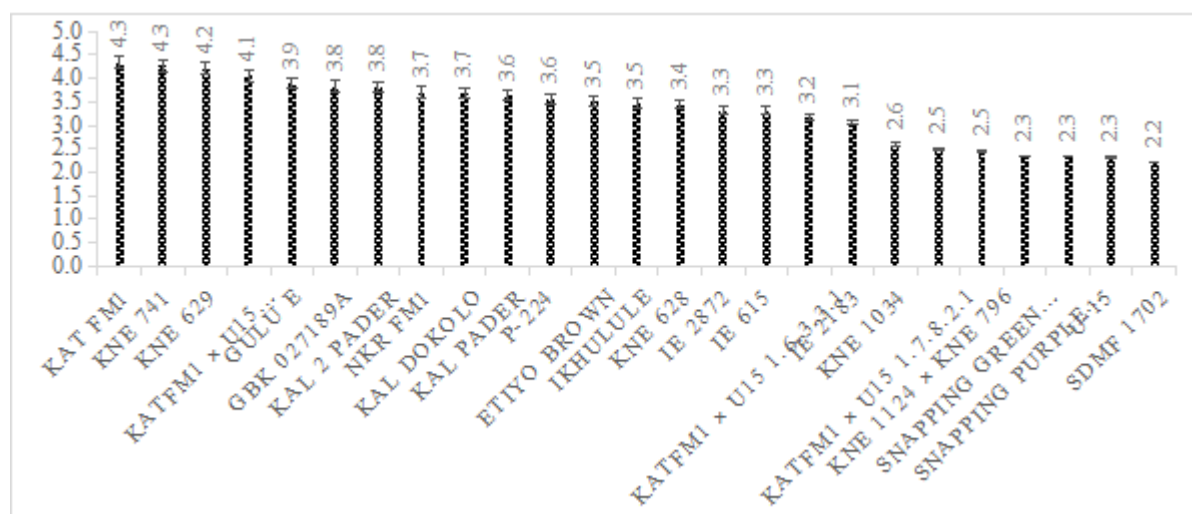


Figure 4.2: Mean variety scores on a scale of 1-5 of 25 varieties in ATC, Bomet County.

Kal 2 pader a local variety stood out to be best in both ATCs, this is a local variety. Local varieties such as Kal 2 Pader, Ikhulule and Otiyo Brown are well adapted to the local environments. The farmers pointed out that the variety can easily survive extreme temperature and rainfall patterns and therefore receiving better preferences. Kal 2 Pader however was the best as it was high yielding, matured early and had resistance to birds. GRAIN and the Alliance for Food Sovereignty in Africa (AFSA), (2018) supports this after

an experiment was done in Uganda. Farmers' preferred local varieties because of their resilience, taste and local preferences such as cultural and spiritual significance. GBK 027189A is a released variety in Kenya mostly for rift valley regions. These was depicted by its ability to have high yields of 1300 kg Ha⁻¹ and 900 kg Ha⁻¹ in Nakuru and Bomet respectively. Gulu E and P-2224 are commercial variety that its ability to resist diseases. Other varieties that caught the attention of farmers was SDFM-1702, Snapping Green early and Snapping-Purple variety.

In ATC-Nakuru, variety SDFM-1702 an advanced breeding line, had short height, takes long to mature and has a spreading nature with high number of tillers. It had high preference to farmers who kept livestock, they marveled at its ability to produce more feed to cattle. They therefore recommended that the variety was suitable for livestock feed and should be improved as a fodder crop. KNE 741, a commercial variety was among the best varieties selected in ATC Bomet, because of its high yielding ability and early maturity. Maturing early is considered as an ability to escape drought. The medium height of this variety also makes it to escape lodging and easy operations such as harvesting and weeding. Such a characteristic was important to farmers from Bomet. In Nakuru however the genotypic performance was different as was expected because the variety had an earlier maturity of 80.5 days (Table 6) which made it more susceptible to birds and blast disease. Farmers evaluated the variety based on what they saw and since most of them saw the damage due to disease and birds, hence the least score in Nakuru. In Bomet, the same variety scored among the best. This is because, the plot was carefully guarded against birds using a scarecrow and physically chasing them away. KatFM1xU151.6.6.3.1.1 was high yielding and had high number of tillers, early maturing, highly uniform and with good grain qualities. Such was desirable to farmers in both ATCs. Snapping-Purple variety had high preference in both ATCs. This is not only because of its highly snapping ability during harvesting but also it is resistance to pests including birds and diseases such as blast. The variety is also high yielding with 800 Kg ha⁻¹ and 900 Kg ha⁻¹ in Nakuru and Bomet ATCs, respectively. Snapping green early has an ability to snap very early, high yielding with 900 Kg Ha⁻¹ in both environments. The variety also has numerous tillers a contributing factor to the high yield. Farmer based selection was dependent on the attributes of each variety most importantly the high yielding traits. Snapping green early also has an ability to escape drought by maturing early.

Table 4.2: Finger millet trait ranking based on focused group discussion (FDGs) at Agricultural Training Centre in Nakuru and Bomet

	B.R	E.M	T.A	B.F	H.Y	L.R	Uni	Market	D.T	R.D	G.T	G.C	F/S	P/S	Rank
B.R		B.R	T.A	B.F	H.Y	L.R	Uni	B.R	D.T	B.R	B.R	B.R	B.R	6	7
E.M			T.A	B.F	H.Y	L.R	Uni	Market	E.M	E.M	E.M	E.M	E.M	5	8
T.A				T.A	T.A	T.A	Uni	T.A	D.T	T.A	T.A	T.A	T.A	10	2
B.F					B.F	B.F	Uni	B.F	D.T	B.F	B.F	B.F	B.F	9	4
H.Y						H.Y	Uni	H.Y	D.T	H.Y	H.Y	H.Y	H.Y	8	5
L.R							Uni	L.R	D.T	L.R	L.R	L.R	L.R	7	6
Uni								Uni	Uni	Uni	Uni	Uni	Uni	12	1
S.A								S.A	S.A	S.A	S.A	S.A	S.A	6	7
Market									D.T	Market	Market	Market	Market	5	9
D.T										D.T	D.T	D.T	D.T	10	2
R.T.D											R.D	R.D	R.D	3	10
G.T												G.T	G.T	2	11
G.C													G.C	1	12
F/S finger														0	13

* B.R=Bird resistance, E.M=Early maturity, T.A= Tillering Ability, B.F= Big finger, H.Y=high Yielding, L.R= Lodging resistance, Uni= Uniformity, S.A= Snapping ability, Market= Marketability, D.T= Drought tolerance, R.T.D= Resistance to Diseases, G.T=Good taste, G.C= Grain colour,

Table 4.3: Mean scores and ranking of varieties on various traits of finger millet in ATC-Nakuru

Variety	High Y	Market	Early M	DRT T	RTD	Taste	F/S fingers	Lodging	Tillering	Uniformity	Bird I	Means	Rank
Kal 2 Pader	4.51	4.29	3.53	3.73	4.12	3.08	4.02	3.98	4.18	4.04	3.8	3.93	1
P-224	4.29	3.92	3.43	3.75	4.14	3.29	4.02	3.71	4.1	4.06	3.94	3.88	2
KATFM1 × U15	4.12	3.65	3.63	3.88	3.94	3.02	4	4.1	4.1	4.14	3.96	3.87	3
1.6.6.3.1.1 GBK 027189A	4.27	4.16	3.69	3.45	3.88	2.88	3.96	4.16	3.82	3.94	3.96	3.83	4
Snapping Green Early	3.8	3.78	3.29	3.59	3.86	3.06	3.88	4.04	3.63	3.98	4	3.72	5
KATFM1 × U15 1.7.8.2.1	4	3.78	3.51	3.82	3.55	2.96	3.94	3.76	3.69	3.73	3.59	3.67	6
KNE 1034	4	3.78	3.37	3.53	3.73	3.24	3.96	3.47	3.63	3.59	3.8	3.65	7
GULU E	3.86	3.76	3.49	3.41	3.59	3.04	3.96	3.94	3.82	3.53	3.59	3.64	8
Snapping- Purple Variety	3.88	4	3.33	3.53	3.84	2.73	3.96	4.06	3.67	3.53	3.41	3.63	9
Ikhulule	4.04	3.78	3.49	3.27	3.61	3.12	3.98	3.82	3.73	3.51	3.2	3.6	10
Kal Dokolo	3.92	3.86	3.43	4.24	3.2	2.78	3.92	3.76	3.75	3.78	2.9	3.6	11

Kal Pader	3.78	3.39	3.22	3.35	3.61	2.86	3.92	3.78	3.51	3.75	3.59	3.52	12
KATFM1 × U15 1.6.3.3.1	3.59	3.45	3.55	3.82	3.41	2.82	3.9	3.63	3.57	3.45	3.47	3.52	13
SDFM-1702	3.61	4.55	3.25	3.53	3.49	2.9	3.94	3	3.45	3.25	3.69	3.52	14
IE 2872	4.02	3.31	3.45	3.41	3.37	2.57	3.94	3.67	3.55	3.76	3.1	3.47	15
U-15	3.57	3.57	3.31	3.43	3.22	2.49	3.96	3.82	3.55	3.63	3.1	3.42	16
KNE 1124 × KNE 796	3.67	3.43	3.08	3	3.2	3.04	4.02	3.39	3.65	3.61	3.37	3.4	17
NKR FM1	3.24	2.9	3.65	3.1	3.04	2.76	4	3.73	3.67	3.9	3.04	3.37	18
KNE 628	3.33	3.24	3.45	3.18	3.45	2.24	3.88	3.65	3.59	3.35	3.12	3.32	19
KNE 629	3.06	3.18	3.02	2.94	2.98	2.82	4.02	3.76	3.37	3.2	3.35	3.25	20
Etiyo Brown	3.39	3.27	3.24	3.22	3.2	2.43	3.9	3.33	3.29	3.12	3.12	3.23	21
KAT FM1	2.9	2.75	3.24	2.67	2.88	2.22	4	3.82	3.45	3.33	2.55	3.07	22
IE-615	2.8	2.84	2.63	2.75	2.73	1.96	3.92	3.24	3.2	3.51	3.33	2.99	23
IE-2183	2.84	2.71	2.98	2.76	2.73	2.25	3.88	3.14	3.24	3.25	3	2.98	24
KNE 741	2.33	2.9	2.86	2.65	2.78	2.39	3.98	3.33	3.16	2.76	2.27	2.86	25
Mean	3.63	3.53	3.32	3.36	3.42	2.76	3.96	3.68	3.61	3.59	3.37	3.48	
S.E.M	0.1070	0.0977	0.0512	0.0823	0.0843	0.0703	0.0093	0.0613	0.0529	0.0667	0.0887	0.23	

*High Y= High yielding, Market=Marketability, Early M= Early Maturity, DRT T= Drought Tolerance, RTD= Resistance to diseases, F/S finger= Folded/Straight finger, Bird I= Resistant to bird infestation.

Table 4.4: Mean scores and ranking of varieties on various traits of finger millet in ATC-Bomet

Variety	High Y	Early M	Drought T	RTD	Marketab ility	F/S finger	Lodging	Tillering	Uniformity	Mean	Rank
KATFM1 × U15 1.6.6.3.1.1	5	4.83	4.83	2.33	4.83	4.83	3.33	4.17	4.83	4.33	1
KNE 1034	4.83	3.83	4.83	2.5	4	4.83	3.33	4.83	4.83	4.2	2
KNE 741	4.83	4.67	4.83	2.17	4.67	4	4.67	3.83	4.83	4.28	3
GULU E	3.5	3.17	3.83	2.5	4.33	3.67	4.67	4.5	4.83	3.89	4
KATFM1 × U15 1.7.8.2.1	4.67	3.5	4.83	2.17	4.83	4.83	3.33	3.5	4.83	4.06	5
GBK 027189A	4.5	2.17	4.5	2	4.5	4.17	3.33	4.5	4.83	3.83	6
Kal 2 Pader	4.33	2.33	4.67	2.33	3.67	4.67	3.33	4.33	4.67	3.81	7
Kal Pader	3.33	2	4.67	3.33	3.67	3.67	3.33	3.83	4.83	3.63	8
KNE 629	2.33	1.67	4.17	2.33	2.67	4.67	4.67	3.83	4.67	3.44	9
U-15	3.83	3.5	4.83	2.17	3.67	3.83	3.33	4.17	4.83	3.8	10
Snapping- Purple Variety	4.67	4.17	4.5	2	4.67	4.67	1.67	4.17	4.67	3.91	11
NKR FM1	3.33	3.33	4.67	2	3.5	3	4.67	4	4.83	3.7	12
Kal Dokolo	3.5	3.33	4.67	3.5	3.33	3.67	3.33	3.33	4.5	3.69	13
P-224	3.5	3.67	3.17	3.33	3	4.67	3.33	4	3.33	3.56	14
Etiyo Brown	2.83	3.17	4.5	2.17	3.33	3.33	3.33	4	4.83	3.5	15

IE-615	2.83	3.33	4.5	2.33	2.33	2.67	3.33	4	4.5	3.31	16
Ikhulule	3.67	3.67	4.83	2.17	3.33	3.67	2.33	2.67	4.83	3.46	17
KATFM1 × U15 1.6.3.3.1	3	2.83	4.17	3	2.33	2.33	3	3.67	4.17	3.17	18
IE 2872	3.5	3.83	4.83	2.17	3.33	3.5	1.33	3.83	3.5	3.31	19
IE-2183	3.17	3.17	4.17	2.17	2.33	3	2	3.33	4.17	3.06	20
KNE 1124 × KNE 796	2	3.17	1.83	2.17	2	3.67	3.5	2.5	2.5	2.59	21
KNE 628	2	1	3	2	1.33	1.33	4.67	3.5	3.33	2.46	22
Snapping Green Early	1.67	1.5	2.67	3	1	2.67	3.33	2.67	2.5	2.33	23
KAT FM1	2	2.5	3.17	2	1.33	3	3	2.33	3.17	2.5	24
SDFM-1702	1.5	1.5	3	1.67	0.33	3	3	3.17	2.83	2.22	25
Means	3.37	3.03	4.15	2.38	3.13	3.65	3.33	3.71	4.23	3.44	
Standard error of mean	0.5411	0.5733	0.5898	0.5715	0.3801	0.3469	0.4004	0.3991	0.5547		

*High Y= High yielding, Market=Marketability, Early M=Early Maturity, DRT T=Drought Tolerance, RTD=Resistance to diseases, F/S finger= Folded/Straight finger, Bird I= Resistant to bird infestation.

Table 4.5: Means of height, maturity and yield t/ha in comparison with the scores and ranking of finger millet varieties scored at ATC- Nakuru

Variety	Height	Maturity	Yield t/ha	Scores	Rank
Kal 2 Pader	110.9 ± 17.2	96.5 ± 9.2	1.2 ± 0.22	3.9	1
KATFM1 × U15 1.6.6.3.1.1	84.3 ± 9.7	86.0 ± 9.7	1.0 ± 0.10	3.9	1
P-224	81.5 ± 7.3	95 ± 6.1	1.5 ± 0.15	3.9	1
GBK 027189A	95.8 ± 20.1	90.7 ± 6.1	1.3 ± 0.17	3.8	2
KATFM1 × U15 1.7.8.2.1	88.4 ± 8.4	108.3 ± 17.4	1.5 ± 0.24	3.7	3
Snapping Green Early	81.3 ± 3.8	92.0 ± 6.4	0.9 ± 0.07	3.7	3
GULU E	82.7 ± 5.6	111.3 ± 1.2	0.9 ± 0.06	3.6	4
Ikhulule	67.5 ± 3.6	96.7 ± 9.0	1.3 ± 0.42	3.6	4
Kal Dokolo	86.3 ± 7.9	92 ± 2.5	1.4 ± 0.14	3.6	4
KNE 1034	77.7 ± 4.1	106.7 ± 7.5	1.73 ± 0.20	3.6	4
Snapping-Purple Variety	88.3 ± 16.9	99.1 ± 7.6	0.8 ± 0.17	3.6	4
IE 2872	68.3 ± 12.5	111 ± 1.2	1.9 ± 0.11	3.5	5
Kal Pader	98.8 ± 19.2	86.3 ± 8.0	1.2 ± 0.21	3.5	5
KATFM1 × U15 1.6.3.3.1	82.5 ± 9.7	103 ± 7.0	1.2 ± 0.03	3.5	5
SDFM-1702	63.2 ± 5.1	107.3 ± 5.8	0.37 ± 0.17	3.5	5
KNE 1124 × KNE 796	86.9 ± 2.1	105.0 ± 2.1	1.2 ± 0.03	3.4	6
NKR FM1	89.1 ± 20.2	114.3 ± 2.4	1.2 ± 0.01	3.4	6
U-15	89.2 ± 3.6	99.3 ± 7.5	1.2 ± 0.08	3.4	6
KNE 628	98.1 ± 13.5	81.0 ± 2.1	1.5 ± 0.13	3.3	7
Etiyo Brown	94.2 ± 11.8	93.7 ± 5.8	1.4 ± 0.21	3.2	8
KNE 629	91.3 ± 2.3	104 ± 4.7	2.1 ± 0.05	3.2	8
KAT FM1	76.1 ± 13.1	89.7 ± 3.5	1.8 ± 0.29	3.1	9
IE-2183	68.5 ± 10.2	101.6 ± 5.2	1.3 ± 0.34	3	10
IE-615	107.5 ± 4.9	93.3 ± 4.8	1.5 ± 0.09	3	10
KNE 741	82.7 ± 4.2	96.2 ± 16.4	0.7 ± 0.11	2.9	11
MEANS	85.1 ± 2.18	97.2 ± 1.82	1.3 ± 0.08	3.5 ± 0.06	5.28

*Means of varieties ± standard error of the mean.

In general, Nakuru had the highest means in plant height, number of days to maturity and yield in t/ha as compared to ATC-Bomet. Varieties in Nakuru ATC had the highest mean

height (0.85 m) with a longer maturity period (97.2 days) and high yield of (1300 kg Ha⁻¹) compared to Bomet ATC which had medium height of 0.57 m, lower maturity days of 95.7 days and 0.9 t/ha yield. The best performing varieties in ATC-Nakuru; Kal 2 Pader, P-224, KatFM1xU151.6.6.3.1.1, GBK 027189A, Snapping green early had a higher yield ranging from 1.18 ± 0.0757 t/ha to 1.29 ± 0.0757 t/ha respectively (Table 6.7) while in Bomet ATC. KatFM1, KNE 741, KNE629, KatFM1xU151.6.6.3.1 and Gulu E, GBK 027189A and Kal 2 pader yield ranged from 0.5 ± 0.0506 t/ha to 1.02 ± 0.0506 t/ha respectively (Table 7). KNE 629 and IE-2872 had the highest yield in both environments with 2.13 t/ha and 1.49 t/ha. IE-2872 was highest in ATC- Bomet with 1.57 t/ha. The yield could be affected by weather. In Bomet, the yield was quite low 900 kg Ha⁻¹ due to high rainfall that settled in the finger millet plots.

This interfered with the performance of the varieties leading to low yields. In Nakuru high yield was observed despite the high infestation of birds in the region. This is because of the favorable conditions of the short season period favored by cool and humid climate.

Table 4.6: Agronomic traits of finger millet varieties scored at ATC-Bomet and the ranking

Variety	Height	Maturity	Yield t/ha	Scores	Rank
KAT FM1	62.7 ± 4.09	89.3 ± 11.3	0.5 ± 0.09	4.3	1
KNE 741	57.2 ± 1.74	63.3 ± 4.70	1.0 ± 0.09	4.3	1
KNE 629	51.0 ± 0.68	104.3 ± 2.96	1.5 ± 0.17	4.2	2
KATFM1 × U15 1.6.6.3.1.1	46.3 ± 2.40	102.3 ± 4.48	0.8 ± 0.18	4.1	3
GULU E	55.3 ± 2.90	83.3 ± 12.17	0.7 ± 0.09	3.9	4
GBK 027189A	62.3 ± 5.04	104.7 ± 6.33	0.9 ± 0.09	3.8	5
Kal 2 Pader	72.8 ± 1.92	105.7 ± 5.69	1.0 ± 0.10	3.8	5
Kal Dokolo	52.8 ± 5.08	85.7 ± 10.20	0.9 ± 0.21	3.7	6
NKR FM1	64.3 ± 4.66	77.3 ± 4.37	0.8 ± 0.11	3.7	6
Kal Pader	65.0 ± 7.63	105.3 ± 2.96	0.8 ± 0.11	3.6	7
P-224	49.3 ± 4.09	99.3 ± 1.66	0.9 ± 0.44	3.6	7
Etiyo Brown	58.0 ± 7.21	100.3 ± 0.67	0.8 ± 0.14	3.5	8
Ikhulule	66.3 ± 3.52	103.3 ± 1.76	0.9 ± 0.02	3.5	8
KNE 628	43.0 ± 1.80	108.0 ± 3.51	0.9 ± 0.13	3.4	9
IE 2872	67.0 ± 3.51	83.0 ± 5.13	1.6 ± 0.07	3.3	10
IE-615	46.7 ± 2.40	98.3 ± 3.92	1.3 ± 0.02	3.3	10
KATFM1 × U15 1.6.3.3.1	49.5 ± 7.05	104.0 ± 3	1.2 ± 0.13	3.2	10
IE-2183	46.0 ± 2.00	92.7 ± 7.26	0.7 ± 0.04	3.1	12
KNE 1034	63.3 ± 7.31	97.0 ± 1.00	0.9 ± 0.11	2.6	13
KATFM1 × U15 1.7.8.2.1	59.7 ± 2.60	110.0 ± 9.50	0.8 ± 0.18	2.5	14
KNE 1124 × KNE 796	49.5 ± 7.05	78.7 ± 4.48	1.0 ± 0.13	2.5	14
Snapping Green Early	65.3 ± 2.60	103.7 ± 6.35	0.9 ± 0.18	2.3	15
Snapping-Purple Variety	54.7 ± 0.33	100.3 ± 2.33	0.9 ± 0.03	2.3	15
U-15	59.0 ± 5.50	89.7 ± 10.20	0.9 ± 0.04	2.3	15
SDFM-1702	47.3 ± 3.38	104.3 ± 1.67	0.6 ± 0.10	2.2	16
MEANS	56.9 ± 1.6	95.8 ± 2.3	0.9 ± 0.1	3.3 ± 0.1	8.64

*Means of varieties ± standard error of the mean

4.6 Discussion

The farmers had a high preference for the uniformity of finger millet (3.91). The response was that non-uniform varieties have difficulties in management causing increased labor. Uniformity provides ease of management activities such as ease of harvesting, weeding and spraying for the control of weeds. Farmers also had a high preference for drought-tolerant varieties (3.75) especially in Bomet as reported by Owere *et al.* (2016) who stipulated that height of 1 ± 0.2 m, high tillering ability and drought-tolerant varieties are most preferred by farmers. This is because with a height of 1 m operations such as harvesting and weeding is easy. Tillering ability and big fingers were directly linked to high-yielding qualities. The higher the number of tillers the higher the plant will produce and the bigger the finger length and number the higher the no of seeds a panicle could carry. Hadjichrisdoulou (1985) and Sadreddine (2016) reported that to select the high-yielding varieties for breeding purposes in multi-environments, one should consider tillering as an important trait. The farmers had a major interest in big fingers as compared to folded or straight fingers. They observed that the bigger the fingers whether they have a straight or folded type could yield more seeds. Therefore, they preferred big-fingered varieties. The folded varieties also had an advantage over the straight varieties. They pointed out that the folded-finger variety could easily escape disease as compared to the straight type of varieties. This however did not seem to directly affect the traits that led to high yield, therefore, scoring less compared to other traits.

The farmers assessed the kernel colour of the varieties and used it to determine the marketability of the varieties. The colour of the grain was either reddish-brown, brown and white seeded. The scores were then used to calculate the percentages and presented in figure 4. The most preferred traits of finger millet were the reddish-brown colour in Bomet while in Nakuru farmers also preferred the red and brown seeded varieties. It was most preferred because it fetches high prices in the market. The farmers were able to point the fact that it could blend well with other flours such as cassava and sorghum. Kanyenji and Oduori (2007) found that the brown and red seeded varieties fetch good prices in the market. Another preference for farmers was the brown grain seeded varieties, which had similar comments only that it fetched slightly lower prices as compared to the reddish-brown. These two varieties were also said to be resistant to blast disease because of the bitter taste they contained. The white grained was said to be sweeter and more suitable for brewing. Ravikumar and Seetharam (1993) reported that the white seeded had a higher content of proteins and lower phenols and tannins which can easily attract microorganisms such as fungi. Taste and bird resistance were not scored in Bomet, this is

because the farmers were not aware of the differences that exist within the varieties in respect to the two traits.

Farmers experienced difficulties in the identification of blast disease in the varieties and the only way they could score is by checking the mature heads. Most farmers had not known that it was a disease and had suspected of birds or other pests such as shoot fly. Farmers also had minimal knowledge on differentiating an attack by shoot fly and the blast disease. Also, the compact-headed varieties were found to be resistant to blast disease as compared to the open and straight finger types. Farmer's knowledge of blast disease was minimal. The farmers could not differentiate the disease when it was on the leaf. On the head attack, the compact fingers projected a higher preference as compared to the straight fingers. This result shows similarity with (Takan, 2004). The study provided a need for extension services in the value addition of finger millet, processing and market information. There were also other socio-economic challenges expressed by farmers such as labor-intensive farming practices that include weeding, post-harvest handling of finger millet, insufficient information of improved genotypes, insufficient supportive agencies among others which were pointed out by Pudasaini *et al.* (2016). For the promotion and utilization of finger millet, capacity building is necessary for farmers and agricultural extension workers (Mgonja *et al.*, 2017). The varieties had different characteristics due to genotype by environment interaction which was evident in the performance and traits of the finger millet (Kebede *et al.*, 2019).

Due to the pandemic of COVID-19, the research could not be conducted in Koibatek-ATC as it was among the major sites of the study.

4.7 Conclusion and recommendation

Farmers preferred uniform varieties with high tillering ability, drought-tolerant, varieties with big fingers and snapping ability. Kat FM1xU151.6.6.3.1.1, Kal-2-Pader, Ikhulule, P-224, Gulu-E, Snapping-green-early and GBK-027189A were the preferred varieties by farmers from Nakuru, while KatFM1, KNE 741, KNE-629, Kat FM1 x U15-1.6.6.3.1, U-15 and Kal-Dokolo were the most preferred varieties for Bomet. The farmers also expressed the lack of knowledge on blast disease affecting finger millet. The less knowledge on improved varieties was also a major concern among the areas. Further research should be done on Farmer Participatory and Varietal Selection of finger millet in various regions of Kenya to increase the knowledge of improved finger millet varieties and possibly adoption.

CHAPTER FIVE
CHARACTERIZATION OF DIVERSITY AND PATHOGENECITY OF BLAST
(*Pyricularia grisea*) AFFECTING FINGER MILLET IN KENYA

Abstract

Pyricularia grisea characterization is a prerequisite for species differentiation and understanding of the pathosystem, evolution and diversity. This study aimed to determine morphological variation, pathogen virulence and molecular diversity of *P. grisea* isolates. Five isolates from infected heads of finger millet were collected from Bomet-ATC, Nakuru-ATC, KALRO-Alupe, ATC-Koibatek and KALRO-Makueni in 2019. The samples were cultured in the lab for both characterization and spore suspension preparation. Data on morphological characterization included colony diameter, colour and shape of conidia. Pathogenicity test was done in the greenhouse in a randomized complete block design using KNE 741 a susceptible genotype and disease data scored. Molecular characterization involved the use of seven SSR markers. Data analyses included the use of software such as AUDPC, Power Maker, GeneAlex and Darwin. Results showed that *P. grisea* isolates had different growth patterns concerning colour, colony diameter and conidia shape. There was a significant variation in conidia shape with all showing grey to brown colour on Oat Meal Agar (OMA) media with 2-4 septate conidia. The isolate from Machakos was the most aggressive in growth with the colony covering 4.57 cm after 7 days and had fully mature spores at 20-day with 14.57 µm conidia diameter while isolate from Bomet had a very slow growth with 4.57 µm conidia diameter. The pathogenicity test revealed that all environments were significantly different ($P < 0.01$) virulence on the tested genotype. Neck blast was scored at physiological maturity was prominent in Koibatek and Bomet strains while leaf blast was severe in Bomet and Alupe strains. In all environments, Bomet isolates strongly expressed symptoms of both neck and leaf blast with AUDPC range of >80 . Molecular analysis showed that the effective number of alleles ranged from 1.30 (MGM 437) -1.99 (*Pyrm* 61-62) with an average of 1.71. Polymorphic information content varied between 0.20-0.37 for primers MGM 437 and *Pyrm* 61-62 respectively. *P. grisea* gene diversity ranged between 0.23 and 0.49. Shannon's information index varied between 0.39-0.69. Factorial and phylogenetic analysis revealed that *P. grisea* isolates were diverse with no geographical grouping. Analysis of molecular variance indicated diversity occurred within populations (87%) as opposed to among populations (13%). The high *P. grisea* variability found in the study is a clear indication of the pathogen high sexual recombination among strains collected in major growing areas of Kenya.

5.2 Introduction

Finger millet, an important cereal crop grown in most parts of South Asia. In Africa, it is grown in the subhumid areas including Kenya, Ethiopia, Malawi, Tanzania, Uganda, Zambia and Zimbabwe. Besides its high nutritional value, finger millet is highly resilient to harsh climatic conditions. Finger millet blast disease, caused by *Pyricularia grisea* (Teleomorph; *Magnaporthe grisea* (T.T Herbert, (M. E Barr) is the most economically important disease of finger millet (Mgonja *et al.*, 2007). It is known to cause significant losses in yield and utilization of finger millet. Worldwide losses of above 50% yield have been reported in finger millet and above 30% in rice production (Esele, 2003; Prajapati *et al.*, 2013). Blast infects finger millet at all stages from seedling stages all through panicle formation (Sreenivasaprasad *et al.*, 2004). Effect on the panicle on susceptible genotypes is drastic and may lead to total seed loss of the entire finger millet crop (Gashaw *et al.*, 2014). Muimba, (2018) reported that favorable weather conditions (temperature of 25 °C and 80% humidity) precede infection of blast diseases which starts when a three-celled conidium lands on a leaf surface. This leads to the formation of an appressorium which later forms a penetration peg, punctures the cuticle allowing entry to the epidermis. Formation of lesions then follows which later spreads to the whole plant through the epidermis forming diamond-shaped grey lesions with brown or black margins. Infection from the leaves begins from the tip backward. The disease has a wide range of hosts especially grasses and sedge species including rice (*Oryza sativa*), wheat (*Triticum aestivum*), pearl millet and foxtail millet (*Setaria italica*). Blast affects production and utilization of these crops leading to a substantial decrease in production in Southern Asia, Eastern and Southern Africa (Takan *et al.*, 2012).

Finger millet is a small-grained cereal that is widely cultivated in arid and semi-arid areas of East and South Africa and Southern Asia. Nutritionally, it contains 7-14% protein, iron, calcium, phosphorus, carbohydrate, zinc and gluten-free amino acids such as methionine, leucine, isoleucine, phenylalanine among others (Kumar *et al.*, 2016). *Eleusine coracana* is not only a food crop but also an important source of food security to marginal areas. Finger millet provides solutions to alleviating ‘hidden hunger’ affecting worldwide populations by providing essential micronutrients such as zinc (Underwood, 2000). It can also be utilized as a crop with a wide range of genetic resources providing resilience to the changing climatic conditions. With all these benefits, finger millet is affected by many diseases such as root rot, smut, streak, mottling virus and blast disease. *Eleusine coracana* is tolerant to most of these diseases however blast disease is the most devastating and destructive leading to losses in yield and poor utilization by farmers (Ramakrishnan *et al.*, 2016).

Resistance breakdown over time due to pathogen variability interferes with the breeding objective of developing resistant genotypes (Kariaga *et al.*, 2016). The morphological and genetic diversity of *P. grisea* population is important as it offers durable resistance to the losses caused by this pathogen (Kariaga *et al.*, 2016). Characterization of *P. grisea* is important in understanding evolution, diversity and pathogenicity. Biodiversity-ecosystem functioning studies control s numerous ecosystem processes such as detection, identification and distribution of the fungi. The effect of fungal biodiversity has been used in approaches such as metagenomics, metatranscriptomics and metabolomics (Frąc & Hannula, 2019). Pathotypes of *P. grisea* in rice, pearl millet and foxtail millet have been studied and identified, however, fewer studies have been done on the morphological and molecular characterization of blast disease in finger millet (Takan *et al.*, 2012). These studies have shown considerable variation morphologically in terms of mycelia growth, colour and colony production of *P. grisea* (Gatachew *et al.*, 2014). Molecular markers have been used to indicate the diversity of pathogenic populations. The use of SSR markers have been used to evaluate pathotype genetic diversity because of their high sexual reproduction recombination potential, co-dominance, locus-specific, multi-allelic and they occur in abundance for all species. This study therefore aimed at characterization of *P. grisea* collected from different finger millet growing regions of Kenya using morphological and genetic features of the pathogen strains and their pathogenicity test to reveal the most virulent pathogen. The determination of the most virulent strain will help to come up with an effective management strategy against the pathogen.

5.3 Materials and methods

5.3.1 Isolation of *Pyricularia grisea*

Four samples each of diseased tissues (leaves and panicle) were picked from five different counties (Bomet-ATC, Baringo (ATC-Koibatek), Busia (KALRO-Alupe), Machakos (KALRO-Makueni) and Nakuru- ATC) and ported to Egerton biotechnology laboratory for isolation of the fungus using Tuite (1969) and Aneja (2005) standard tissue isolation procedure with minor modifications. The margins of the infected tissues were cut in triangular shapes of 5-10 mm and surface sterilized with 0.5% sodium hypochlorite solution and dipped in sterile distilled water for 2 seconds to saturate the specimen and dealcoholize. The tissues were then placed in glass plates with filter paper to dry the excess water. They were then plated on growth media containing Oatmeal Agar amended with 60 mg/l Neomycin sulphate to avoid bacterial contamination (Tredway *et al.*, 2003) and incubated at $25 \pm 1^\circ\text{C}$ in artificial light on a 12 h

light/dark photoperiod for 15–25 days for sporulation and growth of the fungi (Aneja, 2005). Pure colonies were obtained from each region through five subsequent sub-culturing to generate six pure *Pyricularia grisea* isolates from each environment to form a total of 30 samples.

5.3.2 Microscopic characterization of *Pyricularia grisea* isolates

A section of 0.5 cm of the young sporulated fungus was picked using a micro-pin placed on a drop of water on a piece of slide to allow for classical characterization using binoculars microscope after staining with lacto phenol cotton blue and images observed using image analyzer software. Morphological observations were taken based on colony, conidia and conidiophore morphology; Colony diameters of each isolate (mm), surface texture, pigmentation, mycelial growth on different solid media, type of margin, shape, colour, size (length and width) septation of the conidia (Gashaw *et al.*, 2014). Micrographs were taken to show the typical spore morphology of mycelial colour, type of margin and sporulation of *P. grisea* isolates (Barnett & Hunter, 1960). Monoconidial isolates of pure culture fungi were then maintained on in agar slants at 4 °C. Mother cultures were also preserved in a freezer as reference cultures (Khosravi *et al.*, 2019).

5.3.3 Pathogen spore preparation

Spores and conidia from 14-day cultured fungi were harvested by flooding five plates representing the five regions with sterilized distilled water and scrapping the growth by a spatula and placed on glass petri dish (Getachew *et al.*, 2014). A spore solution of 10 µl was pippered and placed on a haemocytometer and the number of spores counted on the chambers A, B, C, D and E. The numbers found were then used to determine the number of spores per ml by multiplying the value by 2000. The spore suspension was then adjusted to desired concentration of 1×10^5 spores/litre with the help of hemocytometer using the formula of $C1V1$ and 0.01 % Tween 20 a surfactant which amends the properties of the carrier to ensure it dissolves. The suspension was then sieved through a double layer of muslin sleeve as it was poured in calibrated hand sprayers.

5.3.4 Planting, inoculation and scoring of disease on susceptible KNE 741

Test plants were planted in a CRD design with three replicates representing the five regions and three pots for control. Eighteen small pots measuring 20 cm diameter and 40 cm height were filled with sterile soil autoclaved at 121 Pa pressure and 21°C for 15 minutes. DAP of 15.5 g was added per pot. Three seeds from the most susceptible variety (KNE 741) were then

sown in the pots and allowed to germinate and grow for two weeks. A spore suspension of *P. grisea* strains from each of the five regions (Bomet, Nakuru, Koibatek, Alupe and Makueni) were used to do the pathogenicity test. Five calibrated hand sprayers were used to spray the pathogen strains to the susceptible variety. The pots were then covered with a parchment bag for 48 hours to create humidity required for the growth of the pathogen. After a period of 7 days the symptoms were recorded from each variety and the most virulent pathogen determined on disease severity. Each observed symptom was assigned group I- III where; I-Highly pathogenic, II-moderately pathogenic III- mild pathogenic.

Disease severity rating (DSR) (% damage) was done on the first four leaves (flag) where five plants were randomly tagged per plot. Disease severity on tagged plants were recorded at tillering, flowering, and physiological maturity stages on KNE 741 using modified Cobb scale (Kiran *et al.*, 2017). Leaf blast was scored based on percent surface area of the infected leaves was evaluated as *P. grisea* severity was used as an indication of severity as described in chapter three section 3.3.4 (Table 3.3). Neck blast severity was based on the relative lesion size on the neck a 1 to 5 progressive rating scale will be used where, 1 = no lesions to pin head size of lesions on the neck region, 2 = 0.1 to 2.0 cm size of typical blast lesion on the neck region, 3 = 2.1 to 4.0 cm, 4 = 4.1 to 6.0 cm, and 5 = >6.0 cm size of typical blast lesion on the neck region as in chapter 3 section 3.3.4 (Figure 3.1).

5.3.5 DNA Isolation

Pure cultures of *P. grisea* strains grown on Oat Meal Agar (OMA) for 5 days were used. DNA extraction was done using a modification of the Dellaporta *et al.* (1994) protocol. The modifications included use of a single SDS buffer (Modified SDS Buffer Components; 20 g SDS, 100 ml of 1M Tris-HCl (pH 8.0), 50 ml of 0.5M EDTA (pH 8.0), 20 g PVP, 10 g Sodium Sulphite and 82 g Sodium Chloride), omission of the sodium acetate, potassium acetate and mercaptoethanol. 70% ethanol was used instead of 80% and subsequent extraction procedure modified as follows: 50-70 mg of fungal mycelia was scrapped from the petridish and placed in a 2 ml microcentrifuge tube. 1000 µl of 2% SDS buffer was added and placed in a water bath at 65 °C for 1 hour. Centrifugation was then done at 20784 g force for 10 minutes. 750 µl of the supernatant was then pipetted out and placed on a sterile empty 2 ml microcentrifuge tube and an equal amount of Chloroform: Isoamyl Alcohol (CIA) 24:1 added. This was then centrifuged at 20784 g force rpm for 10 min. An aqueous solution of 600 µl was then picked, placed on a fresh

tube and an equal amount of cold isopropanol added to precipitate the nucleic acids. This was then incubated in a freezer (-20°C) for 12 hours then centrifuged at 20784 g force g for 10 min.

After centrifugation, the supernatant was carefully decanted leaving a pellet in the tubes. 700 µl of 70% alcohol was added and centrifuged at 20784 g force for 10 minutes. Ethanol was carefully decanted leaving the pellets. The pellets were then air-dried for 30 minutes, 100 µl of sterile *ddH*₂O added and samples incubated at 65 °C for 1hr to dissolve the pellets. The samples were then stored at 4°C.

5.3.6 DNA confirmation of the DNA by gel electrophoresis.

The bands of 30 isolates of *P. grisea* collected from the five environments were confirmed for presence and absence of DNA. The gel was then visualized under a UV transilluminator (Vilber Lourmat).

DNA confirmation was done using a 1% agarose prepared using 1X sodium borate and pre-stained with Gelred dye. The gel was placed in an agarose gel tank (Model CBS Scientific) containing sodium borate. DNA of 5 µl was mixed with 3 µl of lading dye and loaded on the gel wells. The gel was then run for 1 hour at a voltage of 100 volts and a current of 500 mA using an EC 1000 XC Power Pack and CBS Scientific MGU-502T gel tray. The gel was then visualized under a UV transilluminator (Vilber Lourmat).

5.3.7 DNA quantification and purity check

DNA quantification was also done using a Nanodrop 2000c spectrophotometer (Thermo Scientific). The measurements of DNA purity were shown by the concentration in ng/µl and absorbance 260:280 ratio.

5.3.8 SSR genotyping

Seven SSR markers were used for analyzing SSR diversity in *P. grisea* sourced from MGM database (<http://ibi.zju.edu.cn/pgl/MGM/index.html>) (Table 5.1).

PCR was performed in a 10 µl reaction volume containing 6 µl of master mix (One Taq Quickload 2x MM), 1 µl of forward and backward primers, 0.5 µl of 25Mm MgCl₂ (Promega), 1.5 µl of *ddH*₂O and 1 µl of DNA template. Amplification was done in an AB2720 thermocycler (Applied Biosystems) using following conditions: Initial denaturation at 94°C for 5 minutes, 45 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for MGM coded primers for 1 min (*Pyrms* at 61°C), extension at 72°C for 2 min and final extension for 10 min at 72 °C. The

PCR products were run on a 2% agarose gel. Amplicons were visualized under UV light and the fragment sizes scored based on a 100 bp/1Kb molecular ladder.

Table 5.1: Primers used with annealing temperature, forward and backward primer-sequences

Name	AT	Forward primer (3'-5')	Backward primer (5'-3')
MGM200	55	AAGCGTAAATGGCTCAATGC	GCTGATGTTGTTGCTGCTGT
MGM436	55	GACCTTTATCGGATGCGTGT	CACACAGTGGCCATCTAACG
MGM437	55	GCCCCTCAATAGATCGTCAA	ACTGCGGCATTTTAACCTGT
MGM451	55	TCTCAGTAGGCTTGGAATTGA	CTTGATTGGTGGTGGTGTG
MGM454	55	GCAAATAACATAGGAAAACG	AGAAAGAGACAAAACACTGG
<i>Pyrms</i> 15-16	61	TTCTTCCATTTCTCTCGTCTTC	CGATTGTGGGGTATGTGATAG
<i>Pyrms</i> 61-62	61	GAGGCAACTTGGCATCTACC	TGGATTACAGAGGCGTTCG

*AT=Annealing Temperature

5.3.9 Data analyses

Area Under Disease Progress Curve (AUDPC) was used to estimate the severity of leaf and head blast disease in susceptible KNE 741 finger millet variety. It was computed using Wilcoxon *et al.* (1975) formula;

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

Where; y_i = disease severity % on the i th scoring, t_i = number of days from sowing to i^{th} scoring, n = total numbers of scoring t_{i+1} is second assessment date of two consecutive assessment and $y_{(i+1)}$ is disease severity on assessment date $(i+1)$.

The data on severity was subjected to Analysis of variance for green house experiment was computed using PROC GLM in Statistical Analysis Software (SAS institute Inc; Cary, 2002) using the following model;

$$Y_i = \mu + R_j + \varepsilon_i \dots\dots\dots \text{Equation 2}$$

Where, Y_i is the overall observation, μ is the overall mean, R_j is the i^{th} observation due to variety and ε_i is the random error term (Equation 1).

Diversity data was generated by scoring for the presence (1) or absence (0) of amplification on all the gels producing a score matrix. Marker polymorphism was quantified in terms of polymorphic information content (PIC), major allele frequency, observed number of

alleles and effective number of alleles using Power marker software version 3.25 (Liu & Muse, 2005). Diversity of *Pyricularia grisea* involved calculation of Nei's genetic diversity indices and Shannon's Information Index executed in PowerMarker software (Table 3). Phylogenetic analysis and grouping of isolates involved factorial and dendrogram construction using Neighbor Joining method in Darwin software, with 1000 bootstraps to identify the differences among species (Perrier & Jacquemoud, 2015). A Principal Coordinate Analysis (PCA) was also done using Darwin software. Analysis of diversity within and among population was computed using AMOVA in GenAlEx software version 6.5 (PE, 2012).

5.4 Results

5.4.1 Cultural and morphological characteristics of *Pyricularia grisea*

a) Colony colour

There was variation in colony colour of all the pathogens collected from Bomet, Alupe, Koibatek, Nakuru and Makueni regions. Colour variation occurred mostly 3 to 5 days and 5-14 days at initial and later stages respectively. From 15-25 days most pathogens had varied grey and black colour on both the front and the backside of the petri-dish (Figure 5.1)

Isolate 1 (Alupe) was white at initial growth and finally changed to grey colour at mid-stage from day 15-25 it was greyish-white in colour. The colony had smooth margins with 5 rings observed only at the front of the petri-dish. It had a unique appearance of red pigmentation during its growth which disappeared at later stages. Blast isolate 2- (Bomet) was white at the initial stages of growth which turned to a grey colour at the final stages with a buff colour observed at the back of the petri-dish. It had smooth margins with 6 rings observed on the front and the back of the petri-dish. Blast isolate 3- (Koibatek) had numerous variations in colour. White and red pigmentation were observed during the initial stages of growth. The colour later changed to greyish white at the final stages. It had smooth margins with 4 rings observed only at the front of the petri-dish. Blast isolate 4- (Makueni) had grey colour at the initial stage of growth on both sides of the petri-dish which later changes to a totally black colour. It had no rings both at the back and the front of the petri-dish with irregular margin as it grows. Blast isolate 5- (Nakuru) had a clear white colour at the initial stages of growth with smooth margins and 5 centric rings. It finally changed to greyish white colour at later stages (Figure 5.1: Table 5.4).

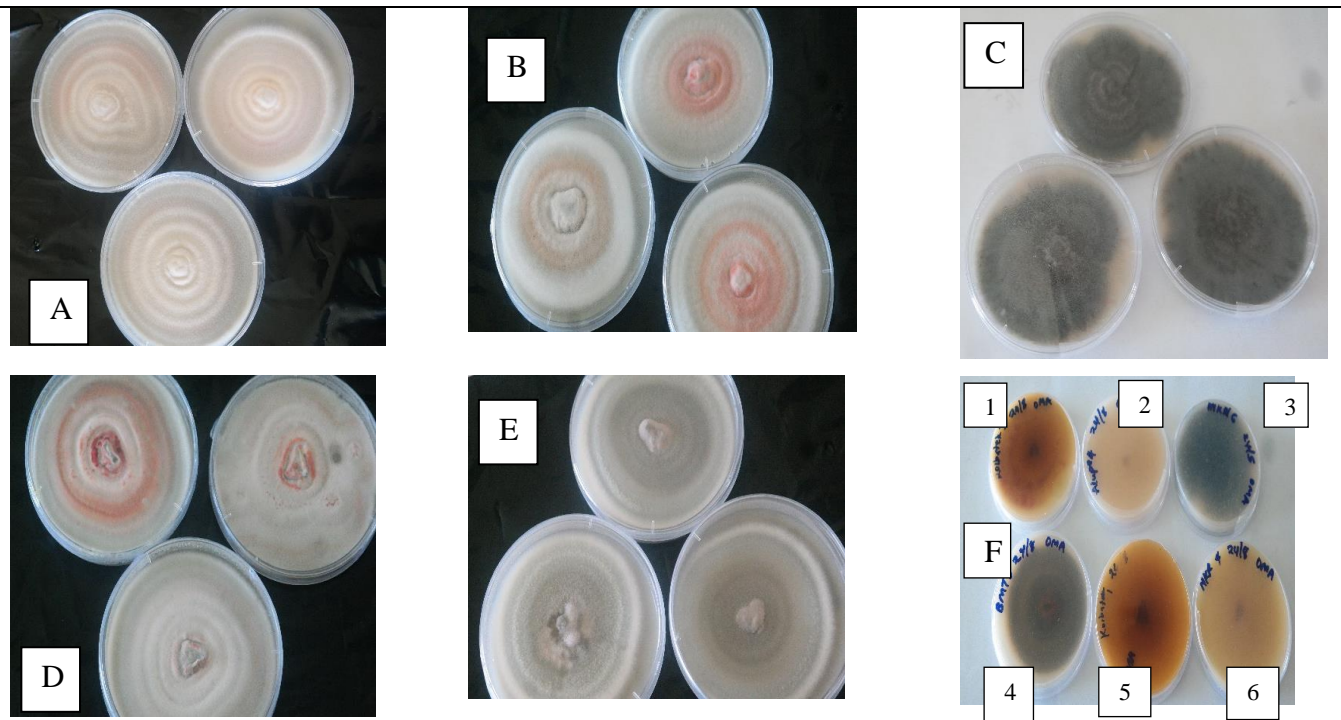


Figure 5.1: 10-day *Pyricularia grisea* isolates on Oat meal Agar colour differentiation collected from different environments. Where A-Alupe isolate, B-Nakuru isolate, C-Makueni isolate, D-Koibatek isolate and E- Alupe isolate. F- Photographs taken from the back side of the petri-dish; where 1 and 5- Koibatek isolate, 2- Alupe isolate, 3- Makueni isolate, 4-Bomet isolate and 6- Nakuru isolate.

b) Colony diameter and spore observation under the microscope

Colony diameter was measured on a 3rd, 5th and 7th day period and growth incubated in conditions of 25 ± 1 °C for 10 days compared on all the five sites. On the 3rd day *Pyricularia grisea* from Bomet colony diameter had the highest mean (27.8) which was significantly different ($P < 0.05$) from those of Makueni, Alupe, Koibatek and Nakuru. On the 5th day, colony diameter from Bomet, Makueni, were not significantly different ($P < 0.05$). Pathogen strains from Koibatek and Nakuru did not differ significantly ($P < 0.05$). Colony diameter from Alupe was not significantly different from all the sites. On the 7th day, colony diameter from Bomet (81.0) had the highest growth and therefore different significant from those from other environments. Alupe, Koibatek, and Makueni isolates had no significant difference ($P < 0.05$). Nakuru had the lowest mean (72.1), however it was not significantly different ($P < 0.05$) from those of Makueni, Koibatek and Alupe (Table 5.2).

Table 5.2: Colony diameter of *P. grisea* incubated at 25°C±1 on oat meal agar for 10 days period and spore diameter (µm, Magn x40) at 20-day growth observed under a microscope.

Environment	Colony diameter			Spore diameter
	3-day	5-day	7-day	(µm) Magn x40
Alupe	17.0 ^b ± 0.9	31.3 ^{ab} ± 1.6	75.0 ^{ab} ± 2.6	5.34 ^b ± 0.24
Bomet	27.8 ^a ± 2.2	36.3 ^a ± 2.1	81.0 ^a ± 1.1	4.57 ^b ± 0.46
Koibatek	20.7 ^b ± 0.5	27.6 ^b ± 1.4	76.6 ^{ab} ± 2.0	4.95 ^b ± 0.22
Makueni	17.2 ^b ± 2.1	36.0 ^a ± 2.0	75.0 ^{ab} ± 2.6	6.37 ^a ± 0.25
Nakuru	20.8 ^b ± 1.4	29.6 ^b ± 0.8	72.1 ^b ± 1.7	4.92 ^b ± 0.37
LSD (5%)	0.5	0.54	0.66	0.79
CV%	14.6	10.05	5.14	6.30
R ²	75.7	68.5	53.3	99.10

Means of three replicates per environment ± standard error of the mean. Means followed by the same letters are not significantly different (P<0.05).

c) Conidia characteristics

Microscopic observation of the spore diameter for *P. grisea* was measured and the results indicated that there was a significant difference (P<0.01) in spore sizes on the 20 days cultures. *Pyricularia grisea* from Makueni had the highest mean of 6.37 µm. The spores were long, pyriform with four septa at 20 days culture, more mature in terms of growth. Nakuru had two septate conidia with a rounded shape. The septae had separate margins. Alupe had two mediumly sized pyriform spores with a rounded apex. Bomet had the lowest 4.57 µm with 2-4 septate conidia which had a smooth margin at the apex. (Figure 5.2). The spores had the highest diameter of 5.34 µm when fully mature at 25 days. There was no significant difference in spore sizes from Alupe, Koibatek, Nakuru and Bomet at full maturity (Table 4.2). All *P. grisea* isolates from all the environments had pyriform shapes with varied apex; round/sharp and round/flat, number of cells; from 2-4 celled conidia of various sizes and shapes. The middle cells were larger compared to the apex and the base cells. There was also varied growth from being large and fully mature at 20th day (Isolate 4) to slow development of spore characterized by small and immature spore (Isolate 5) (Figure 5.2).

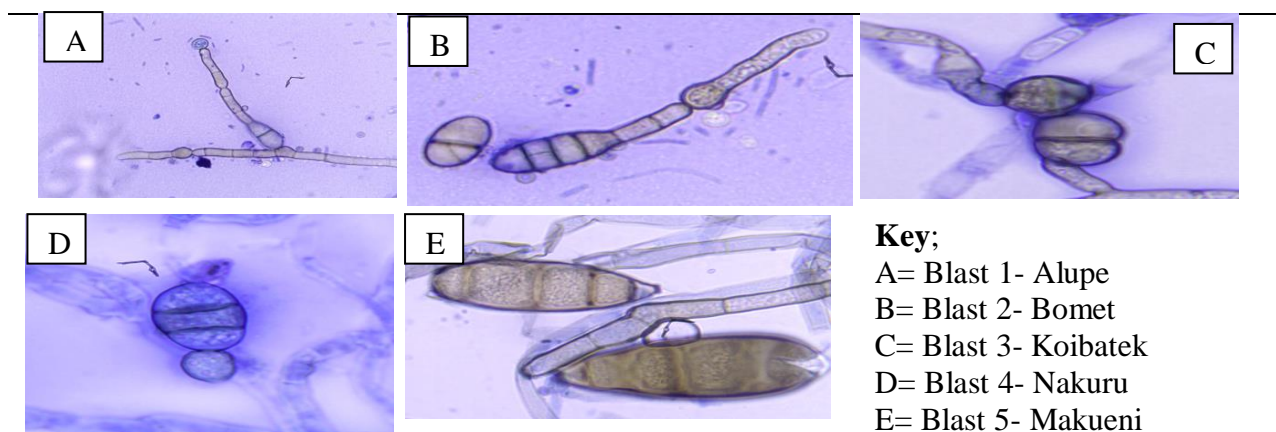


Figure 5.2: Micro-images of a twenty-day culture of *P. grisea* isolates; Magn x40 collected from different growing regions of Kenya

5.4.2 Pathogenicity test

There was variation in growth patterns, shape and colour of *Pyricularia grisea* collected from the five regions. The effect of the *P. grisea* from various sites on test plants indicated the pathogenicity levels of the pathogen. The symptoms were noted 7-20 days after inoculation as small white lesions which grew into diamond shaped by the 10th day, greyish white symptoms with brown spots. Later spots developed to be black at the 15th day and leaf necrosis by yellowing which was typical from the apex as it progressed downwards at the 20th day. These symptoms were a clear indication of *P. grisea* especially when compared to control which was not sprayed. *Pyricularia grisea* from Bomet and Makueni had the highest virulence (Highly pathogenic- Group I) while those from Alupe and Nakuru and finally Koibatek *P. grisea* had the least virulence (Mild pathogenic- Group III) (Figure 5.5).

a) Virulence of pathogens strains from selected regions

Pathogen strains from different regions had different indication of the symptoms and infection to the susceptible variety KNE 741. From the ANOVA table, environment was significant ($p < 0.005$) for both leaf and neck blast severity (Table 5.3).

Table 5.3: ANOVA table for leaf and neck blast severity for KNE 741.

Source of variation	df	LAUDPC	NAUDPC
Environment	5	1259.09***	1309.93***
Rep	1	36.75	36.75
Error		28.78	7.35
CV		7.53	4.00
R ²		97.77	99.44

***Significant at $P < 0.01$, df =Degree of Freedom, AUDPC= Area Under Disease Progress Curve

LAUDPC= Leaf severity values, NAUDPC=Neck severity values

Mean separation from the sites revealed that *Pyricularia grisea* significantly differed ($P < 0.01$) in leaf and neck blast severity. Strains from Bomet, Alupe, and Makueni had higher severity values (> 80) and did not differ significantly ($P < 0.01$) while in Koibatek and Nakuru were not significantly ($P < 0.01$) different in leaf severity. A lower mean was recorded on the control (< 20). Pathogens from Alupe, Makueni, Koibatek and Nakuru had no significant difference ($P < 0.01$) on leaf severity. Neck severity appeared more on pathogen picked from Koibatek and Bomet and therefore they were not significantly different ($P < 0.01$) while Alupe and Makueni had the same mean on neck blast severity (Figure 5.3).

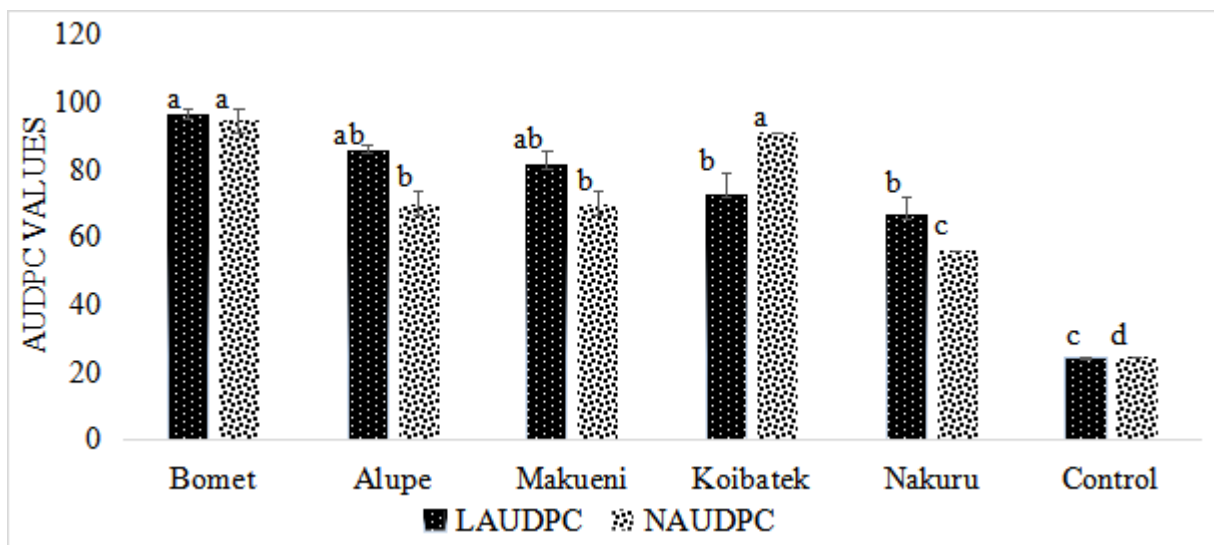


Figure 5.3: Comparison of leaf and neck severity of *Pyricularia grisea* on KNE 741. Means with the same letters are not significantly different ($P < 0.01$)

b) Relationship between neck and leaf blast

Pearson's correlation coefficient indicated that there was a positive correlation of leaf and neck blast since a one-unit increase in leaf blast with all the factors held constant led to an increase of 0.9003 with 88.2 % of neck blast (Figure 5.4).

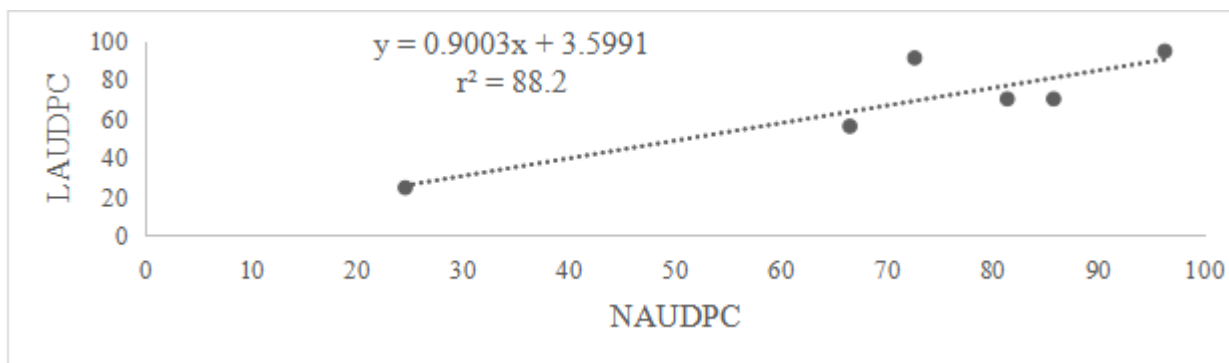


Figure 5.4: Relationship between neck and leaf blast of *P. grisea* sprayed on susceptible variety (KNE 741)

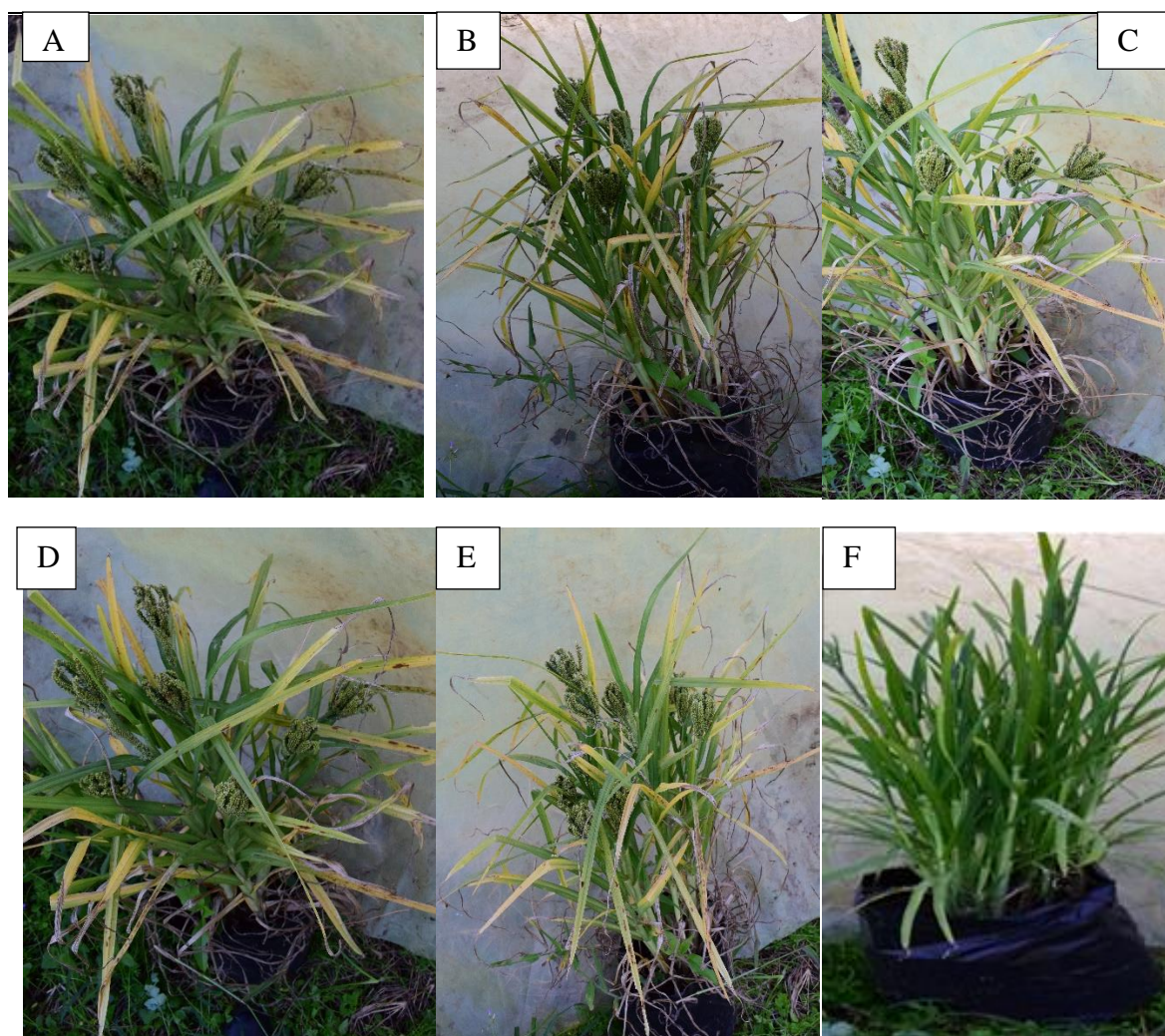


Figure 5.5: Effect of *P. grisea* isolates on susceptible KNE 741 variety. Where letters represent the pathogens sprayed on susceptible KNE-741: A= Bomet isolate, B= Makueni Isolate, C= Alupe isolate, D=Nakuru isolate, E=Koibatek isolate and F=Control (Non-inoculated)

Table 5.4: Morphological characterization of *Pyricularia grisea* from five selected counties of Kenya

Isolate	Location	Colony morphology	Colony colour	Shape of conidia	Pathogenicity	Pathogenicity group
Blast-1	KALRO-Busia, Busia county	Smooth margins with 5 circular rings observed on the front of the petri-dish	White and brown in colour with buff colour at the back	Pyriform very small	Moderate	II
Blast-2	ATC-Bomet Bomet county	Smooth margins with rings observed on both front and back side of the petri-dish	White which finally turns to grey in colour	Pyriform- Large	High	I
Blast-3	ATC-Koibatek, Baringo county	Smooth margin with rings observed only on the front of the petri-dish	Brownish grey in colour	Pyriform- medium	Mild	III
Blast-4	KALRO-Makueni, Machakos county	Circular in growth with irregular margins	Grey which finally turns to black colour	Pyriform- Very large	High	I
Blast-5	ATC- Nakuru county	Round with smooth margins and rings at the front	White which finally turns to grey	Pyriform- Medium	Moderate	II

5.4.3 Molecular characterization of *Pyricularia grisea* from five selected major growing areas of Kenya

a) Confirmation of the DNA by gel electrophoresis

The bands of 30 isolates of *P. grisea* collected from the five sites were confirmed to be present after visualization under a UV transilluminator (Vilber Lourmat) (Figure 5.5)

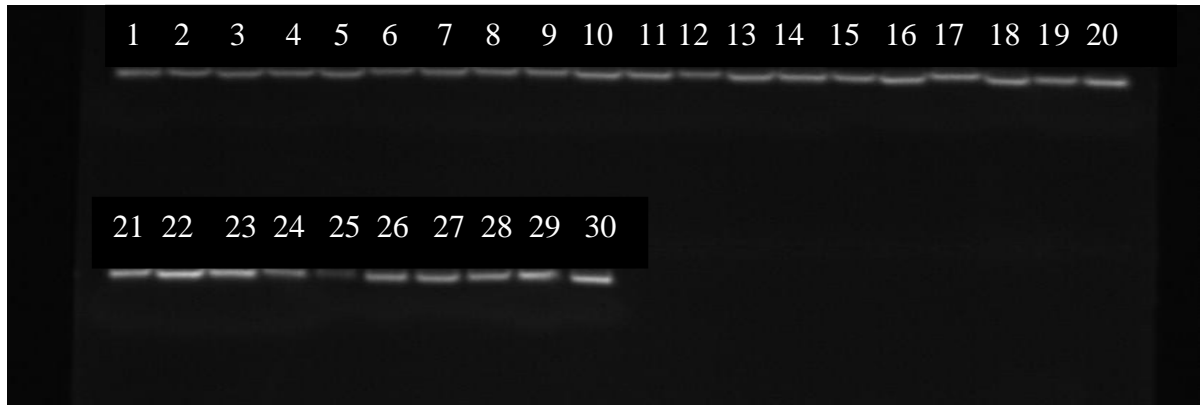


Figure 5.5: The presence of *P. grisea* isolates ranging from isolate 1-30 showing presence of DNA bands as viewed under a UV transilluminator

b) Marker polymorphism and genetic diversity of *P. grisea* isolates collected in the five major growing areas of Kenya

A simple numeric scoring was used to denote the presence (1) and absence (0) of allele for each locus (Table 5.6; Appendix 9). The observed allele number was two for all the selected primers indicating that only one locus was amplified by all the markers. Effective number of alleles $\{A_E=1/(1-H_{exp})\}$ ranged from 1.3006 (MGM 437) to the highest (1.9912) (*Pym* 61-62). Major allele frequency ranged from 0.5333 to 0.8667. Polymorphic Information Content (PIC) varied between 0.2044 and 0.3739 for primers MGM 437 and *Pym* 61-62 respectively. When *P. grisea* diversity was assessed, gene diversity ranged between 0.2311 and 0.4978 with a mean of 0.4. Shannon's Information index varied between 0.3927 and 0.6909 with a mean of 0.5848 (Table 5.6).

c) Factorial and phylogenetic analysis of the strains of *P. grisea* collected in various regions

Factorial analysis revealed that the samples from all the five environments clustered randomly with no distinct pattern observed (Appendix 10). Most isolates clustered as individuals showing that they were genetically distinct while a few clumped together. The phylogenetic analysis grouped the samples into two main clusters and six sub-clusters.

Cluster 1 comprised of 2 sub clusters. Sub-cluster 1 comprising of isolates 2, 3, 4 and 5 from Nakuru, 3 and 4 from Alupe and 3 and 6 from Koibatek which appeared as duplicates and isolate 6 from Bomet which clustered as a distinct individual. Sub cluster two had only two isolates Makueni 1 and Alupe 6 which appeared distinct. Cluster II comprised of 4 sub clusters. Sub cluster I comprised of isolate 4 and 5 from Bomet which appeared as duplicates and isolate 1 from Koibatek. Sub cluster II comprised of isolate 1 and 2 from Bomet and 2 and 5 from Koibatek which appeared as duplicates and isolate 5 from Alupe appeared as distinct. Sub cluster III comprised of distinct isolates 6 and 1 from Makueni and Nakuru respectively. Sub cluster IV had 5 duplicate isolates Makueni (2, 3, 4 and 5) and distinct isolate 1 from Alupe, 3 from Bomet and 6 from Nakuru. There was no particular pattern deduced from the grouping, however, some strains from different regions were observed to cluster in same sub clusters as duplicates with few appearing distinct. (Figure 5.6).

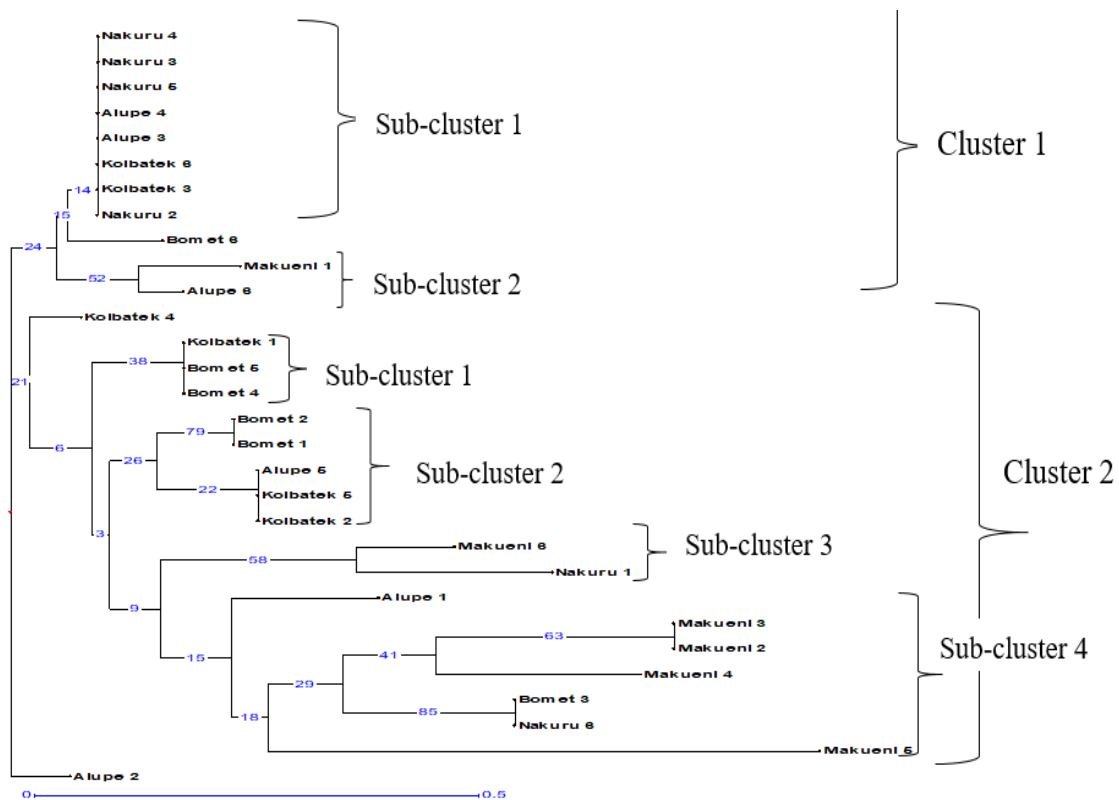


Figure 5.6: Phylogenetic tree showing *Pyricularia grisea* diversity constructed using Neighbour joining method

Overall, phylogenetic analysis revealed that *P. grisea* isolates from the studied regions were genetically diverse within the isolate population as opposed to geographical differentiation.

5.4.3.4 Analysis of Molecular variance (AMOVA)

Results of AMOVA (Table 5.6) revealed that huge diversity within the *P. grisea* isolate populations (87%) compared to among the population (13%) with a P value of 0.053.

Table 5.5: AMOVA table of *P. grisea* with 30 samples collected from 5 regions

Source	df	Ms	Est.var.	%	P.value
Among Pops	5	2.233	0.193	13	0.053
Within population	24	1.235	1.285	87	

*Est.var-Estimated variance and %-Percentage populations.

Table 5.6: Summary statistics of genetic diversity indices of 30 samples of *Pyricularia grisea* in the five selected environments.

Marker	Sample Size	Observed	Effective	Major		Gene Diversity	Shannon's
		Allele Number	Number of Alleles	Allele Freq.	PIC		Information Index
MGM 200	30.0000	2.0000	1.3846	0.8333	0.2392	0.2778	0.4506
MGM 436	30.0000	2.0000	1.9651	0.5667	0.3705	0.4911	0.6842
MGM 437	30.0000	2.0000	1.3006	0.8667	0.2044	0.2311	0.3927
MGM 451	30.0000	2.0000	1.6423	0.7333	0.3146	0.3911	0.5799
MGM 454	30.0000	2.0000	1.9651	0.5667	0.3705	0.4911	0.6842
PYRM 15-16	30.0000	2.0000	1.7241	0.7000	0.3318	0.4200	0.6109
PYRM 61-62	30.0000	2.0000	1.9912	0.5333	0.3739	0.4978	0.6909
Mean	30.0000	2.0000	1.7104	0.6857	0.3150	0.4000	0.5848
Std. deviation		0	0.2850	0.1345	0.0682	0.1081	0.1202

5.5 Discussion

5.5.1 Morphological diversity of *Pyricularia grisea* in major finger millet growing areas in Kenya

Pyricularia grisea isolates collected from Alupe, Bomet, Makueni, Koibatek and Nakuru showed high variation both morphologically and genetically. There was variation in colony colour, colony diameter and structure in growth patterns both at the Petri dish and microscopically. Description of the *Ascomycete* fungi has been described by Conidia and conidiophore morphology as the main characteristics of the fungi (Choi *et al.*, 2013). The

difference colour variation in colour may be due to different growth stages of the spores which tend to vary with blast isolate and patterns of growth. Meena (2005); Getachew *et al.* (2014); Shahriar *et al.* (2020), revealed that *Magnaporthe grisea* tends to vary due to sexual hybridization which shows variability in form and colour at a different asexual stage of the fungi. Colony structure on the microscope revealed significant morphological differences in the structure of conidia and conidial appendages of *P. grisea* from different regions. *Pyricularia grisea* had several flared pigmented conidiophores which are aseptate. The findings are similar to those reported earlier by a study done by Klaubauf *et al.* (2014) who noted that the fungi have septate conidia of varying shapes. This could be due to the effects of different environments and ecological conditions under which the various *P. grisea* were collected which could have influenced the size, septation and form of the conidia. Similarly, Getachew *et al.* (2013) reported similar findings and noted that the environment affects the growth of the fungi size and shape. Conidia appendages aid in attachment to substrates, dispersal of spores and acclimation to new environments which affects their variability in shape, mode of development, colour and infectivity. Klaubauf *et al.* (2014) also noted that different isolates differ in colour and colony shape and size which also is a determinant of their variability in shapes, mode of development, colour and infectivity to the host plant. The pathogen from Bomet and Makueni had higher growth on the petri-dish compared to those of Nakuru, Koibatek and Alupe. This could be due to the pathogen's aggressiveness to mature faster and produce leading to more colonization on the host plant, this type of pathogen tends to gain resistance faster and can break down easily hence gaining resistance. These findings are in conjunction with the Saleh *et al.* (2014) who studied the origin, diversity and, dispersion of rice blast fungi and found that faster evolution and resistance of *Magnaporthe grisea* is linked to the faster growth of the pathogen.

5.5.2 Pathogenic diversity of *Pyricularia grisea* in major finger millet growing areas in Kenya

Pathogenicity test, which was a measure of the virulence of the pathogen collected from five different major growing areas showed that there is variation in symptoms of various *P. grisea*, this, could be due to the existence of diversity that existed within the pathogens and their expression on the host. This was because an attack on the vegetative cycle of the plant can easily be translocated on the neck. Similar to the findings, Ghatak *et al.*, 2013 observed the aggressiveness of *P. grisea* and reported the epidemics of leaves

during the early stages of the crop cycle leads to a high probability for neck infections during the reproductive stage. This is due to the numerous population shifts occurring. However, this differs with genotype resistance to the pathogen since various resistant genotypes will tend to show hypersensitivity, therefore, leading to no more spread of the disease unlike susceptible genotypes such as KNE 741 have a high probability of expressing both types of blast disease.

5.5.3 Genetic diversity of *Pyricularia grisea* in major finger millet growing areas in Kenya

Genetic variability is important for proper understanding of blast mechanisms and the development of strategies for the control of most fungal diseases. Few studies have focused on the genetic variability of *M. grisea*. The use of RAPD by Kumar and Singh, (2010) confirmed the variability and virulence complexity of *Magnaporthe grisea*. Takan *et al.* (2012) used AFLP technology to show genetic variation patterns and adaptive divergence of host-specific forms of *M. grisea* and the use of MGR- RFLP by Viji *et al.* (2000), distinguished rice and finger millet blast fungi in India. Recent studies include the use of SSR markers (Jagadeesh *et al.*, 2020; Ngermuen *et al.*, 2019; Yadav *et al.*, 2019). Similar to this study is Anjum *et al.* (2016); who evaluated finger millet using SSR markers to show genetic variability in *P. grisea*.

In this study, genetic diversity indicated the existence of different strains collected from different environments. The average number of the observed and effective number of alleles reported in the study (2 and 1.71). This finding was slightly lower than that reported by Babu *et al.* (2013) (6.18). This variation in the number of alleles has been reported in other studies (Fujita *et al.*, 2009; Kaye *et al.*, 2003; Zheng *et al.*, 2008). This could be due to few numbers of markers and samples, the nature of MGM markers which were more specific to rice than finger millet and possible difference in the level of genetic variation in different areas (Salem *et al.*, 2009).

Magnaporthe (MGM) markers had lower scores of PIC and genetic diversity compared to *Pyrm.* Among the MGM markers, MGM 454 scored high PIC (0.3705) and genetic diversity (0.4911), while MGM 437 had the lowest 0.2044 and 0.2311 for PIC and genetic diversity respectively. MGM 454 has a higher motif number (ct-29) compared to MGM 437 (tct-11) (<http://ibi.zju.edu.cn/pgl/MGM/index.html>). Higher repeat motif is linked to the coverage area of the primer which directly impacts primer amplification and detection (Wang *et al.*, 2009). The PIC and genetic diversity values in this study were similar to those

reported by Anjum *et al.* (2016) for marker MGM 454 with PIC and genetic diversity of 0.40, 0.32 respectively as compared to PIC of 0.08 and genetic diversity of 0.08 respectively for marker MGM 437.

Markers *Pyrm* 15-16 had PIC values of 0.3318 and 0.3739, respectively while *Pyrm* 61-62 had 0.785 and 0.760 respectively. These values were lower than those reported by Babu *et al.* (2013). The higher values reported by Babu *et al.* (2013) could be because the isolates of *M. grisea* used were from a combination of finger millet, foxtail millet and rice. Anjum *et al.* (2016) who incorporated the use of the same markers as those in the study recorded a 0.59 and 0.54 on *Pyrm* 15-16 and *Pyrm* 61-62 respectively from isolates of *P. grisea* from finger millet. The higher values reported by Babu *et al.* (2013) could be true since their study used rice, foxtail millet and finger millet while in this study only finger millet was used. Genetic diversity for *Pyrm* 15-16 and *Pyrm* 61-62 was 0.42 and 0.49 respectively which was almost similar to the findings of Anjum *et al.* (2016) who evaluated *P. grisea* isolates using the same markers and found *Pyrm* had (0.59 and 0.54). Babu *et al.* (2013) reported higher values 0.803 and 0.780 for the same markers. The higher value reported could be due to the genetic diversity of the pathotypes collected from different crops while this study is in contrast to Anjum *et al.* (2016) who used *P. grisea* isolates collected from finger millet only. These variations in values with the same pathogen assessed are due to host-pathogen specificity and adaptation of the pathogen to a particular host which in turn could have an influence on the variability of the pathogen hence higher values recorded (Murakami *et al.*, 2008).

The results on factorial analysis showed that the samples from all the five environments (Alupe, Bomet, Makueni, Koibatek and Nakuru) clustered randomly with no distinct pattern observed. For example, Cluster 1 is comprised of 2 sub-clusters. Sub-cluster 1 comprising of isolates 2, 3, 4 and 5 from Nakuru, 3 and 4 from Alupe and 3 and 6 from Koibatek which appeared as duplicates and isolate 6 from Bomet, which clustered as a distinct individual. This finding was in line with other studies on finger millet who found out that there was geographical differentiation between strains (Rebib *et al.*, 2014), suggesting that the non-existence of sexual reproduction among strains of *P. grisea*, hence pathogen changes with time only occur through evolution (Bengtsson, 2003; Fry, 2015).

Phylogenetic analysis revealed that *P. grisea* in the study regions were diverse with no geographical grouping with some strains from different regions observed to cluster in the same sub-clusters as duplicates with few appearing distinct. This observation was similar to

the findings of Longya *et al.* (2020) who also failed to deduce regional differentiation of rice *P. grisea* in Thailand. Similar findings have been documented by Anjum *et al.* (2016), Kumar and Singh, (2010) who noted that there is always limited variation within regional strains of finger millet blast.

Analysis of molecular variance indicated there was huge diversity within *P. grisea* isolates (87%) and low diversity among the selected regions (13%). This implies that the majority of the observed variation in the *P. grisea* was due to genetics rather than geographical differences. The pathogen genetic diversity variation poses a challenge in the development of the management and control of the pathogen (Mia, 2013). Similarly Kumar *et al.* (1999); Rebib *et al.* (2014) reported a huge genetic diversity which occurred within populations as opposed to among populations of *Magnaporthe grisea* of rice. They noted that this variation was mainly linked to the varied genetic make-up of the pathogen in rice.

5.6 Conclusion and recommendations

The findings of this study showed that there were differences in the finger millet blast (*Pyricularia grisea*) strains existing in the five major finger millet growing area in Kenya (Alupe, Bomet, Makueni, Koibatek and Nakuru). The key variations were associated with morphology, pathogenicity and genetic diversity. The morphological test revealed that the pathogen from Bomet and Makueni were similar to those from Alupe, Nakuru and Koibatek, although they were more virulent than the latter. The pathogenicity test showed that the environment plays a significant role in the physical appearance of the pathogen which is mainly associated with rainfall, temperature and humidity of the regions. Molecular diversity showed that there was large variation within the isolates as opposed to among the isolates indicating the possibility of finding the same strains of the pathogen in different environments as opposed to having the same strains in the same environment. Blast populations collected from five sites were genetically diverse and the relationship among them can be identified by the use of specific SSRs for the selected pathogen. From this study, it is clear that *P. grisea* diversity is important in disease management strategies, disease dynamics and host-pathotype understanding which can lead to the development of resistant finger millet hosts. Therefore there is a need for more studies to be done on the *Pyricularia grisea* affecting finger millet in more areas of Kenya using different types of markers. It is also important to further do the sequencing of the isolates of blast disease affecting finger

millet and documented in Kenya for further host-pathotype understanding and proper management strategy of the blast fungi.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

The inherent ability of a plant to withstand a disease-causing organism is defined as resistance. Host plant resistance is the simplest, practical, effective and economical method of controlling blast disease in the marginal areas of Kenya. It saves time energy and money that could otherwise be used in other conventional methods such as spraying with harmful chemicals. The use of resistant genotypes can only ensure the crops are protected from harmful pathogens such as fungi for a very long period. From this study genotypes IE-2183, Snapping Purple, KNE-1124 x KNE-796, KATFM1 x U-15-1.6.6.3.1.1, Gulu-E, KNE 1034, SDFM 1702 and GBK-127189A had highest resistance and above average yield, hence can be recommended for use in enhancing resistance of the susceptible genotypes through breeding.

Farmer participatory and variety selection is important in the identification of traits and finger millet varieties that can easily be adopted by farmers. Highly recommended traits by farmers have been used to develop varieties that are easily adopted and that suit the ecological conditions of the area. Breeders benefit from such information as they develop improved varieties of finger millet. Farmer preferred traits such as snapping ability and uniformity are important for the management of finger millet in the farmers' field while the most preferred genotypes selected in various areas can be released and made accessible for farmers for adoption. This will bridge the gap between scientists and farmers.

Understanding the host-pathogen relationship is most important in the management of fungal pathogens such as *Pyricularia grisea*. An aggressive strain of a pathogen causes severe disease on plants leading to a total loss in yield. A disease only results from an interaction of both the plant resistance gene and the pathogenicity genes in the pathogen. Understanding the genotype of a pathogen helps to understand its aggressiveness which is important in the management of the pathogen. Pathogens from areas where the blast is more virulent included Makueni and Bomet. It is important to note the genotypes that survive these strains have a good mechanism for resistance and therefore can be recommended in the future breeding program. The blast populations collected from five sites were genetically diverse and the relationship among them can be identified by the use of specific SSRs for the selected pathogen.

6.2 Conclusions

The findings of the study showed that blast disease was most severe in Koibatek than Nakuru and Bomet. This was due to the high humid conditions and high temperature at Koibatek favouring the growth and multiplication of the fungal spores leading to higher infection rates. Genotypes Snapping-Purple, IE-2183, GBK-127189A, Gulu-E, KATFM1 x U15-1.6.6.3.1, KNE-1124 x KNE-796 were the resistant varieties across all environments. Greenhouse results confirmed that IE-2183 and Snapping-Purple had resistance to leaf and head blast. Farmer's preference on traits of finger millet included high tillering, drought-tolerant, big fingers and snapping ability. These qualities are highly related to yield. The snapping ability of finger millet made the farmers appreciate the current innovations of harvesting the finger millet with had rather than the traditional way of harvesting using a knife. The varieties preferred by farmers included Gulu-E, Kal 2 pader, GBK-127189A and KATFM1 x U15-1.6.6.3.1.1 (high yielding and resistant to blast disease), Snapping green early (preferred due to the white grained seeds and snapping quality) and Ikhulule (red, tasty and high yielding). Pathogens collected from Alupe, Nakuru, Koibatek and Makueni regions showed variability morphologically, virulence and molecular diversity. *Pyricularia grisea* from Makueni County was the most aggressive in terms of growth patterns such as colony diameter and shape of the conidia. Pathogenicity test showed that *Pyricularia grisea* isolates from Makueni and Bomet Counties were the most virulent on the susceptible genotype KNE-741 causing the most serious infections on the neck. The molecular diversity of *Pyricularia grisea* revealed that the effective number of alleles ranged from 1.3 (MGM-437) to the highest 1.91 (*Pyrm* 61-62). Polymorphic information content (PIC) ranged from 0.2-0.3, while Shannon's diversity index varied from 0.3-0.6. AMOVA indicated variation within *P. grisea* populations (87%) as opposed to among populations 13%.

6.3 Recommendations

Based on above objectives the following recommendations were derived from the study;

- i. Evaluation of more finger millet varieties to identify resistant and high yielding genotypes that are better than the current commercial varieties such as Gulu-E, P-224, U-15 and NKR-FM1 and KNE 629.
- ii. Resistant genotypes such as Snapping Purple, IE-2183, KNE-1124 x KNE-796, KATFM1 x U-15-1.6.6.3.1.1, Gulu-E, KNE 1034, SDFM 1702 and GBK-127189A can be used in breeding programs to enhance resistance.

- iii. In various environments, the following genotypes were recommended; Snapping purple, IE-2183, GBK127189A, Gulu-E and Kal 2 Pader for Nakuru. KatFm1 x U15 1.6.6.3.1.1, IE 2872, Snapping purple, KatFm1 x U15 1.7.8.2.1 for Koibatek and Snapping purple, IE 2183, GBK 127189A, Gulu E and IE 615 for Bomet.
- iv. More farmer participatory trials should be done in many other counties in Kenya to identify challenges of finger millet farmers and disseminate knowledge of finger millet innovations done through research to enhance adoption of under-utilized crops such as finger millet.
- v. Sequencing and further molecular work should be done on *P. grisea* isolates to understand the host-pathotype environment which is helpful in identifying stable resistant genotypes and further provide solutions to breeding programs.
- vi. High yielding genotypes such as Ikhulule, KAT-FM1, GBK-027189A, IE-2872, P-224, SFM 1702, KNE 629 and Gulu-E are stable across environments and should be recommended for use in Baringo, Bomet and Nakuru counties.

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APPENDICES

Appendix 1: Combined DMRT table for the environments and seasons; season 1- short rain season (2019) and season 2-long rain season (2020) showing finger number, finger length, height, tillers, days to maturity, kernel weight, Biomass, yield, harvest index, leaf and neck blast AUDPC of 25 finger millet genotypes.

Environment	Finger number	Finger length	Height	Tillers	Laudpc	Naudpc	Days to maturity	Kernel weight	Biomass	Yield t ha⁻¹	HI
Nakuru	5.87 ^b	6.76 ^b	82.69 ^a	8.15 ^a	55.23 ^b	51.73 ^a	92.33 ^b	2.84 ^a	2.58 ^a	1.41 ^b	0.61 ^b
Koibatek	5.45 ^c	5.69 ^c	54.72 ^b	6.63 ^b	59.63 ^a	53.81 ^a	96.69 ^a	2.73 ^b	2.07 ^b	1.20 ^c	0.73 ^b
Bomet	6.91 ^a	7.13 ^a	54.68 ^b	6.56 ^b	53.76 ^b	48.71 ^b	94.06 ^{ab}	2.85 ^a	2.55 ^a	3.60 ^a	1.76 ^a
DMRT	0.2602-	0.2320-	2.542-	0.3761-	3.006-	3.106-	2.912-	0.0825-	0.2162-	0.1598-	0.1582-
(P<0.05)	0.2739	0.2442	2.676	0.3756	3.164	3.270	3.065	0.8691	0.2276	0.1682	0.1665
Season											
1	6.75 ^a	7.02 ^a	61.47 ^b	9.37 ^a	39.42 ^b	45.06 ^b	98.01 ^a	3.18 ^a	2.42 ^a	0.93 ^b	1.64 ^b
2	5.38 ^b	6.03 ^b	66.60 ^a	4.83 ^b	72.99 ^a	57.40 ^a	90.35 ^b	2.42 ^b	2.38 ^a	3.21 ^a	0.40 ^a
DMRT	0.2124	0.1894	2.0760	0.2913	2.454	2.516	2.377	0.6741	0.0416	0.1305	0.1291
(P<0.05)											

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity, DTM=Days to maturity, Yield t= Yield t ha⁻¹, HI= Harvest Index. Means under the same letters are not significantly different (p<0.05)

Appendix 2: Combined mean table for varieties, yield and yield parameters.

No.	Variety	F.N	F.L	Height	Tillers	Laudpc	Naudpc
1	KatFm1×U151.6.6.3.1.1	4.84 ^h	6.07 ^{e-g}	60.36 ^{c-e}	6.33 ^{e-i}	48.27 ^{h-j}	43.02 ^{f-i}
2	KatFm1 × U15 1.6.3.3.1	5.50 ^{f-h}	6.09 ^{e-g}	59.21 ^{d-e}	6.12 ^{g-i}	60.84 ^{a-d}	51.74 ^{c-f}
3	KatFm1 × U15 1.7.8.2.1	6.29 ^{a-f}	6.70 ^{c-f}	65.41 ^{a-e}	6.85 ^{c-h}	59.08 ^{b-g}	51.59 ^{c-g}
4	KNE 1124 × KNE 796	6.11 ^{c-f}	7.09 ^{bc}	65.26 ^{a-e}	6.14 ^{g-i}	48.27 ^{ij}	51.16 ^{c-g}
5	Snapping Green Early	5.87 ^{c-g}	5.79 ^g	57.95 ^{ef}	5.95 ^{hi}	60.74 ^{a-d}	60.22 ^{b-d}
6	KNE 629	5.65 ^{e-h}	6.06 ^{e-f}	68.92 ^{a-c}	6.65 ^{d-i}	51.15 ^{f-i}	47.59 ^{e-f}
7	P-224	5.82 ^{d-g}	6.64 ^{c-f}	63.03 ^{a-e}	6.73 ^{c-h}	66.72 ^{ab}	41.86 ^{f-i}
8	SDFM-1702	6.16 ^{c-f}	6.92 ^{b-d}	61.17 ^{b-e}	10.45 ^a	58.99 ^{b-g}	40.64 ^{g-i}
9	KNE 1034	6.04 ^{c-g}	6.99 ^{c-d}	67.15 ^{a-d}	5.58 ⁱ	65.14 ^{ab}	50.93 ^{c-g}
10	NKR FM1	7.16 ^a	9.26 ^a	66.83 ^{a-e}	6.87 ^{c-h}	61.11 ^{a-d}	71.43 ^a
11	Gulu E	6.09 ^{c-f}	5.69 ^g	63.93 ^{a-e}	7.59 ^{cd}	48.93 ^{h-j}	44.70 ^{f-h}
12	Ikhulule	6.71 ^{a-d}	6.20 ^{e-g}	60.83 ^{b-e}	7.36 ^{c-g}	61.88 ^{a-d}	47.96 ^{e-g}
13	KNE 741	6.77 ^{a-c}	6.62 ^{c-f}	65.80 ^{a-e}	6.29 ^{f-i}	70.11 ^a	61.00 ^{bc}
14	U-15	6.32 ^{a-f}	5.95 ^{f-g}	64.82 ^{a-e}	7.74 ^{cd}	49.94 ^{f-i}	46.78 ^{fg}
15	KAT FM1	5.61 ^{f-h}	6.05 ^{e-g}	63.36 ^{a-e}	7.54 ^{c-f}	64.23 ^{a-c}	61.10 ^{bc}
16	Snapping-Purple variety	7.05 ^{ab}	6.79 ^{c-e}	62.68 ^{bc}	9.24 ^b	45.14 ^{ij}	33.19 ⁱ
17	KNE 628	6.18 ^{c-f}	6.08 ^{e-g}	61.45 ^{b-e}	7.25 ^{c-g}	60.04 ^{a-f}	66.16 ^{ab}
18	Kal Pader	6.07 ^{c-f}	6.30 ^{c-g}	69.76 ^{ab}	7.52 ^{c-f}	52.95 ^{d-i}	50.09 ^{d-g}
19	Kal 2 Pader	5.16 ^{gh}	5.95 ^{f-g}	67.07 ^{a-e}	6.71 ^{c-i}	58.35 ^{b-h}	45.96 ^{fg}
20	GBK 027189A	6.55 ^{a-e}	6.83 ^{b-e}	72.24 ^a	7.56 ^{c-e}	40.88 ^j	35.37 ^{h-i}
21	IE-615	6.31 ^{a-f}	6.78 ^{c-e}	67.03 ^{a-e}	7.93 ^c	54.55 ^{c-h}	48.62 ^{e-g}
22	Otiyo Brown	5.91 ^{c-g}	6.30 ^{c-g}	63.77 ^{a-e}	6.89 ^{c-h}	58.89 ^{b-g}	64.96 ^{ab}
23	IE-2183	5.69 ^{e-h}	7.56 ^b	53.83 ^f	6.68 ^{c-i}	54.16 ^{c-i}	43.94 ^{f-h}
24	IE 2872	6.08 ^{c-f}	5.96 ^{f-g}	63.63 ^{a-e}	7.60 ^{cd}	54.02 ^{c-h}	57.50 ^{b-e}
25	Kal Dokolo	5.81 ^{d-g}	6.48 ^{c-g}	64.25 ^{a-e}	6.30 ^{e-i}	51.61 ^{d-g}	63.31 ^{ab}
DMRT (P<0.05)		0.81-	0.66-	7.55-	1.03-	8.68 -	8.90-
		1.02	0.84	9.52	1.29	10.94	11.22

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity.

Appendix 2 continued...

No.	Variety	DTM	KW	Biomass	YieldT	HI
1	KatFm1 × U151.6.6.3.1.1	100.44 ^a	3.00 ^{a-c}	2.51 ^{b-e}	1.66 ^g	0.73 ^d
2	KatFm1 × U15 1.6.3.3.1	93.66 ^{ab}	2.53 ^{h-j}	2.39 ^{b-g}	2.89 ^a	1.27 ^{a-d}
3	KatFm1 × U15 1.7.8.2.1	99.94 ^a	2.93 ^{a-f}	2.39 ^{b-f}	2.34 ^{b-e}	0.90 ^{b-d}
4	KNE 1124 x KNE 796	90.41 ^{a-c}	2.73 ^{c-h}	3.04 ^{ab}	1.91 ^{d-g}	0.88 ^{b-d}
5	Snapping Green Early	94.94 ^b	2.93 ^{a-f}	2.12 ^{d-g}	1.87 ^{e-g}	0.95 ^{b-d}
6	KNE 629	94.17 ^{ab}	2.94 ^{a-e}	2.26 ^{c-g}	2.26 ^{b-f}	0.94 ^{b-d}
7	P-224	90.70 ^{a-c}	2.64 ^{g-i}	2.52 ^{b-f}	2.68 ^{ab}	1.00 ^{a-d}
8	SDFM-1702	97.05 ^{ab}	2.98 ^{a-c}	2.09 ^{d-g}	1.76 ^{fg}	0.90 ^{b-d}
9	KNE 1034	91.61 ^{a-c}	2.94 ^{a-e}	2.25 ^{c-g}	2.01 ^{d-h}	1.08 ^{a-d}
10	NKR FM1	93.11 ^{ab}	2.96 ^{a-d}	3.63 ^a	2.05 ^{d-f}	1.11 ^{a-d}
11	Gulu E	91.11 ^{a-c}	2.30 ^j	2.18 ^{c-g}	2.58 ^{a-d}	1.36 ^{ab}
12	Ikhulule	98.33 ^{ab}	2.73 ^{c-f}	2.46 ^{b-f}	2.94 ^a	1.42 ^{a-c}
13	KNE 741	82.65 ^c	2.91 ^{a-f}	2.31 ^{b-g}	0.55 ^h	1.20 ^{a-d}
14	U-15	94.00 ^{ab}	3.01 ^{a-c}	2.39 ^{b-g}	1.83 ^{e-g}	0.88 ^{b-d}
15	KAT FM1	94.00 ^{ab}	3.15 ^a	2.25 ^{c-g}	1.83 ^{e-g}	0.84 ^{b-d}
16	Snapping-Purple variety	95.71 ^{ab}	2.80 ^{b-h}	1.72 ^g	1.57 ^g	0.85 ^{cd}
17	KNE 628	94.17 ^{ab}	2.88 ^{a-f}	2.82 ^{b-d}	2.05 ^{c-g}	0.92 ^{b-d}
18	Kal Pader	95.94 ^{ab}	2.94 ^{a-e}	2.27 ^{c-g}	1.71 ^g	1.33 ^{cd}
19	Kal 2 Pader	99.88 ^a	2.72 ^{c-i}	2.93 ^{bc}	1.80 ^{e-g}	0.85 ^{b-d}
20	GBK 027189A	99.88 ^a	2.66 ^{e-i}	2.78 ^{b-d}	1.81 ^{e-g}	0.75 ^{cd}
21	IE-615	93.33 ^{ab}	2.56 ^{g-j}	2.15 ^{e-g}	2.44 ^{a-e}	1.26 ^{a-d}
22	Otiyo Brown	96.44 ^{ab}	3.05 ^{ab}	1.92 ^{fg}	2.33 ^{b-e}	1.05 ^{a-d}
23	IE-2183	94.05 ^{ab}	2.45 ^{ij}	1.89 ^{fg}	1.81 ^{f-h}	0.79 ^{b-d}
24	IE 2872	88.05 ^{bc}	2.67 ^{d-i}	1.99 ^{e-g}	2.63 ^{ab}	1.50 ^{ab}
25	Kal Dokolo	92.11 ^{ab}	2.81 ^{b-g}	2.70 ^{b-e}	2.76 ^{ab}	1.51 ^a
DMRT (P<0.05)		8.50-	0.2389-	0.6260-	0.4615-	0.1260-
		10.72	0.3011	0.7890	0.5818	0.1589

*D.T.M=Days to maturity, KW= Kernel weight, YiledT= Yield tonnes ha⁻¹, H.I= Harvest Index

Appendix 3: Season 1 ; ANOVA table for plant stand, plant vigor, height, finger length, finger number, tillers, no of days to maturity, LAUDPC, HAUDPC, KW, biomass and yield of finger millet varieties evaluated under field conditions

Sov	df	LAUDPC	HAUDPC	Height	F.L	F.N	Tillers	DTM	1000KW	Biomass	Yieldt	H.I
Environment	2	32.75	933.08**	37184.60***	178.92***	204.18***	228.73***	378.85*	14.71***	15.44***	9.40***	0.092***
Variety	24	529.32***	1168.70***	243.44**	8.91***	8.20***	17.97***	220.61**	1.17***	4.06***	0.39***	0.018***
Environment*Variety	48	119.57***	260.49**	208.89**	7.87***	8.54***	14.46***	302.09***	0.43***	0.36***	0.19***	0.002
Rep(Block)	14	130.27**	77.70	379.99***	1.87*	3.23*	9.54***	277.46***	0.093	0.153	0.09	0.007***
Error		46.34	120.59	113.51	0.89	1.65	2.76	97.15	0.091	0.089	0.058	0.002
CV		17.26	25.12	17.32	13.57	19.01	18.04	10.04	9.46	12.35	25.55	4.05
R²		77.89	73.59	86.39	89.22	83.4	84.92	65.32	87.04	92.90	83.38	78.9

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity, DTM=Days to maturity, Yieldt= Yield t Ha⁻¹, HI= Harvest Index. . *** = p<0.001, ** =p<0.01 and * =p<0.05 respectively.

Appendix 4: Season 2; ANOVA table for height, finger length, finger number, tillers, no of days to maturity, LAUDPC, HAUDPC, KW, biomass and yield of finger millet varieties evaluated in field conditions

Sov	DF	LAUDPC	NAUDPC	Height	F.L	F.N	Tillers	DTM	KW	Biomass	Yield t	HI
Environment	2	4326.24***	4483.45***	13589.79***	59.19***	2.98***	37.75***	308.08*	9.47***	12.88***	33.57***	14.84***
Variety	24	930.91***	1050.72***	216.17*	2.79***	1.83**	2.68**	152.53**	0.08	0.04	0.02***	0.21***
Env × Variety	48	1460.73***	1610.42***	154.83	3.71***	0.86*	3.68***	176.91***	0.18	0.06***	0.22***	0.24***
Rep(Block)	14	1708.25***	2504.10***	175.62	3.51***	1.81**	1.68	112.60	0.21	0.01	0.18**	0.09**
Error		229.75	158.76	124.16	0.92	0.41	1.15	70.69	0.14	0.03	0.06	0.03
CV		20.47	21.77	16.72	20.45	12.02	18.03	9.21	15.25	10.38	13.16	13.03
R²		81.40	87.86	72.58	76.09	67.82	69.60	61.30	63.71	89.2	90.35	91.22

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity, DTM=Days to maturity, Yieldt= Yield t Ha⁻¹, HI= Harvest Index. . ***= p<0.001, **=p<0.01 and *=p<0.05 respectively.

Appendix 5 : Season 1; DMRT table for height, finger length, finger number, tillers, no of days to maturity, LAUDPC, HAUDPC, KW, biomass and yield of finger millet varieties evaluated under field conditions in the three environments

Environment	LAUDPC	NAUDPC	Height	F.L	F.N	Tillers	DTM	KW	Biomass	Yieldt	HI
Nakuru	39.77a	41.89b	85.37a	6.59b	5.45c	11.14a	98.44ab	3.57a	2.78a	1.29a	1.22a
Koibatek	38.67a	48.39a	41.87c	5.67c	6.18b	7.68c	100.24a	2.69c	1.92c	0.57c	1.15c
Bomet	39.89a	46.52a	57.22b	8.74a	8.69a	8.75b	95.76b	3.25b	2.56b	0.94b	1.18b
DMRT	2.21-2.32	3.68-3.87	3.50-	0.31-	0.42-	0.82-	3.15-	0.09-	0.09-	0.078-	0.0157-
(P<0.05)			3.68	0.32	0.45	0.86	3.31	0.10	0.10	0.082	0.0166
Mean	39.44	45.49	61.47	6.98	6.75	9.21	98.10	3.18	2.42	0.942	1.183

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity, DTM=Days to maturity, Yieldt= Yield t Ha⁻¹, HI= Harvest Index. Means under the same letters are not significantly different at p<0.05.

Appendix 6 :Season 2; DMRT table for height, finger length, finger number, tillers, no of days to maturity, LAUDPC, HAUDPC, KW, biomass and yield of finger millet varieties

Environment	LAUDPC	NAUDPC	Height	F.L	F.N	Tillers	DTM	KW	Biomass	Yieldt	HI
Nakuru	70.39b	64.77a	85.37a	5.07a	5.47a	6.72a	88.89b	2.07c	1.82a	2.72a	1.92a
Koibatek	82.73a	59.27b	41.87c	5.32a	5.32a	5.78b	94.40a	2.78a	1.09b	1.68b	1.08b
Bomet	68.94b	49.55c	57.22b	3.68b	5.08b	5.33c	92.42a	2.43b	1.80a	1.46c	1.24c
DMRT	4.89-5.15	4.06- 4.28	3.50-	0.31-	0.21-	0.35-	2.71-2.85	0.11-	0.05-0.06	0.083-	0.059-
(P<0.05)			3.68	0.33	0.22	0.36		0.12		0.087	0.063
Mean	74.02	57.87	66.61	4.69	5.29	5.94	91.24	2.43	1.57	1.95	1.41

*F.N=Finger number, F.L= Finger Length, LAUDPC= Leaf blast severity, NAUDPC= Neck Blast severity, DTM=Days to maturity, Yieldt= Yield t Ha⁻¹, HI= Harvest Index. Means under the same letters are not significantly different at p<0.05.

Appendix 7: Chi- square table for finger millet traits scored by farmers in ATC-Bomet.

	H.Y	E.M	DT	RTD	MKT	B.F	RTL	T.A	UNF
Chi-square	35.56	30.18	28.15	5.52	37.33	26.80	17.31	19.18	32.66
df	24.00	24.00	24.00	24.00	24.00	24	24	24	24
Asymp.Sig	0.06	0.17	0.25	1.00	0.04	0.31	0.83	0.74	0.11

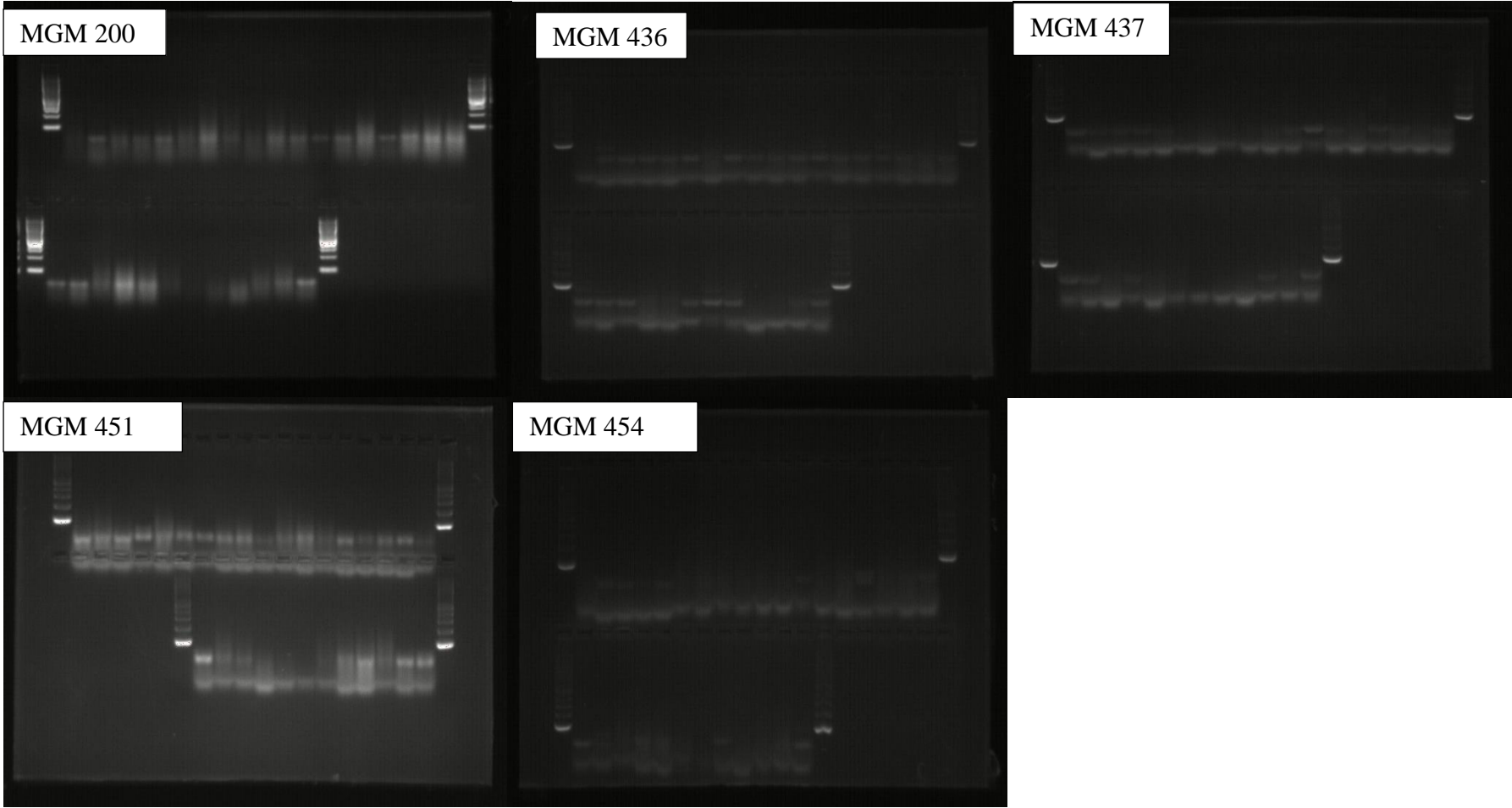
*H.Y= High Yielding, E.M= Early Maturity, D.T= Drought Tolerance, RTD= Resistance to Diseases, MKT= marketability, B.F= Big fingers, R.T.L= Resistance to lodging, T.A= Tillering Ability, UNF= Uniformity and Asymp.Sig=Asymptotic significance.

Appendix 8: Chi- square table for finger millet traits scored by farmers in ATC- Nakuru.

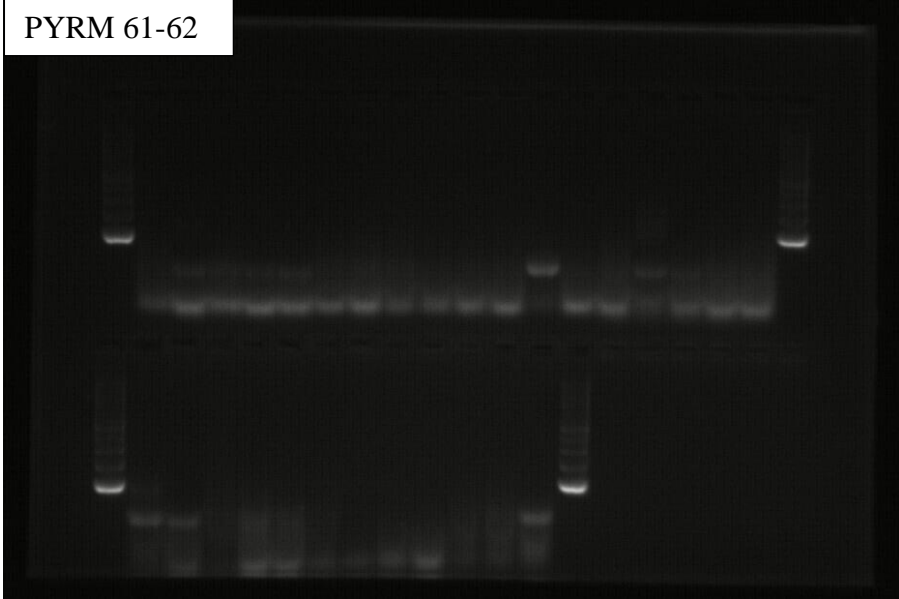
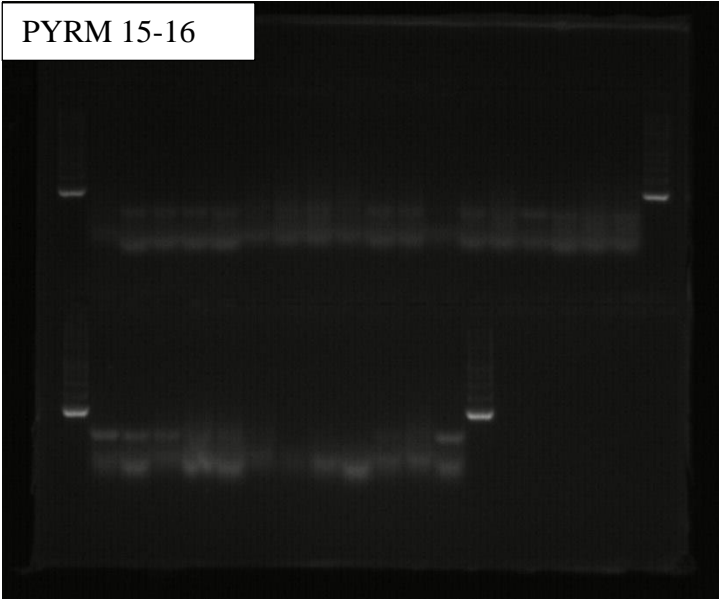
	H.Y	E.M	DT	RTD	MKT	B.F	RTL	T.A	UNF	B.R	G.T	F/S F
Chi-square	219.63	92.91	115.49	148.25	149.59	98.19	82.23	72.66	100.66	119.92	65.19	85.40
df	24.00	24.00	24.00	24.00	24.00	24	24	24	24	24	24	24
Asymp.Sig	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*H.Y= High Yielding, E.M= Early Maturity, D.T= Drought Tolerance, RTD= Resistance to Diseases, MKT= marketability, B.F= Big fingers, R.T.L= Resistance to lodging, T.A= Tillering Ability, UNF= Uniformity, B.R= Bird Resistance, G.T= Grain Taste, F/S F= Folded/ Straight fingers, R.B.I= Resistance to Bird Infestation df= Degree of freedom and Asymp.Sig=Asymptotic significance

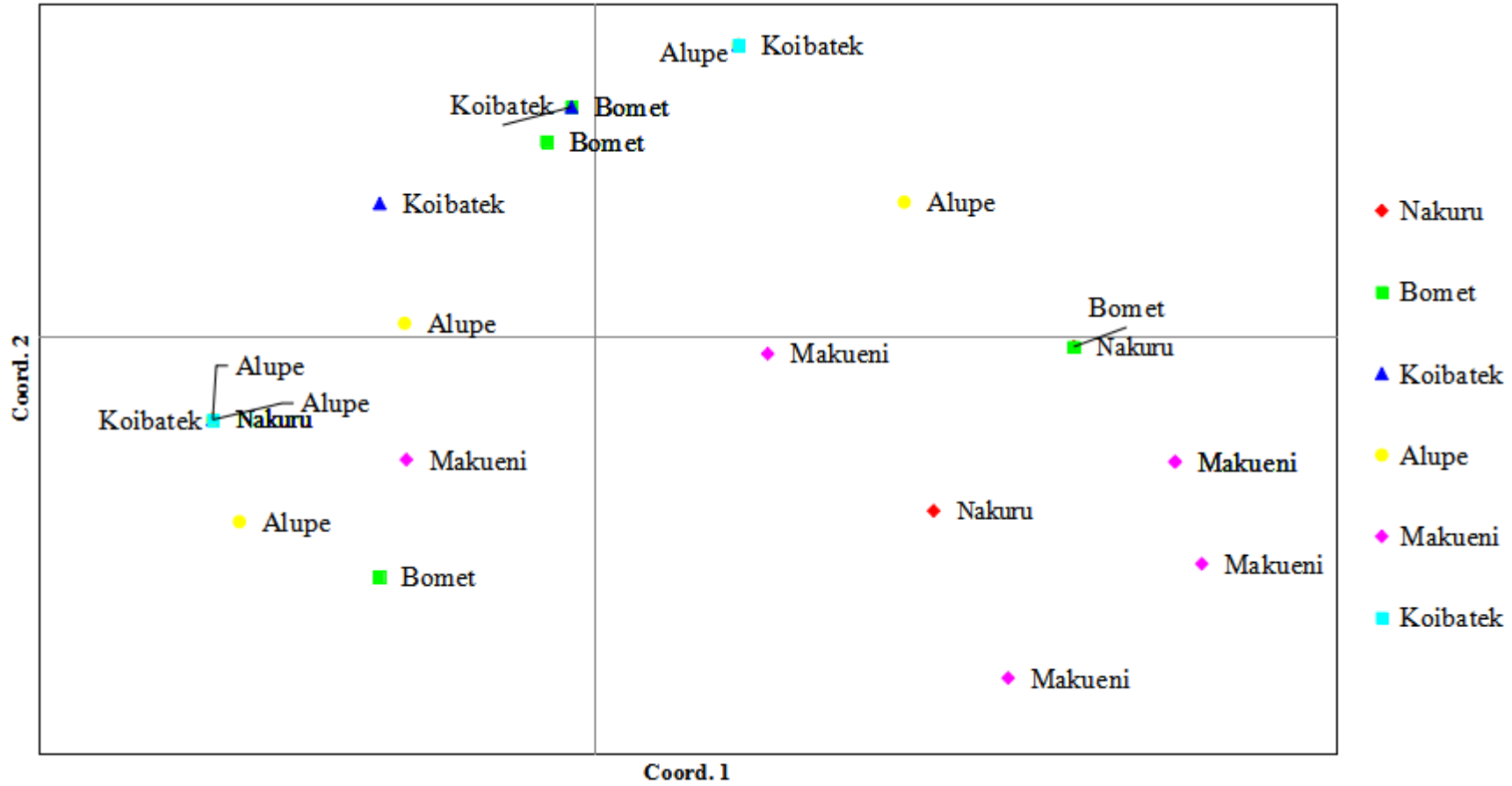
Appendix 9: Performance of the SSR Markers used in the study



Appendix 9. Performance of the SSR Markers used in the study continued...



Appendix 10: Factorial analysis of *P. grisea* collected from the five regions





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This is to Certify that Miss. Jayo Manyasi Tracyline of Egerton University, has been licensed to conduct research in Baringo, Bomet, Nakuru on the topic: Host Plant Resistance and Characterization of Blast disease (Pyricularia grisea) in Selected Finger Millet (Eleusine coracana L.) Genotypes in Kenya for the period ending : 03/April/2021.

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Full Length Research Paper

Farmer preference for selected finger millet (*Eleusine coracana*) varieties in Rift Valley, Kenya

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Lack of awareness and information on the traits of orphan crops such as finger millet is a major constraint to finger millet production. Farmer participatory and varietal selection (FVPS) is an efficient method of achieving productivity through enhancing adoption of improved high yielding varieties. A study was conducted in two major growing areas in central Rift Valley, Agri-ecological zone III (ATC-Nakuru and Bomet), to assess the level of awareness and farmer preference of twenty-five finger millet varieties. Farmer participatory variety selection was conducted at physiological maturity of the finger millets. One hundred farmers assessed and scored their preferred traits and varieties in each site. The scores were ranked on a scale of 1-5 in Focused Group Discussions (FDGs) and analyzed using Kruskal Wallis H-test of non-parametric data using Statistical Package for Social Science (SPSS) while scores collected on variety traits were used to construct a Pair-wise ranking table to find the best traits selected by farmers. The results showed that farmers preferred high yielding varieties with qualities such as uniformity, drought tolerance, tillering ability, big fingers, lodging and folded or straight fingers. They appreciated the snapping varieties for the ease of harvesting using fingers instead of traditional cutting using a knife. Kal 2 Pader (3.9), P-224 (3.9), KatFM1xU151.6.6.3.1.1 (3.9), GBK 027189A (2.8), Snapping green early (3.7) and KatFM1xU151.7.8.2.1 (3.7) were the most preferred varieties while in AEZ III, Bomet ATC KatFM1 (4.3), KNE 741 (4.3), KNE629 (4.2), KatFM1xU151.6.6.3.1 (4.1), Gulu E (3.9), GBK 027189A (3.8) and Kal 2 pader (3.8) were the most preferred varieties in ATC Nakuru. In both sites KatFM1xU151.6.6.3.1.1 (4.0), Kal 2 pader (3.85) and GBK 027189A (3.8), Gulu E (3.75) and P-224 (3.75), were ranked the best. The farmers expressed their interest in accessing the seeds of these improved varieties. FVPS provides a platform for identification of the most preferred traits of finger millet and knowledge dissemination of improved varieties to farmers.

Key words: Finger millet, farmer participatory variety selection (FVPS), farmers preferred traits and varieties.

Full Length Research Paper

Characterization of diversity and pathogenicity of *Pyricularia grisea* affecting finger millet in Kenya

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Pyricularia grisea characterization is a prerequisite for species differentiation and understanding of the pathosystem, evolution and diversity of species. The aim of this study is to determine the morphological variation, pathogen virulence and molecular diversity of *P. grisea* isolates. Five isolates from infected heads of finger millet were collected from Bomet, Nakuru, Baringo, Busia and Machakos counties in 2019. The samples were cultured in the lab for both characterization and spore suspension preparation. Data on morphological characterization included colony diameter, color and shape of conidia. Pathogenicity test was done in the greenhouse in a randomized complete block design using KNE 741, a susceptible genotype and disease data scored. Molecular characterization involved the use of seven SSR markers. Data analyses included use of softwares such as AUDPC, Power Maker, GeneAlex and Darwin. Results showed that *P. grisea* isolates had different growth pattern with respect to color, colony diameter and conidia shape. Pathogenicity test revealed that all sites had significant different ($P < 0.01$) virulence on the test genotype. Neck blast, scored at physiological maturity was prominent in Kolbatek and Bomet strains while leaf blast was severe in Bomet and Alupe strains. Molecular analysis showed that ENA ranged from 1.30 (MGM 437) -1.99 (*Pyrm* 61-62) with an average of 1.71. PIC varied between 0.20-0.37 for primers MGM 437 and *Pyrm* 61-62, respectively. Factorial and phylogenetic analysis revealed that *P. grisea* isolates were diverse with no geographical grouping. AMOVA indicated diversity occurred within populations (87%) as opposed to among populations (13%). The high *P. grisea* variability found in the study is a clear indication of the high sexual recombination among strains collected in major growing areas of Kenya.

Keywords: Diversity, morphology, pathogenicity, *Pyricularia grisea*