DISTRIBUTION AND SOCIO-ECONOMIC IMPACT OF BLAST PATHOGEN, MOLECULAR CHARACTERIZATION OF ASSOCIATED FUNGI AND SCREENING FOR DISEASE TOLERANCE IN KENYAN FINGER-MILLET (*Eleusine coracana*)

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DOCTOR OF PHILOSOPHY

(Biotechnology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2023

Distribution and Socio-economic Impact of Blast Pathogen, Molecular Characterization of associated Fungi and Screening for Disease Tolerance in Kenyan Finger Millet (*Eleusine coracana*)

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology of the Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

To the memories of my late parents Jane Jeniffer Akong'o Odeph and O.J.E McOdum. The sacrifices you made on my behalf laid the strong foundation that has seen me through the stormy weather in the course of my journey.

ACKNOWLEDGEMENTS

My journey to earn a PhD has been windy and long and I have had the gift of dependable people around me. I want to thank everyone who contributed to this journey in whichever way. Numerous people have ceaselessly supported my course over the years. I want to thank you all from the bottom of my heart. I would however want to mention a few people whose continuous support and encouragement gave me the strength to plough on. My husband Charles Ocharo and our children Mephibosheth, Jayden, Jeremy, Gayle and Jade have been affected in all manner of ways by this quest for knowledge. Your patience with me as I burnt the midnight oil and love has been awesome. In you, I derived the power to stay on and succeed. I have a profound feeling of gratitude to my dear siblings Rose, Dale, Jackie, Robert and Austin. You have never left my side and so you remain very dear to my heart. Your heightened sense of optimism and belief in my ability has been my source of inspiration and strength during the most difficult times. You have been my greatest cheerleader, my go-to person during my work. I salute you all for always being there for me. My colleagues at JKUAT as well as my friends within and without academia have been very resourceful in so many different ways and I also want to acknowledge your support. Your unending support made life bearable during difficult moments while your constant assertion was a stimulus that spurred me on. I single out Richard Rotich, Agnes Omire and Tofick Wekesa, for their symbol of sacrifice and selfless service to me. Your altruistic deeds bring true meaning to selflessness.

This work would not amount to much without funding from the National Research Fund (NRF). I wholehearted want to thank you for your generous financial support that enabled me to carry out this research work to completion. I am also much obliged to my supervisors Dr Agnes Kavoo, Dr Wilton Mbinda and Dr Cecilia Mweu for deeming me fit as one of their students. Their diligence, ingenuity, acuity and valuable inputs and suggestions in the course of the research, writing of manuscripts and final compilation of my thesis cannot be overlooked. I have greatly gained from

your knowledge pool and thorough evaluation. I am equally grateful to Prof. Steven Runo for allowing me access to some of the facilities and or equipment at the Plant Transformation Laboratory (PTL) at Kenyatta University. I would also like to appreciate the impressive role of my colleagues and technicians at the Institute for Biotechnology Research, Botany, Zoology and Horticulture departments at JKUAT and friends. Your unending support made life bearable during difficult moments while your constant assertion was a stimulus that spurred me on.

To the almighty God who has been my rock and my fortress, my deliverer and my strength; an ever-present help in time of need.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
BAP	6-Benzylaminopurine
CTAB	Cetyl Trimethyl Ammonium Bromide
DLA	Detached leaf Assay
DNA	deoxyribonucleic acid
GBK	Gene Bank of Kenya
GPS	Global Positioning System
ICRISAT	International Crops Research for the semi-arid tropics
ITS	Internal Transcribed Spacer
MAFFT	Multiple Alignment using Fast Fourier Transform
MAMP	Microbe associated molecular pattern
OMA	Oat Meal Agar
PAMP	Pathogen associated molecular pattern
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
QTLs	Qualitative traits loci
SPSS	Statistical Package for Social Science
ROS	Reactive Oxygen Species

ABSTRACT

Finger millet is an underutilized, climatic resilience grain crop with significant nutraceutical value and agricultural diversification prospects. Blast disease impedes its production and productivity causing a yield lose of 10-50% in Kenya. The disease is widely distributed and its impact occurs in all finger millet growing areas of Southern Asia and Eastern Africa. Sustainable finger millet blast management approaches in Kenya are threatened by inadequate farmers' knowledge and poor blast management techniques used that eventually impact on their socio economic status. Besides, information on occurrence, severity and distribution of blast in Kenya is not well established.This is coupled with limited information on the genetic diversity of fungal species associated with the blast pathogen. This information forms a basis on which long term sustainable blast management approaches that meet farmers preferences can be laid. This study aimed at assessing farmers' knowledge, practices and social economic effects of finger millet blast andthe disease occurrence, severity and distribution. The study further sought to characterize the genetic diversity of the fungal species associated with blast infected tissues, and screening for host tolerance of different varieties of Kenyan finger millet. Survey studies were done to assess farmers knowledge, practices and socio-economic impact on blast disease occurrence, distribution and severity. A semi-structured questionnaire, through face-to face interviews was administered to 325 farmers from five Counties in Kenya namely Bungoma, Busia, Kisii, Makueni and Machakos in 2018 and 2019. The data collected was analyzed using the SPSS statistical software version 25. Disease Occurrence was based on either presence or absence of infected plants in the field. Severity assessessment was based on an incidence score of low (0-29)=1, moderate (30-69%)=2 and very severe (>70%). Disease distribution was based on spatial disease spread on the farms. Plant tissues exhibiting blast disease symptoms were collected and placed in sterile bags for morphological and molecular characterization using internal transcribed spacer (ITS) and 28S rDNA regions. The blast pathogenic agent was then isolated and used for pathogenicity assays to observe the disease symptoms and effect of the pathogen on growth parameters such as plant height, leaf area index, chlorophyll a, chlorophyll b and carotenoid contents. This study showed that 90% of the farmers recognized blast disease as the greatest constraint to finger millet production with 51% of respondents reportinghigher severity in the dry season. Majority (72%) of farmers in Bungoma county were able to relate blast disease to the type of finger millet variety planted. Hand weeding was perceived as the most effective blast management practice by 99% of the respondents although it was identified as a very laborious task. Additional management practices chosen by 60% of respondents included ash treatment, uprooting infected plants, and herbicide application once a season. A conclusion was drawn that farmers in all the counties surveyed have limited knowledge on blast disease and the management practices used are basic. Blast disease occurred in all the counties visited with Machakos (56%) having the least leaf blast severity while Busia county (82%) had the highest disease severity in all the plant parts (leaf,neck and finger). Fifty five isolates were obtained from finger, leaf and neck with a total of 11 genera for 28SrDNA and 10 together with two unnamed fungi for ITS. Phylogenetic studies

revealed two trees with similar topologies for both regions and the isolates were assigned to three classes of Phylum Ascomycota, namely Eurotiomycetes, Dothiodeomycetes and Sordariomycetes. There was a significant difference ($p \leq 0.05$) in varietal difference in leaf area index, plant height and photosynthetic pigments contents in presence of both pathogens. Genotype GBK 043050 (GBK 42) had a disease severity score of 3.6 and 3 in presence of *C. lunata* and *P.oryzae* respectively that was equivalent to a disease reaction of moderately susceptible. GBK 043050 (GBK 42) and Engundi varieties were not significantly different at ($p \leq 0.05$).Thus, the two varieties were deemed to be tolerant to both pathogens. In conclussion, the study recommends a concerted d focus on improving farmers' adoption of best blast management practices, identifying the possible role played by fungal species associated with blast infected plant tissues during finger millet blast pathogenesis, and developing blast-tolerant finger millet varieties with farmer preferential traits. It is recommended that capacity building be done for all farmers from all the Counties surveyed.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Finger millet is an essential crop extensively grown by subsistence farmers living in Sub-Saharan Africa and Asia as a dietary staple (Bal *et al.*, 2020). Taxonomically, it is a self-pollinating allotetraploid (2n = 4X= 36) found in the Poaceae family and genus *Eleusine.* The crop derived its name from the shape of the seed head which is made up of several spikelets that appear like human fingers (Sood *et al.,* 2019). It is largely grown on marginal plots of land either as pure or mixed stands by a minority of farmers as it is considered a 'poor person's crop' and may be sold to provide additional income (Onyango, 2016). Presently, finger millet ranks fifth most important cereal after wheat, maize, rice and barley and fourth among other millets after sorghum, pearl millet and foxtail millet (Sood *et al.*, 2019). Globally, finger millet production is approximately 4.5 million tons of grain of which Africa produces nearly 2.5 million tons (Onyango, 2016). The crop is climate-resilient, drought-tolerant and can adapt to different types of tropical soils, flourishing under wide altitudinal ranges of between 500-3000 metres above sea level (Sood *et al.*, 2019). The grain has a long shelf-life, unmatched multiplication rates, minimal viability loss and damage by storage pests, unlike most cereals (Opole, 2019). The nutritional and health benefits of finger millet play a crucial role in the diets of diabetics, convalescing persons, and pregnant and lactating mothers, since the grain is gluten-free, rich in proteins, vitamins, minerals, fibre content and energy compared to other cereals (Maharajan *et al.*, 2021).

Despite the essential role played by finger millet for food security and livelihoods, its productivity is relatively low in Kenya (0.5 t/ha) compared to the potential yield of the crop in farmer-managed field trials (1.5 t/ha) and under on-station studies (3.8 t/ha) (Handschuch & Wollni, 2016). This low productivity is attributable to a range of biotic and abiotic stresses and socio-economic constraints common in small-scale production systems in Kenya. Finger millet blast caused by the ascomycetous fungus

Pyricularia oryzae (syn. *Magnaporthe oryzae*), is the most devastating disease of finger millet causing serious yield losses of between 20-50% threatening global finger millet production. (Mbinda *et al.,* 2021). *P. oryzae* is a hemibiotrophic ascomycetous fungus that initially infects the host in a biotrophic way as it develops a bulbous, branched network of hyphae that rapidly spreads with the host and then finally converts to a necrotrophic phase obtaining nutrients from dead cells (Yadav *et al.,* 2019). The mode of reproduction for the pathogen is exclusively asexual, which enables it to expeditiously produce very many asexual spores during suitable environmental conditions that swiftly colonize its hosts (Masaki, 2020). This explains why the finger millet blast disease incidences have escalated in the recent past due to the tendency of the pathogen to occasionally spring from one host to another coupled with increased global trade and climate change (Langner *et al.,* 2018). This quick spread of asexual spores intensifies the problem of food supplies that could feed hundreds of millions of people globally.

Finger millet blast disease infection occurs at all stages of growth with the finger blast being the most damaging leading to the non-grain formation and or shrivelled grain with very low weight. Finger blast can attack one, few or all the fingers on the panicle (Takan *et al.,* 2012; Shahriar *et al.,* 2020). The host range for this pathogen is rather extensive including over 50 cultivated and wild monocots such as rice, foxtail millet, barley, and wheat among others (Gladieux, , *et al.,* 2018a). However, for individual isolates, the host range is limited and is divided into distinct pathotypes based on compatibility including *Oryza*, *Setaria*, *Eleusine*, *Triticum* and *Panicum* which are pathogenic on rice, foxtail millet, finger millet, wheat and rye-grass respectively. The host range of each pathotype is somewhat restricted to a plant genus (Chung *et al.,* 2020; Asuke *et al.,* 2021).

In East Africa, the finger millet blast disease causes yield losses of between 10-68% with disease infection being more intensified by favourable weather conditions (sub-humid to humid) and the presence of alternate hosts such as wild grasses. Due to alternate host compatibility, the disease tends to occur throughout the year, especially in endemic areas (Manyasa *et al.,* 2019). Typical disease symptoms include the

appearance of diamond-shaped lesions on the leaves that rapidly enlarge and fuse, finally causing complete drying of infected leaves (Takan *et al.,* 2012; Shahriar *et al.,* 2020). Such yield losses threaten the already weak food security situation and the livelihood of the resource-poor smallholder farmers in Kenya.

Plant tissues are colonized by highly diverse microorganisms whose pathogenesis may be suppressed or aided by the presence of other microbes. These microbial populations are pervasive among plant species and are believed to coevolve within the plant in a mutually dependent manner (Card *et al.,* 2016). Their collaboration is unclear due to the complex web of relationships within plant tissues like disparities brought about by microbial communities and plant genotypes. It is important therefore to understand their relationships and behaviour during disease outbreaks since they share the same biological niche as the pathogenic microbe in question. Discerning which microbial species are present, how and when they occur and the crucial role they play during these interactions is key (Busby *et al.,* 2015). Careful behavioural study of these microbes when the pathogen appears and when the disease occurs is a point of paramount significance (Latz *et al.,* 2018). Insight into the fungal species associated with finger millet blast pathogen is crucial because it can advise researchers on the influence of these fungi on the overall disease impact and potential use as biocontrol agents for the blast (Nganga *et al.,* 2022). Thus, periodic pathogen surveillance to determine their occurrence and population variations with time would form a good platform for the effective management and control of finger millet blast disease.

Finger millet blast disease management has incorporated the use of cultural methods and resistant varieties without much success. Moreover, most cultivars grown by the farmers have shown disease susceptibility two to three years after release due to the high genetic variability nature of the fungus in the field. The application of systemic fungicides in blast control has been effective to some extent but this is a very expensive option compared to the economic return of finger millet for the already resource-poor farmer (Mgonja *et al.,* 2013). Besides, extended use of these agrochemical compounds has led to the development of pesticide-resistant fungal

pathogens posing potential risks to human health, food safety, the ecosystem, soil fertility and water quality. Due to this, there is a huge global demand for pesticide-free food that is both safe and nutritious (Sekar *et al.*, 2018). A more feasible approach in finger millet blast management is to employ host plant resistance. However, information on genomic data useful for further improvement of finger millet is limited compared with other major crops such as rice hindering efforts for further enhancement (Ceasar *et al.*, 2018). It is therefore critical to understand the population dynamics of the fungus as a first step towards developing region-specific disease control strategies.

This study was therefore designed to assess the farmers' knowledge, practices and socio-economic impact on finger millet blast disease, evaluate the occurrence, distribution and severity of finger millet farms, determine the fungal species diversity present in finger millet blast infected tissues, and assess for host tolerance of the blast pathogen in some finger millet varieties in Kenya. Results from the study would help develop better strategies to mitigate the effects of the disease in different counties in Kenya from a more knowledgeable point of view.

1.2 Problem statement

Food and nutrition insecurity in Kenya is quite alarming with studies showing that almost a third of the population is inherently food insecure with malnutrition playing a fundamental role in infant mortality (ICRISAT, 2021a). Finger millet is a potential cereal crop for bridging this gap due to its excellent nutritional characteristics, climate resilient farming and agricultural diversification (Vetriventhan *et al.*, 2020). However, finger millet blast disease caused by *P. oryzae* is the greatest economic constraint to finger millet cultivation causing serious grain loss of 20-50% in Kenya. As a result, farmers are diverting from its cultivation in preference for more competitive crops such as maize which produces higher yield and is less labour intensive compared to finger millet, causing a further decline (Onyango, 2016). The expeditious production of scores of asexual spores by the fungus is a good survival mechanism for thriving especially during favourable weather conditions. This

promotes a massive spread of blast disease to the detriment of the smallholder farmers' livelihood and poses a serious threat to food security in Kenya (Masaki, 2020).

Majority of finger millet farmers in Kenya have limited knowledge of blast infection and the crop protection measures they apply are anecdotal with a limited scientific basis. Besides, they prefer to cultivate local unimproved finger millet varieties that are blast susceptible hence contributing to low yields (Owere *et al.*, 2014; Onyango, 2016). Even though the blast disease menace has received substantial interest as the greatest constraint to finger millet production, there is still limited information on the occurrence, distribution, severity, farmers' preferences, knowledge and socio-economic impact, and the diversity of fungal species associated with blast-infected tissues in Kenya.

1.3 Justification

The achievement of sustainable finger millet production relies upon effective blast disease control measures. This can be achieved by the utilization of resistant cultivars developed through an integrated approach where farmers' preferences are also taken into consideration (Mbinda & Masaki, 2021). The assessment of the occurrence and severity of finger millet blast is necessary to determine the geographic distribution and disease state in the country to prioritize research on finger millet blast disease both locally and regionally. Identification of fungal species associated with blast-infected finger millet tissues is one way of diversifying finger millet blast disease control. However, the potential of this approach has not been exploited so far in the pursuit of blast disease management. This information would be a good source of knowledge on the fungal species coexisting with the blast pathogen infected tissues. To highlight this concealed area, this study sought to examine farmers' perceptions, practices and socio-economic impact of finger millet blast, document the distribution, occurrence and severity of blast disease in the farms, characterize fungal species associated with the blast-infected tissues and finally evaluate the tolerance of some Kenyan finger millet varieties to the disease. The information generated from

this study would be useful to plant breeders for establishing a more diversified approach toward the mitigation of finger millet blast disease. The information will also be useful to policy makers in advising farmers on best blast management practices including adoption of farmer preffered tolerant varieties.

1.4 Objectives of the study

1.4.1 General objective

To assess the distribution and socio-economic impact of the blast pathogen, characterize the associated fungal pathogens and screen for tolerance to the disease in different varieties of Kenyan finger millet.

1.4.2 Specific objectives

i. To assess farmers' knowledge, practices and socio-economic impact of finger millet blast disease in selected agro-ecological zones in Kenya.
ii. To evaluate the occurrence, distribution and severity of the blast pathogen from selected agro-ecological zones in Kenya.
iii. To determine the genetic diversity of fungal isolates from blast-infected finger millet tissues using ITS and 28S rDNA molecular markers.
iv. To assess host tolerance of the blast pathogen in some finger millet varieties in Kenya.

1.5 Null hypotheses

i. Farmers in the selected agro-ecological zones in Kenya are not aware of the socio-economic impact of blast disease and its management practices.
ii. There is no occurrence, distribution and severity of blast pathogen from selected agro-ecological zones.
iii. There are no fungal pathogens associated with finger millet blast
iv. There is no variation in the host tolerance to different varieties of Kenyan finger millet.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a climate-resilient highly nutritious crop cultivated in arid and semi-arid regions of developing countries. It is one of the most important small millets and the oldest domesticated cereal crop in East Africa and the Southern parts of India (Dida *et al.,* 2021). The crop derived its name from the shape of the seed head which is made up of several spikelets that appear like human fingers (Sood *et al.,* 2019). It is largely grown on marginal plots of land either as pure or mixed stands by a minority of farmers as it is considered a 'poor person's crop' and may be sold to provide an additional income (Sood *et al.,* 2019). It can tolerate adverse agro-climatic conditions and a diversity of soils unlike maize, wheat and rice thus playing a significant role as a food security crop in times of drought. Due to the small size of the seed, it is resistant to attack by post-harvest pests and thus can stay for a long period without damage (Lenne *et al.,* 2007; Gupta *et al.,* 2017; Dida *et al.,* 2021).

2.1.1 Finger millet origin, botany, and agroecological requirements

Finger millet is of two kinds: the wild type which is known as *Eleusine coracana* subsp. *africana* commonly considered an aggressive colonizer and the cultivated form referred to as *Eleusine coracana* subsp *coracana* (Dida & Devos, 2006). The origin and domestication of finger millet was in Africa particularly, western Uganda and the Ethiopian highlands of Eastern Africa over 5000 years ago (Hilu *et al.,* 1979). The crop was likely transported to India about 3000 years ago consequently becoming its secondary centre for diversity. The evolution of cultivated finger millet may have been due to the selection and domestication of a large-grained mutant of the wild *E. coracana* subsp. *africana* as revealed by cytological, morphological and molecular data (Dida & Devos, 2006).

Millets are generally categorized into two main groups: major and minor millets based on their grain size. Sorghum and Pearl millet form the major millets. The minor millets are also known as small millets and they include finger millet (*Eleusine coracana* (L.) Gaertn.), Kodo millet (*Paspalum scrobiculatum* L.), foxtail (*Setaria italica* L.), proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinochloa crus-galli* (L.) P. Beauv. and *Echinochloa colona* (L.) Link) and Little millet (*Panicum sumatrense* Roth. Ex. Roem. and Schult.), teff (*Eragrostis tef* (Zucc.) Trotter), fonio (*Digitaria exilis* Stapf and *D. iburua* Stapf), job's tears (*Coix lacrma-jobi* L.), guinea millet (Brachiaria deflexa (Schumach.) C.E. Hubb. Ex Robyns, = *Urochloa deflexa* (Schumach.) H. Scholz), and brown top millet (*Brachiaria ramosa* (L.) Stapf. = *Urochloa ramosa* (L.) T.Q. Nguyen) these different species of millets may not be closely related but they all belong to the Poaceae family and may be separated by tribe or subfamilies. A total of nine different genera of small millets have been identified within the Poaceae family. The taxonomic classification of both minor and major millets is shown in Figure 2.1(Vetriventhan *et al.,* 2020).

Finger millet is a highly versatile crop and can do well in a wide range of environmental and climatic conditions. It can thrive well at higher elevations than most tropical cereals and tolerates salinity better than most cereals. In addition, it can be cultivated as a dryland crop with limited fertilizer use in areas where annual rainfall is 500mm or below thus it is a highly valued crop by subsistence farmers. Areas experiencing low rainfall and low relative humidity during seed ripening and maturation are the most suitable for regeneration. Finger millet is equally adapted to a wide range of tropical soils such as red lateritic, sandy loams and black heavy vertisols. It is a biannual crop that can be found at altitudes between 2500-3000 metres above sea level in the Himalayas (Sood *et al.,* 2019; Dida & Devos, 2006; Onyango, 2016).

2.1.2 Finger millet economic importance

The nutritional content of the seed is superior to most other cereals thus they provide a good source of quality protein and nutrition (Gupta *et al.,* 2017). For instance, some finger millet genotypes contain a calcium content of as high as 450mg/100g. This can be exploited for use as preventive medicine for osteoporosis. The straws are used as animal fodder and it contains about 60% digestible nutrients (Kumar *et al.,* 2014). The seed coat also contains sufficient amounts of phytochemicals such as dietary fibre and polyphenols. The presence of high polyphenol levels indicate high anti-oxidative processes which are essential for anti-cancer and anti-diabetic activities. The high fibre content consequently maintains slow digestion and balanced blood sugar (Gupta *et al.,* 2017). Due to these superb qualities, finger millet plays a significant role in the diet and livelihood of the subsistence farmers in Kenya (Opole, 2019).

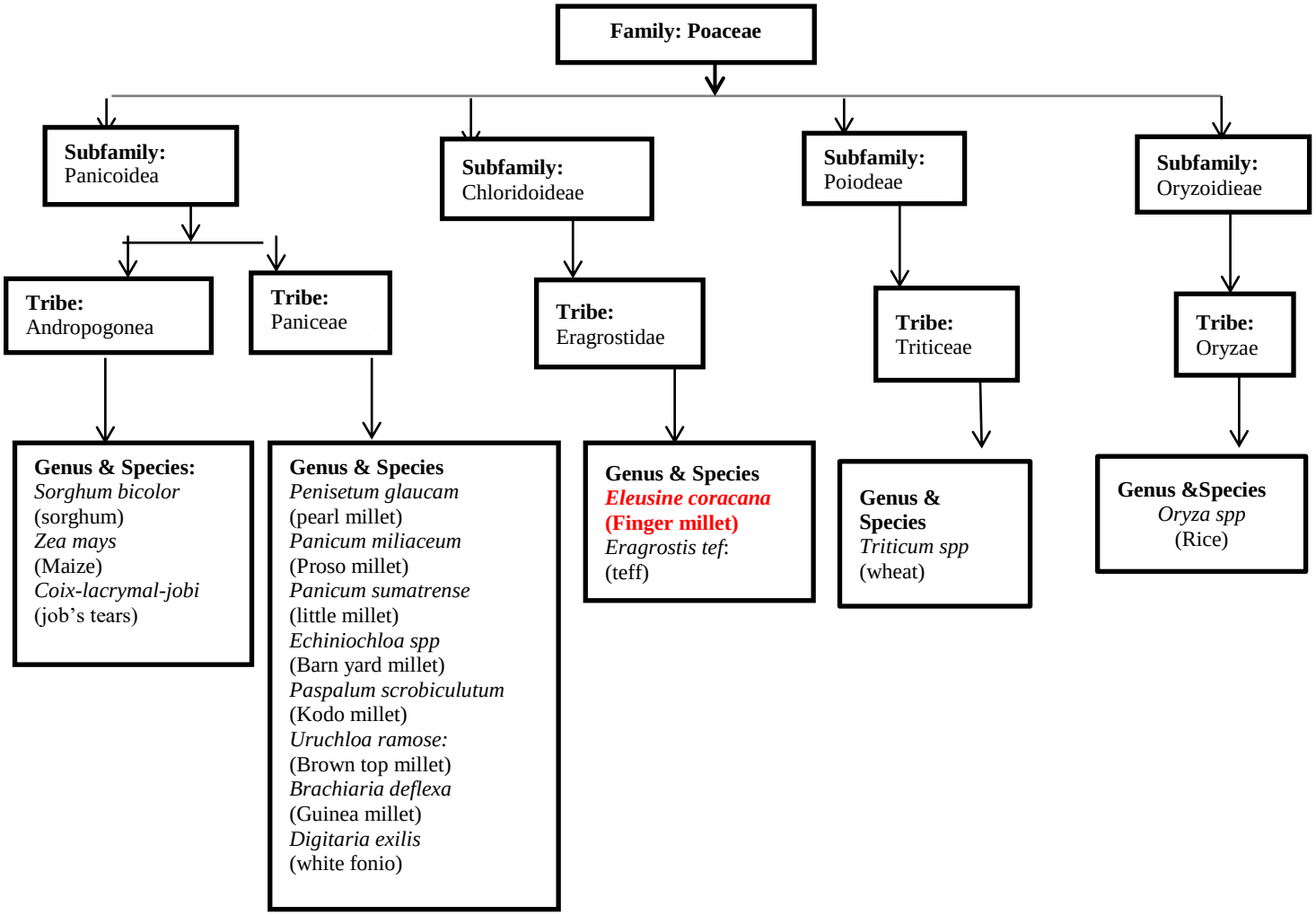

Figure 2.1:Taxonomic classification of some cereals such as rice, wheat and maize, major millets(sorghum and pearl millet) and minor millets.

Key: Finger millet (*Eleusine coracana*) which is highlighted in red.

2.1.3 Finger millet production

Finger millet crop is cultivated widely under several local names such as ragi in India, *wimbi* in Kenya, *ulezi* in Tanzania, *telebun* in Sudan, *Tamba* in Nigeria, *Takuso* in Ethiopia among others (Ramashia *et al.,* 2019). The production and productivity levels vary from one country to another. Most reports on millets production are based on lumped data for all the different types of millets grown rather than on individual types of millets. Thus, a more comprehensive description of the production area and total production for finger millet is limited both locally and globally (Onyango, 2016; Gebreyohannes *et al.,* 2021). Recent studies have shown that global finger millet production is at least 4.5 tons of grain with Africa producing more than half the total amount (2.5 million tons) while India produces 1.2 million tons annually(Ceasar *et al.,* 2018). Approximately, 60% of the globally produced finger millet is in Africa and is mainly cultivated in Ethiopia, Kenya, Uganda, Nigeria, Malawi, Tanzania, Uganda, Zambia and Zimbabwe (Ramashia *et al.,* 2019). In addition, finger millet accounts for at least 85 % of all the millets produced in India thus making India the world's largest producer of finger millet (Sakamma *et al.,* 2018). In Kenya, there are several types of millet cultivated but again their production statistics data are grouped together. Finger millet in Kenya is mainly cultivated in Nyanza, Eastern, Rift valley, Western, Central and Eastern regions as shown in. for the years 2002-2003. According to data on Table 2.1, the Eastern province produced more than half of the total finger millet yield in both years suggesting that the region has a very high potential for finger millet production (Onyango, 2016).

Although this crop plays a significant role as a dietary staple, the area under cultivation has greatly declined which could soon lead it to extinction even among the largest producers like India (Grovermann *et al.,* 2018). A similar trend is also observed in the western region of Kenya where finger millet used to be a predominant cereal crop but is currently grown on marginal plots by a few farmers. In Kenya, although the area under the crop increased significantly from 2005-2007, a sharp decline to 53,155 ha in 2008 was observed probably due to the cultivation of

other competitive crops like maize, erratic weather or disease (Table 2.1). This did not affect the yield because a bumper harvest was realized leading to a production of 626,856 bags (Table 2.1) of finger millet which was much higher than the previous year (Ministry of Agriculture, 2010). In the succeeding five years (2010-2014), millet production recorded a 37.8% increase in production from 1,431,311 bags in 2013 to 1,972,810 bags in 2014 in Kenya (Table 2. 1). This could be related to increased awareness due to the promotion and adoption of drought-tolerant/high-yielding varieties. Indeed, in the year 2010, the Eastern region had the greatest production where finger millet crop area rose to 67,709, followed by Nyanza 22,169. Whereas Central, North Eastern and Nairobi had the least with 112ha, 100ha and 0 ha respectively out of a total production area of 104,576 ha from seven regions (formerly provinces) in Kenya namely Rift valley, Nyanza, Central, Western, Eastern, North Eastern and Nairobi. Notably, the Western region which previously was one of the major growing areas had a crop area of only 4,833ha (Ministry of Agriculture, 2010). However, the crop acreage declined by 17.5 % in 2014 to a dismal 138,829 ha from 168,291 ha in 2013 (Table 2.1).This decline in finger millet crop area in the Western region of Kenya was also noted by Handschuch & Wollni, (2016). Despite this decline, the millets continued to flourish in the marginal areas of the Eastern and Nyanza regions of Kenya having produced 45,211 and 12,973 metric tons of finger millet respectively in the same year (Ministry of Agriculture Livestock and Fisheries, 2015). Over time, production and acreage under millets production in Kenya have been oscillating between high and low (Onyango, 2016). To overcome imminent food scarcity as a result of increasing population and industrialization in the years to come, there is an urgent need to increase the acreage of cereal crop cultivation soon (Ceasar *et al.*, 2018).

Table 2.1: Finger millet production statistics in Kenya (2005-2014)

Year		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Area (ha) 000'		92.43	138	128	53	74	104	111	118	168	139
Production	**90kg bags 000'**	661	880	1,329	627	427	599	816	832	1,431	1,973
	Tons 000'	59	79	119	38	56	110	111	138	125	1,443
Average yield 90kgs bag/ha		7.0	6.4	7.3	8.0	6.0	6.0	7.3	7.0	8.5	14.2
Total value (billion Kshs)		1.59	1.5	2.5	1.2	2.93	1.59	1.5	2.5	1.2	2.93

Source: Ministry of Agriculture, Kenya and State Department of Agriculture, Kenya.

2.2 Finger millet production constraints

Finger millet production and productivity face many biotic and abiotic challenges. The overall cultivation process from land preparation all through to harvesting the crop is highly labour intensive posing a serious problem to finger millet production (Owere *et al.*, 2014). Indeed, the high labour requirement greatly escalates the comparative cost of finger millet against other competing crops such as maize in the finger millet growing region. Moreover, the yields per hectare for finger millet are lower than that of maize thus farmers prefer cultivating the more competitive crops leading to a decline in finger millet production. Finger millet farmers also employ elementary farming techniques that are characterized by limited use of improved seeds, fertilizers, and agrochemicals with low-level mechanization (Onyango, 2016). As a result, the soils tend to suffer deficiencies of important nutrients such as nitrogen, phosphorus and zinc which may be compounded by drought leading to stunted growth, reduced finger millet biomass and thus poor yields (Ramakrishnan *et al.*, 2017).

Although finger millet can tolerate salinity to some extent, high salinity levels reduce water content, leaf expansion, plant height, grain weight, finger millet length and width coupled with delayed flowering (Anjaneyulu *et al.,* 2014). Insect pests that attack finger millet have not been reported so far however several bacterial and fungal pathogens are known to affect finger millet. *Xanthomonas campestris pv eleusines* is an example of a bacterial pathogen that affects finger millet. Examples of fungal pathogens include blast (*Pyricularia oryzae*), rust (*Puccinia substriatia*), downy mildew (*Sclerospora graminicola*), seedling and leaf blight (*Helminthosporium nodulosum*), Cercospora leaf spot (*Cercospora pennisetic*) Cylindrosporium leaf spot (*Cylindrosporium species*) and tar spot (*Phyllachora eleusines*). Of all these fungal pathogens, blast disease caused by *Pyricularia oryzae* is the greatest constraint and economically significant pathogen to finger millet production (Owere *et al.,* 2014; Shittu, 2018).

Blast disease attacks the crop at all stages of growth and in some cases can lead to the destruction of the whole plant because most farmers prefer cultivating unimproved varieties that are highly susceptible to finger millet blast isolates. The disease occurs in all finger millet growing areas globally. Significant yield losses of 10-50% (Kenya), 10-80% (Uganda) and 28-36% (India) have been reported although as high as 80-90% yield loss has been recorded in endemic areas (Babu, 2011). Unfortunately, the pathogen causes blast disease to over 50 cultivated and wild monocots including other cereals such as rice, wheat and barley that are of chief importance to food security. Thus, *P. oryzae* is an economically significant fungal pathogen worldwide (Gladieux *et al.,* 2018; Langner *et al.,* 2018). Taxonomically, *P. oryzae* belongs to the Phylum Ascomycota, class Sordariomycetes, Order, Magnaporthales, family Magnaporthaceae and genus *Pyricularia*. It is a biphasic plant pathogen that successfully colonizes living plant tissues for nutrition and continues to subsist on the dead plant to complete its life cycle. Such hemibiotrophic fungi include many plant pathogens that cause huge crop losses posing a major threat to the global economy and food security (Shittu, 2018).

2.3 Morphology of finger millet blast pathogen, distribution and biology

Finger millet blast disease is caused by the fungus *Pyricularia oryzae* (sexual morph *Magnaporthe oryzae*); a fungus that is morphologically similar to *Pyricularia grisea* (sexual morph *Magnaporthe grisea*)(Klaubauf *et al.,* 2014; Couch & Kohn, 2002; Zhang *et al.,* 2016). Molecular techniques have been used to segregate them into two distinct groups namely *P. oryzae* and *P. grisea* using some ortholog genes that could separate them based on evolutionary association. This classification was according to the pathogen responsible for attacking rice, wheat and millet (*P. oryzae*) and *P. grisea* as responsible for causing foliar disease in *Digitaria* species (Couch & Kohn, 2002; Klaubauf *et al.,* 2014; Zhang *et al.,* 2016). These two pathogens are morphologically indistinguishable about conidia, perithecia and ascospores. (Shittu, 2018). *Pyricularia oryzae* is a heterothallic filamentous fungus responsible for blast infection on more than 80 species of both cultivated and wild plants.

The vegetative stage of *P. oryzae* is made up of mycelium, conidia and a perfect stage associated with asci (Dubina *et al.,* 2020). The hyphae are septate and hyaline that turn brown with age. On the agar media plate, growth is observed more on the upper surface while the underside tends to be darker with melanin spots. Three colour variations have been observed on rice flour agar including white, cream and grey (Longya *et al.,* 2020). Conidiophores are made up of two portions; the upper part is simple and septate while the basal portion appears dark (Babu, 2011). Conidia vary in shape from oval, short pyriform, pyriform and long pyriform and are two septate with three cells; the middle being darker and wider while the end cell gives rise to germ tubes (Longya *et al.,* 2020). Thick-walled, olive brown, globose intercalary or terminal chlamydospores regularly occur. The fruiting body of the pathogen is a fertile perithecium, especially under laboratory conditions (Viji & Uddin, 2002; Babu, 2011).

Finger millet blast caused by *P.oryzae* is widely distributed in virtually all the growing regions of the world. This includes finger millet growing countries in Asia for example India, Sri Lanka, Nepal, and Malaya. In Africa, countries affected by

finger millet blast include Kenya, Tanzania, Uganda, Ethiopia, Somalia, Zambia, and Nigeria among others (Figure 2.2).

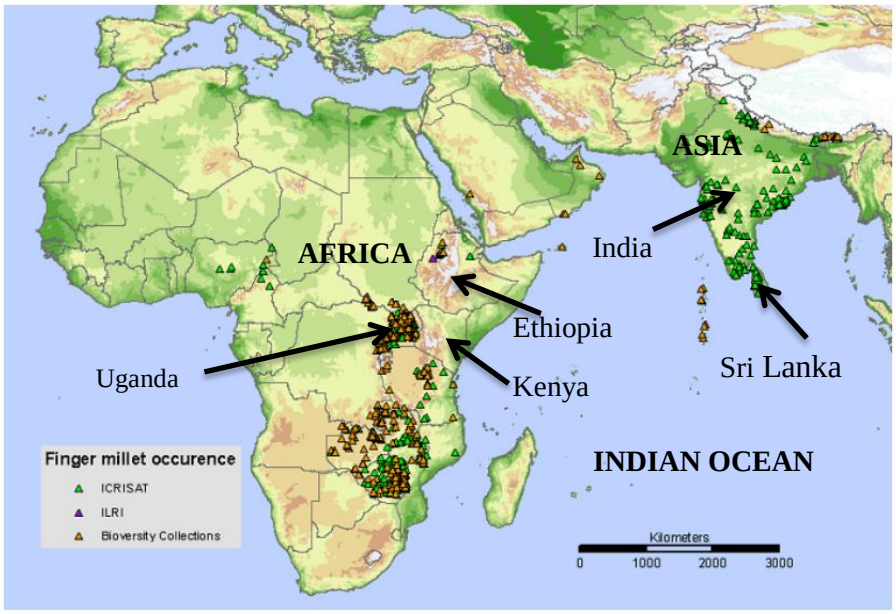

Figure 2.2: Geographical distribution of finger millet accessions.

Key: Photo adapted from (Mathur & Guarino, 2012) The finger millet accessions have been mapped from two continents: Africa and Asia that are the largest producers of finger millet. The distribution map displays sampling sites surveyed by ICRISAT (International Crops Research for the semi-arid tropics); ILRI- International livestock research institute and Bioversity International only. Not all countries that grow finger millet are shown including Kenya.

The pathogen targets different aerial plant parts at all stages of growth (Babu, 2011). Plant pathogens establish their mechanisms to get nutrients from their host plants. *P. oryzae* is a hemibiotrophic pathogen that initially grows as a biotrophic and then, later on, transforms to necrotrophic growth, when lesions become apparent, eventually killing the infected tissues (Wilson & Talbot, 2009). This fungus is seed-borne hibernating in infected crop debris occurring mainly during the rainy season. The disease non-selectively damages the foliage, neck and finger at different stages of growth. The intensity of disease damage is dependent upon the severity of the

disease and the development stage of the plant (Ghimire *et al.,* 2022). Disease symptoms are generally classified as leaf blast, neck blast and finger blast concerning the plant part attacked. The primary symptom (leaf blast) if severe at early seedling, can cause serious damage to the whole plant since many agronomic traits such as number of productive tillers, length of fingers, number of fingers and even yield will be heavily affected (Babu *et al.,* 2013).

Typical leaf blast symptoms are diamond or elliptical shape brown spots with grey centers, neck blast is brown lesions encircling the neck of the plant inducing sterility. Finally, finger blast causes bleached fingers that are either partially or completely dry prompting spikelet sterility. Finger millet is more susceptible to blast disease during low temperatures and higher humidity (> 70%) during the growing season (Ghimire *et al.,* 2022). Management of blast disease is problematic thus a three-pronged strategy seems adequate to contain blast: the breeding of blast-resistant varieties, employing proper crop husbandry and, chemical or biological agents application (Mbinda & Masaki, 2021).

2.4 *Pyricularia oryzae* biology and host infection process

Understanding *Pyricularia oryzae* biology and interactions with its hosts are pivotal to the control of finger millet blast disease. However, this has not been forthcoming because many *P. oryzae* pathotypes that can infect diverse annual and perennial grasses as well as other economically important graminaceous plants such as wheat, millet and barley (Fernandez & Orth, 2018). This fungus is also capable of cross-infection with cereal crops. An example of this is the wheat blast that first occurred in Brazil in 1985 after what appears to be a host jump from a grass-infecting isolate of the fungus (Inoue *et al.*, 2017). The disease has since spread to other countries such as Bangladesh where it now poses a serious threat to wheat production that may likely spill over to India, the second largest producer of wheat (Eseola *et al.*, 2021).

Recent reports have revealed the presence of blast disease in wheat in Zambia (Tembo *et al.,* 2020). All this taken into account together with the devastating effects of blast on rice, finger millet and other cereals suggests that blast disease poses a

serious threat to global food security. Thus, understanding the biology of the blast is the right path toward developing new disease-control strategies. At the same time, the fungus can expeditiously conform to and mutate to emerging resistance to multiple host varieties/ cultivars. This fungus acquires peculiar morphological changes to permeate and attack the host plant cell (Mbinda & Masaki, 2021).

Pyricularia oryzae interestingly colonizes its host's cell. This hemibiotrophic fungus at first creates a biotrophic union where the host's immune system is overwhelmed. The necrotrophic stage then follows where plant cell death is inevitable (Fernandez & Orth, 2018). At the time of infection, the conidium lands on the leaf cuticle and then discharges a sticky mucilaginous substance that enables it to cling firmly to the hydrophobic leaf surface. The conidium then germinates to form a tapered germ tube at one of the topmost cells traversing the leaf surface. During favourable conditions such as the presence of a hydrophobic surface, the tip of the germ tube forms a dome-shaped protrusion called an appressorium (Wilson & Talbot, 2009). Later on, it produces a thick melanin layer on the inside of the appressorium cell wall equipping it with an impermeable surface that limits the discharge of equivalent solutes.

The contents of the conidium are then transferred into the appressorium promoting its further advancement. Consequently, massive turgor pressure is established that eventually forms a penetration peg which bursts the plant host cell cuticle (Fernandez & Orth, 2018). Once inside the host cell, *P. oryzae* diffuses to neighbouring cells via plasmodesmata without changing the host cell walls. At the opportune time, the fungus then profusely sporulates from the diseased lesions allowing the disease to spread expeditiously to neighbouring host plants and their affiliates by wind or water droplets to start another cell cycle (Mbinda & Masaki, 2021). New blast lesions appear 3-5 days post-infection at optimum temperature. Long periods of leaf wetness and night temperature of about 20 °C coupled with high levels of nitrogen in the soil are conducive conditions for blast fungus to thrive (Shahriar *et al.,* 2020). In 21 days post-infection, sexual fruiting bodies termed perithecia are formed. These perithecia contain sac-like structures called asci that carry the ascospores or conidia (Talbot,

2003). Figure 2.3 gives an overview of *P. oryzae* infection process once the conidium lands on the surface of the plant tissues.

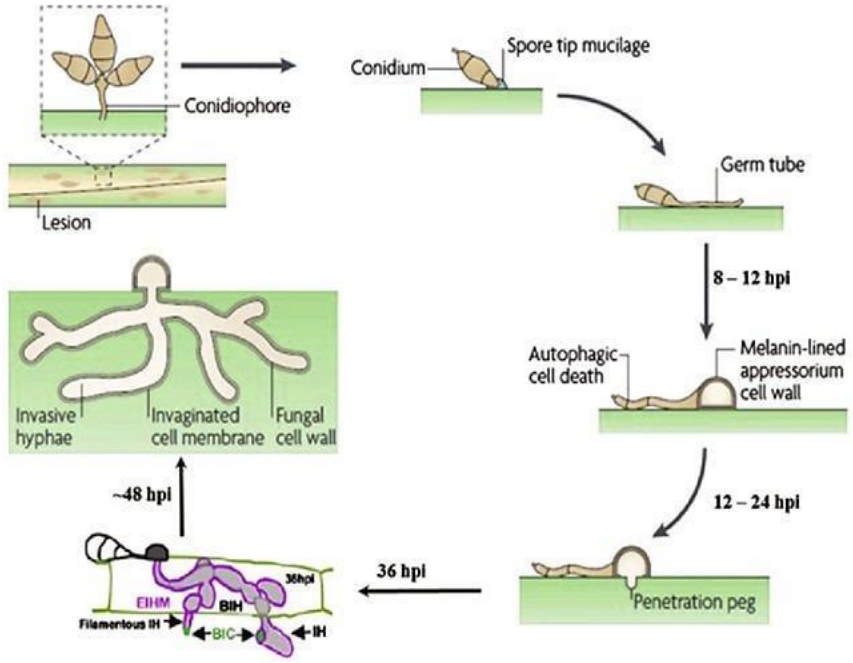

Figure 2.3: Infective life cycle of *P. oryzae* adapted from Wilson and Talbot (2009) and Mosquera *et al.*, (2009).

2.5. Molecular markers and genetic diversity of fungi

The application of molecular technological tools in fungal genetic studies envisages an era of great scientific discoveries. A genetic marker can be defined as a DNA sequence with a specified chromosome location controlling a particular gene or trait (Nadeem *et al.*, 2018). Precise molecular taxon identification is usually fixed upon the marker gene sequencing whose sensitivity, resolution and throughput are regulated by the choice of the marker gene and sequencing platform (Banos *et al.*, 2018). This provides a good mechanism for understanding the population diversity and distribution pattern of fungal pathogens (Shittu, 2018). The Sanger-sequencing technique, for example, is modeled for single taxon identification while Illumina Miseq and third-generation sequencing are suited for community surveys (Banos *et al.*, 2018).

Fungal marker genes vary in length, ability to resolve different fungal groups, phylogenetic power, number of publicly available sequences and available suitable primer sets (Reich & Labes, 2017). DNA markers such as the nuclear ribosomal internal transcribed spacer (ITS), 28SrDNA and 18S rDNA regions have been greatly used in understanding fungal diversity. These three nuclear ribosomal genes have been frequently used in fungal identification especially when fungal morphology overlaps between different organisms (Raja *et al.,* 2017). It is critical to accurately identify fungal species to apply precise crop disease management tools (Kusai *et al.,* 2015). The ITS region is made up of two sections (ITS 1 and ITS 2) which flank the conserved 5.8S region. ITS region is sandwiched between the small subunit gene (18S) at the 5' end and the large subunit (28S rDNA) at the 3' end (White *et al.,* 1990). This region exhibits the highest variation and at the same time evolves the fastest. Thus, it has been identified as the barcode for identifying environmental fungal sequences (Schoch *et al.,* 2012). However, during sequence analysis, great care must be employed to minimize user generate biases since the region is highly hyper-variable (Nilsson *et al.,* 2012).

The ITS region is a robust tool for the identification of fungal community ecology with high taxonomic resolution at the species level (Schoch *et al.,* 2012). It has been widely utilized in classification and population studies involving both pathogenic and non-pathogenic fungi to discern their evolutionary relationships (Shittu, 2018). ITS has played a significant role in segregating *P. oryzae* isolates linked to *Eleusine* species into two from various geographical locations revealing the evolutionary independence between the two (Tanaka *et al.,* 2007).

The large sub-unit region (28S) exhibits two hypervariable domains D1 and D2. It also sufficiently delivers valuable information about species identification in fungi either on its own or in combination with the ITS region (Raja *et al.,* 2017). The 28S region is especially useful in phylogenetic analyses to assess species relationships. The small subunit (18S) displays the least variation among taxa and also evolves the slowest. However, it is more useful when interested in fungal phylogenetic

placement at higher taxonomic levels such as family, order, class or phyla (Zuccaro *et al.,* 2008).

2.6 Genetic diversity of *Pyricularia oryzae*

Genetic diversity studies in plant pathosystem are a critical requirement to understand coevolution in the plant pathosystem. A strategy employed by researchers is to monitor pathogenicity and genetic shifts of the blast fungus in commercial fields. Differences in the pathogenicity of *P. oryzae* were used in the identification of physiological races using differential rice varieties. The term race was used as an indicator of pathogenicity meaning that a change in the race meant a change in the pathogenicity of the *P.oryzae* isolates as well. This differential system has played an effective role in ascertaining the aggressiveness of blast fungus and host genetic resistance globally (Wang *et al.,* 2017). The *P. oryzae* genome has been sequenced and several genes associated with its pathogenesis have been identified. One of the approaches employed in the control of blast disease is the utilization of a host resistance strategy since it is economical and environmentally friendly. This approach, however, is hampered by the complex population structure and high variability nature of the *P. oryzae* in the fields that enables it to quickly overcome the resistant cultivars within a short time of initial placement. This analysis of the genetic structure and variation of the *P. oryzae* is key for disease prediction and forecasting and the determination of the distribution of resistant varieties. This can adequately prevent disease epidemics brought about by host resistance (Xu *et al.,* 2019).

To effectively manage the blast disease good insight into the structure of the blast populations and genetic diversity will play a significant role (Longya *et al.,* 2020). Although the *P. oryzae* pathogen has a wide host range evidenced by the high number of plants that it affects, it mainly exists as a host-specific adapted form adept at infecting a single host. Because of the capricious nature of this fungus, it is composed of several physiological races or pathotypes (Xu *et al.,* 2019). The race of an isolate is figured out subject to the reaction pattern observed on a set of eight

cultivars referred to as standard international differentials. For example, in Brazil, the pathogenic diversity of 85 *P. oryzae* isolates gathered from 14 upland rice cultivars in experimental plots over five years revealed eleven predominant international races. Thus, the knowledge of *P.oryzae* pathogenicity based on pathotype identification is limited only to the reactions of cultivars used as international differentials.

A more exhaustive approach in the analysis of virulence using important local cultivars in place of international differentials is of utmost importance when using genes that display a wide spectrum of resistance to a determined population. In addition to the virulence spectrum, *P. oryzae* population structure can equally be analysed using genetic markers. This approach gives more insight to several aspects of the evolutionary dynamics under field conditions (Silva *et al.*, 2009); Takan *et al.*, 2012). DNA profiling techniques have played a significant role in organizing the *P. oryzae* pathogenic populations into distinct lineages or groups by use of genetic markers such as inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD) and fragment length polymorphism (AFLP).

These markers use universal primers for unknown regions to comprehensively analyse the genetic diversity of the said fungal pathogen. In recent times sequence-related amplified polymorphism (SRAP) markers have been developed that amplify coding regions with primers targeting open reading frames (ORF) (Longya *et al.*, 2020), retro transposon-microsatellite amplified polymorphism (REMAP), repetitive element-based polymerase chain reaction (rep-PCR) and microsatellites or simple sequence repeat (SSR) are more commonly used to exhibit variations in pathogen populations. Although there may seem to be some genetic diversity among *P. oryzae* populations from a given region or country, the isolates within populations are usually categorized into small groups based on genetic similarity from the molecular data (Xu *et al.*, 2019).

2.7 Characterization of fungal community associated with *P. oryzae*

Highly diverse microorganisms whose pathogenesis may either be suppressed or enhanced by the presence of other microbes tend to colonize plant tissues. Such microbial populations are ubiquitous among plant species and are thought to coevolve within the plant in a mutually dependent manner (Card *et al.,* 2016). An obscure understanding of their interactions exists due to the complex web of relationships present within the plant tissues such as types of microbial communities and plant genotypes.

Having good insight into their relationship and behaviour during a disease outbreak is essential as they share the same biological niche as the pathogenic microbe in question (Busby *et al.,* 2015). Discerning which microbial species are present, and how and when they occur including their vital roles during these interactions is important. This approach would thus dictate that the behaviour of the microbial species when the pathogen appears and when the disease symptoms occur is coherent (Latz *et al.,* 2018). This novel microbiome perspective to plant-microbe interactions is a good strategy to plant disease control setting the pace for a more comprehensive understanding of the roles of these fungi, thus leading to improved development of plant disease management practices including finger millet blast.

Presently, there is limited information on the microbial species that coexist with the blast pathogen *P.oryzae* in blast-infected tissues in finger millet crops. However, several studies on the fungal species associated with *P. oryzae* in rice has been done in rice (Nganga *et al.,* 2022); Leewijit *et al.,* 2016; Lapmark *et al.,* 2009). Some of the fungal genera associated with *P. oryzae* in rice isolated in these studies included *Curvularia* spp, *Fusarium* spp, *Penicillium* spp, *Chaetomium* spp, *Colletotrichum* spp, *Bipolaris* spp, *Aspergillus, Nigrospora* spp and *Sarocladium*. Another study revealed a total of 22 and 31 fungal species present in the leaves and stems respectively of rice tissues in Thailand (Seephueak *et al.,* 2019). This group of microbes may directly interact with pathogens in different ways that can transform

how a disease is expressed in the host plant. Such transformative mechanisms may include hyperparasitism, competition or antibiosis (Martín *et al.,* 2015).

Species of *Ampelomyces*, for instance, are mycoparasites that internally colonize powdery mildew hyphae stifling mildew sporulation and eventually killing the parasitized fungal cells (Busby *et al.,* 2015). Insight into the identity of fungal communities associated with foliate diseases forms a good basis for studies on similar work with finger millet crops. This can help researchers perceive the influence of such fungi on the disease impact as well as evaluate their potential usage as biocontrol agents for finger millet blast disease.

The fungal genus *Curvularia* is an economically significant fungal pathogen because it causes diseases to many plants including grasses and cereal plants like finger millet. It is classified in the phylum Ascomycete, family Pleosporaceae and class Dothidiomycetes (Kusai *et al.,* 2015). It is composed of over 40 species that are differentiated from each other based on the morphology of many septa, and colony and conidia morphology. The species may exist as saprophytes, endophytes and pathogens (Santos *et al.,* 2018).

The most common symptom is leaf spots that can be identified by circular necrotic lesions on the leaves of the plant from seedling up to maturity. Saprophytes such as *Curvularia* spp were of great interest in this study because they equally colonize necrotic lesions of blasted plants and quickly advance in growth at the expense of *P. oryzae* which was the target fungal pathogen in this study. The genus *Curvularia* and specifically *C. lunata* was the most frequently isolated fungal pathogen coexisting with *P.oryzae* in blast-infected finger millet tissues in this study. Thus, the role played by these fungal pathogens coexisting with *P. oryzae* was obscure and could only be speculated. Perhaps some of these microbes could be involved in enhancing the aggressiveness of *P. oryzae* during colonization.

For instance, *Epicoccum nigrum*, is a fungal pathogen that has been identified as a beneficial co-inhabiting fungus in sugarcane due to its ability to secrete cell-wall-degrading enzymes that facilitate the successful invasion of the plant cell wall and ultimately cause disease, especially during the saprophytic phase of the fungal cycle (Kubicek *et al.,* 2014). There is a possibility of finger millet blast pathogen forming a pathogen complex rather than just being a single causative agent (*P. oryzae*) since this study identified other fungal pathogenic species from finger millet blast infected tissues with the most frequently isolated being *Curvularia* species. It is against this backdrop that *Curvularia lunata* was utilized in the pathogenicity study since it was the most dominant fungi isolated from finger millet blast infected tissues compared to other fungal species.

2.8 Finger millet tolerance mechanisms toward blast disease

The structure of the finger millet grain: minute size and grain hardness, acts as the major restraint against disease infection and pest infestation (Gupta *et al.,* 2017). Several local and world finger millet germplasm collections have been utilized for the development of improved varieties over the years (Onyango, 2016). It has been revealed that Eastern Africa has a rich source of wild finger millet relatives and landraces that can be exploited to improve finger millet blast-disease resistance. This is because the wild relatives and landraces are abundant, distinct and have coevolved with the pathogen for a long period under non-intensive agriculture (Dida *et al.,* 2021). Sadly, most farmers cultivating finger millet are still using unimproved local varieties that are susceptible to blast (Onyango, 2016).

Comparative genomics has been utilized for studying blast resistance in finger millet to genetically boost finger millet for effective, durable resistance to important diseases (Mbinda & Masaki, 2021). At least 58 functional SSR markers for a good number of important genes that are resistant to blast have been developed. In addition, association mapping analysis using 104 SSRs revealed five QTLs for blast resistance four for finger blast and one for neck blast using the generalized linear model (Babu *et al.,* 2014). Molecular marker-based breeding has played a key role in

the development of blast resistance and the improvement of important agronomic traits in rice and foxtail millet. Indeed, genetic mapping of functional quantitative trait loci (QTLs) using molecular markers promotes marker-assisted breeding for crop improvement for the traits of interest (Ramakrishnan *et al.,* 2016). At present, there is no finger millet variety developed and released based on the marker-assisted-selection technique although the potential of this technique has been observed in other cereal crops such as rice (Mbinda & Masaki, 2021).

It is essential to identify host-resistance factors to help in controlling plant diseases naturally by identifying resistant germplasm, isolating and characterizing resistant genes and molecular breeding cultivars. Thus, it is needful to develop accurate, rapid, and large-scale inoculation methods to breed and develop plant-resistant cultivars (Zhang *et al.,* 2018). Successfully screening large plant populations for disease depends upon the assessment of disease response using accurate and reproducible techniques (Miller-Butler *et al.,* 2018). Whole plant inoculation assays have historically produced accurate and repeatable results when used to test for disease resistance or measure pathogen aggressiveness, but the tests are time-consuming, expensive, and space-intensive in glasshouses (Pettitt et al., 2011). Sometimes, such experiments may be constrained by prevailing unfavourable environmental conditions which may bring out experimental errors or the likelihood of pathogenic isolates escaping to nearby plants.

Recently, cheaper *in vitro* assays to determine pathogen aggressiveness have been utilized effectively for several fungal species (Sakr, 2020; Sakr, 2019). It has also been reported that disease reactions displayed in many in vitro assays on young plants strongly correlated with pathogenicity carried out on mature plants (Sakr, 2020; Aregbesola *et al.,* 2020). Detached Leaf Assay can be used as an alternative or as a complement to whole plant assays. The Detached Leaf Assays (DLA) are fast, low-cost laboratory-based techniques used to screen for resistance to disease of diverse plant pathogens. It allows for fast screening of many genotypes within a relatively small area with regulated conditions, limited planting space and resources (Aregbesola *et al.,* 2020; Miller-Butler *et al.,* 2018; Sakr, 2020).

Plant functional traits such as morphological, phenological, physiological and nutritional influence plant response to environmental factors. The outcome of differences in these traits is superiority in one genotype over the other (Sood *et al.*, 2019). Morphological markers for finger millet blast resistance genotypes can easily be ignored in the process of developing improved prolific finger millet varieties. Thus, it is critical to understand the available germplasm, and the extent of variation present among the accessions and trait relationships. Several studies have been conducted on the variability, heritability and trait association in finger millet (Anuradha *et al.*, 2017; Patro *et al.*, 2018; Owere *et al.*, 2016; Owere *et al.*, 2015). Finger millet head architecture has played a key role in yield improvement and may be a consequence of farmers' selection preference and acceptability (Dida & Devos, 2006). For example, it has been revealed that open-headed or short and/or early maturing genotypes are more susceptible as opposed to incurving type (Owere, 2013). Also noted, is that compact head shape genotypes are somewhat resistant to head/ finger infections but more susceptible to leaf infections. The variations in finger millet head shapes brought about by the inflorescence compactness and shape have given rise to four different races of finger millet namely *Elongata, Plana, Compacta* and *Vulgaries* and several subraces (Sood *et al.*, 2019; Owere, 2013). The race *coracana* bears resemblance to subspecies *africana* and has well-developed central spikes ranging from 5 to 20 in number (Figure 4). The spikes are erect, slender with a length of upto 11cm. The race *vulgaris* inflorescence has incurved or erect spikes (Fig 4C). *Compacta* race has incurved or straight spikes with branched lower finger (Figure 4A). The race *plana* has broader spikelets evenly aligned in two rows along the rachis to give the head a ribbon-like look (Figure 4B). Finally race *elongata* (Figure 4D) has long slim spikes that are incurved at maturity of lengths upto 24 cm (Dida & Devos, 2006).

Figure 2.4: Head morphology of finger millet inflorescence of four races.

Key: (a). compacta, (b). plana, (c). vulgaris and (d). elongata. Photo adapted from (Malambane & Jaisil, 2015).

2.8 Plant defence mechanism against pathogens

Plants can employ various techniques to resist the invasion of a pathogen. These mechanisms can either be structural or metabolic and at the same time can also be pre-formed or induced upon infection (Daayf *et al.,* 2012). The plant develops structural characteristics such as leaf waxes and lignified cell walls that act as physical barriers and hinder the pathogen from penetrating and colonizing its tissues. The pre-formed defences by plants may include the production of toxic substances, phenolics and saponins or the creation of conditions that inhibit the pathogen growth in the plant causing resistance.

Resistance mechanism can also be considered as a lack of recognition between the host and the pathogen (that is, non-host resistance), lack of specific receptors on the host membrane to essential virulence factors of the pathogen or lack of other

substances necessary for the pathogens own growth or development (Daayf *et al.,* 2012). Overall, these defence mechanisms the plant uses whether biochemical or structural are ultimately regulated by the genes of both the host plant and the pathogen (Jibril *et al.,* 2016). The modified concentration of biochemical components in the host plant hampers disease progression. These biochemical or metabolic markers can be exploited for the evolution of 'pathogen-free' plants. Moreover, adequate application of the key metabolites-based metabolic markers can be an open avenue for identifying the candidate gene (Kaur *et al.,* 2022).

Upon plant invasion by a pathogen, a mutual recognition results in a network signal of reactions that stimulate a sequence of highly coordinated defence responses such as increased transcript levels of several genes either during compatible or incompatible interactions. If incompatibility occurs then the infections stop immediately without pathogen multiplication or sporulation. If on the other hand compatibility occurs then the disease will be established. The reactions may end up in cell wall lignification and suberization, *de novo* synthesis of pathogen-related proteins, build-up of phytoalexins and phytoanticipins and production of phenolics or related compounds (El Hadrami *et al.,* 2011; Ros *et al.,* 2005).

Recognition and signalling overflow lead to a pathogen-triggered immunity (PTI) by the plant whereas other elicitors referred to as effectors are intercepted nucleotide-binding domains and leucine-rich repeats (NB-LRRs) are also triggered. This type of recognition by the R gene and defence feedback is called effector-triggered immunity (ETI) (Jones & Dangl, 2006). The two defence mechanisms (PTI and ETI) are interconnected and complement each other together with several other mechanisms that trigger various defence responses to minimize the spread of the pathogen. However, the disparity in strength or timing of signals leads to distinct defence strength (Katagiri & Tsuda, 2010). These include cell wall modification, closure of stomata, production of reactive oxidative species (ROS), hypersensitive reactions, or the production of various anti-pathogen proteins and compounds such as protease inhibitors, chitinases, defensins and phytoalexins (Kaur *et al.,* 2022). For instance, in

rice plants, the activity of phenylalanine ammonia-lyase (PAL) was induced in response to the pathogen *Pyricularia oryzae* (Giberti *et al.,* 2012).

The chlorophyll content is also another biomarker for stressed plants. This is because the concentration of the photosynthetic pigments determines the photosynthetic efficiency of the plant. After all, they play a role in the capture of the light energy that is later converted to chemical energy (Nascimento *et al.,* 2019). Chlorophyll fluorescence has gained popularity as a technique for assessing how plants react to environmental stresses. Plant physiological and biochemical changes in response to stress are connected to altered gene expression. When a stress occurs, it activates some (mostly unknown) initial sensors, which then stimulate cytoplasmic Ca^{2+} and protein signaling pathways, leading to stress-responsive gene expression *(Saibo et al*., 2009). Furthermore, abscisic acid (ABA) accumulation plays an important role in abiotic stress signaling and transduction pathways, mediating many responses. It is well documented that abiotic stresses in general, through regulation of both gene expression and protein turnover, alter the abundance of many transcripts and proteins (Jiang *et al*., 2007).Transcriptome studies have successfully displayed a comprehensive set of gene expression profiles usually by comparing the transcriptome of host plants with and without pathogen infection to determine and identify the genes involved in pathogen response and defence.

RNA-Seq for example has been utilized in rice and foxtail millet to reveal their gene expression profiles after inoculation with rice false smut and foxtail millet rust pathogens (Li *et al.,* 2015). Greenhouse evaluation against diverse pathotypes aids in identifying the stable and likely tolerant finger millet varieties. This study assessed the aggressiveness of *P. oryzae* and *Curvularia spp* on six finger millet genotypes in Kenya namely *Okhale, Black Eastern, Engundi,* GBK 42 (GBK 043050), GBK 17 (GBK 043137), and GBK 21 (GBK 043124), using both detached leaf assay and whole plant assays. Quantitative traits based on growth characteristics such as plant height, leaf length, leaf width, chlorophyll a and b content and carotenoids were evaluated for the six-finger millet accessions. This would give an insight into the level of variability among the six accessions and thus help identify the more tolerant

or susceptible varieties for use in selecting superior accessions or utilized to select parents for finger millet breeding programs.

2.9 Plant-associated molecular defence strategies

Plants are sessile organisms and are constantly exposed to diverse environmental stimuli. The ability to discern the environmental signal and respond accordingly is essential for their survival (Rathore & Ghosh, 2018). These plants solely depend upon both preformed defences and their innate immune system to avert microbial pathogens. The preformed defence consists of the cell wall and cuticle for example, that serve as physical barriers to microbial colonization. The inherent plant immune system is vigilant enough to perceive several generic microbial elicitors which permit the plant to shift from growth and development into a defence mode, repulsing most potentially harmful microbes. These elicitors are vital schemes for pathogen survival and are conserved among pathogens (Rathore & Ghosh, 2018; Newman *et al.,* 2013).

During the growth and development, both plants and pathogens evolve features to fight each other. The plant is armed with complex and swiftly mounted defence mechanisms while their associated pathogens develop counterattacks to subdue those defences leading to competition between the plant and the pathogen (Jones & Dangl, 2006). This defence-suppression kind of interplay between plants and pathogens has been depicted as a zigzag model which proposes a two-branch type of plants' immune response mechanism. The first line of defence displayed by the plant is the recognition of conserved molecules unique to many microbes and is referred to as elicitors or microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs).

Since MAMPs make up the basic structure of pathogens, they are conserved in pathogenic, non-pathogenic and saprophytic microorganisms. The pattern recognition receptors (PPRs) are strategically concentrated on the surface of plant cells to quickly perceive the arrival of MAMPs. This defence mechanism is referred to as MAMP-triggered immunity (MTI)(Ausubel, 2005; Newman *et al.,* 2013). The pathogen-triggered immunity (PTI) initial cellular response comprises the rapid

generation of reactive oxygen species (ROS) and nitrogen oxide (NO), activation of mitogen-activated kinases, expression of immune-related genes, alteration in cell wall architecture and synthesis of pathogenesis-related protein and antimicrobial compounds. ROS and NO possess antimicrobial effects; NO is further responsible for the cross-linking of the polymer present in plant cell wall providing it with strength against pathogen degradation. The PR proteins such as β-1-3 glucanase and chitinase inhibit pathogen multiplication. These initial immune responses are adequate to overcome several microbes or pathogens. Therefore plants deficient in PRR and PTI signalling mechanisms are often more susceptible to pathogens (Dou & Zhou, 2012). The second line of defence employed by the plant is a direct or indirect recognition of a given effector by way of a set of plant resistance (R) gene products resulting in effector-triggered immunity (ETI) commonly referred to as the gene-for-gene interaction (Jones & Dangl, 2006).

The effector-triggered immunity (ETI) is a modified MAMPs-triggered immunity (MTI) response and thus it is a potent defence response (resistance) that facilitates localized cell death referred to as hypersensitive response (HR). Most of the R proteins are intracellular receptor proteins of the nucleotide-binding-leucine-rich-repeat (NB-LRR) type that interact indirectly with the effectors (Newman *et al.,* 2013). The plant defence system can also recognize other plant-derived molecules that induce plant defence reactions. These are known as damage-associated molecular patterns (DAMPs) or microbe-induced molecular patterns (MIMPs) that are triggered due to damaged plant cells. The fungal pathogen *Pyricularia oryzae* for example secretes the endoglucanases (MoCEl12A and MoCEl12B) during infection of rice. These endoglucanases target the hemicellulose of the rice cell wall to produce two specific (disaccharide 31-β-D-Cellobiosyl-glucose and the tetrasaccharide 31-β-D-Cellotriosyl-glucose.

These two oligonucleotides then bind to the immune receptor (OsCERK1) and not the chitin-binding protein (OsCEBiP). This binding act triggers the dimerization of the two immune receptors. These polysaccharide-derived oligosaccharide DAMPs can stimulate rice immune responses during *P. oryzae* infection (Yang *et al.,* 2021).

It has been revealed that *P. oryzae* and many other fungal pathogens secrete an assortment of cell wall degrading enzymes (CWDEs) to target host cell walls for infection. It is unclear whether these CWDEs either target host cell walls for nutrition or destroy them to initiate cell death. It is believed that this process eventually leads to the switch of infection to a necrotrophic stage of this pathogen. Because it is hard to distinguish the exact time course of the biotrophic stage, the early expression of MoCel12A and MoCel12B could help for the acquisition of nutrition or the initiation of cell death (Kubicek *et al.*, 2014).

The identification of new MAMPs is an open avenue for researching potential novel sources of antimicrobial agents. This will provide insight into the molecular and evolutionary mechanisms underlying host-pathogen interactions, and better awareness of the mechanisms by which MAMPs elicit defence responses may have a substantial impact on the improvement of plant health and disease resistance.

2.10 Strategies for management of finger millet blast disease

Management and control of finger millet blast disease are of great concern to both subsistence farmers and researchers worldwide (Mbinda & Masaki, 2021). This is because the *P. oryzae* has an extensive host range; it experiences genetic variation, evolves more virulent pathotypes and has higher chances of host shifts. As a result, proper and swift measures to diagnose and control the devastating effects of the pathogen should be considered (Gladieux *et al.*, 2018; Saleh *et al.*, 2014). Appropriate blast disease management in millets and cereals can be achieved by blending two strategies: prevention of disease development and early detection to contain disease spread. In addition, asymptomatic plants act as a good reservoir of the blast pathogen thus early detection would contain the disease spread to complementary hosts and reduce the inoculum load (Kumar *et al.*, 2021).

Three different approaches are mostly utilized in the control and management of finger millet blast disease namely cultural, biological and chemical control. An integrated approach involving the three methods yields better disease control (Mgonja *et al.*, 2013). However, most of the subsistence farmers growing finger

millet blasts apply low-cost technologies due to their low-income status. These include negligible use of certified and improved seeds, inorganic fertilizers, agrochemicals, improper crop husbandry and low mechanization(Owere *et al.,* 2014). Such agricultural inputs often don't get to the farmer on time probably due to insufficient inputs supply system, weak methods of disseminating information due to insufficient extension services or simply farmers' ignorance (Onyango, 2016).

Efficient chemical control methods such as seed treatment with systemic fungicides or timely foliar spray have displayed positive results. However, these chemicals persist in the soil leading to phytopathogens resistance and at the same time potentially causing harm to plants, animals, humans and the environment at large (Mbinda & Masaki, 2021). The use of biocontrol agents to contain finger millet blast is a more friendly approach compared to chemical control. Efforts to control finger millet blast and improve the growth of the plant using Pseudomonas sp strain MSSRFD41 *in vitro* have revealed positive results. Also, *Bacillus tequilensis* GYLH001 can suppress the growth of *P. oryzae* in the control of rice blast disease (Sekar *et al.,* 2018; Li *et al.,* 2018). Despite this, there is currently no effective bio inoculant formulated and universally approved to adequately control blast disease pathogen. An integrated blast disease management plan would be more feasible for finger millet crop protection against *P. oryzae* (Mbinda & Masaki, 2021).

The unique traits targeted in finger millet breeding include high yield and other elements related to yield. However, these traits are highly controlled by environmental interactions and plant genotypes (Vetriventhan *et al.,* 2020). Thus, conventional breeding methods involving the transfer of sturdy blast resistance within the adopted finger millet germplasm are the explicit objective for most breeding programs. But then, challenges such as linkage drag and erosion, reproductive barriers such as pollen rejection or self-incompatibility and the long time frame required to achieve results compelled researchers to explore newer breeding techniques (Sood *et al.,* 2019). Several genomic resources in both plants and other organisms have been exposed promoting a clear understanding of the physiological; biochemical and overall genetic make-up of the organism in question.

The outcome of this study will thus provide good insight into the basic information about the more superior finger millet genotype in terms of disease tolerance. It is also a good benchmark for studying a large number of finger millet accessions so that those with similar economic traits can be grouped to select parents for a breeding program using the latest genomic tools. This would aid in bridging the research gap between finger millet and other major cereal crops like rice and maize.

CHAPTER THREE

ASSESSMENT OF FARMERS' KNOWLEDGE, PRACTICES, AND SOCIO-ECONOMIC IMPACT OF FINGER MILLET BLAST DISEASE IN SELECTED AGRO-ECOLOGICAL ZONES IN KENYA

3.1 Introduction

Finger millet crop is a significant food security crop with high nutraceutical value and excellent storability (Vetriventhan *et al.,* 2020). However, several factors suppress its potential productivity lowering its average yields to a paltry 500-750 kg/ha against an expectation of 2,500 kg/ha achievable in research conditions (Owere *et al.,* 2014). These impediments can be categorized as socio-economic, biotic, and abiotic factors. Biotic stresses such as blast disease seriously impede finger millet productivity worldwide because of its highly invasive nature and broad host range. The causative agent is a fungus *Pyricularia oryzae* Cavara (*Magnaporthe oryzae* B.C. Couch) (Klaubauf *et al.,* 2014) but there is a likely chance that blast disease is caused by other fungi as well based on results obtained in this study. Adequate farmers' knowledge and attitude toward crop improvement will play a significant role in the achievement of a more sustainable disease management strategy (Mbinda *et al.,* 2021).

Thus, it is necessary to establish farmers' knowledge of plant diseases, their perception of yield damage, methods of control and the potential effectiveness of the available methods (Kansiime *et al.,* 2019). Therefore, this study investigated the farmers' perception of blast disease, their knowledge of yield losses occasioned by the blast, and the type of disease management practices they employ. The outcome of this study will help set up a robust disease management program that would positively impact the income of finger millet farmers in Kenya.

3.2 Materials and methods

3.2.1 Study area and sample collection

The study was carried out in the Machakos, Makueni, Busia, Bungoma and Kisii Counties of Eastern and Western Kenya regions. In Bungoma County, three sub-Counties were sampled namely Kanduyi, Webuye west and Kabuchai. In Kisii County, three sub-Counties were sub-sampled including, Bomachoge chache, Bobasi and Nyaribari chache. In Busia County, three sub-Counties namely Teso south, Nambale and Bunyala. In Makueni the sub-Counties included Kilome, Nzaui, Kibwezi West, Makueni and Mbooni East, while in Machakos County only Mwala sub-County was surveyed. The choice of the two regions (Eastern and Western agro-ecological zones) took cognizance of the diverse farming systems, agricultural potential, diverse livelihood, and marketability access despite having different climatic conditions. The Kenyan Western agro-ecological zone is defined by a humid lower midland climate and high altitude from 900 to 1800 m above sea level or higher. The region is well-enriched with two rainy seasons; long rains occurring from March to May and short rains from October to December. The Eastern agro-ecological zone on the other hand is considered a semi-arid region with limited precipitation where farmers depend on rain-fed agriculture for livelihood. Finger millet is one of the main crops cultivated in this region together with sorghum, green grams and pigeon peas (Manzi and Gweyi-Onyango, 2021).

The purposive sampling technique (Tongco, 2007) was employed based on the high concentration of finger millet production to assess the impact of finger millet production in the areas surveyed. The inclusion criteria were; (a) respondents must be finger millet farmers, (b) they must be of age 18 years and above, and (c) the study was conducted in low and middle-income settings. The exclusion criteria were; (a) ability to speak English or Swahili and (b) non-finger millet farmers etc (Bujang *et al.,* 2017).

The questionnaire (Appendix I) administered in this survey had 49 items with a five-point Likert scale for most items. The Cronbach's alpha coefficient in the null

hypothesis and alternative hypothesis was assumed to be equal to 0.0 and 0.7 respectively. Based on the alpha value fixed at 0.05, the minimum sample size requirement was 14 or 17 to achieve the power of 80% or 90% respectively (Bujang *et al.,* 2018). During the survey period, 325 finger millet farmers were collectively interrogated; 46 from Bungoma, 10 from Machakos, 26 from Makueni, 150 from Busia and 93 from Kisii counties. A systematic random sampling technique was employed to identify respondent households; aiming at 3^{rd}- 4^{th} households in the listed areas.

3.2.2 Questionnaire dissemination and interviews

Data collection was done in June 2019 using a semi-structured questionnaire distributed by trained personnel. A pilot study using the questionnaire was previously conducted in Busia County field station with 150 farmers. The questionnaire was transferred to the Open Data Kit (ODK) program that could transmit data to laptops, smartphones, and tablets. The respondents were interviewed in their local dialects on a face-to-face mutual agreement after seeking their informed consent. The information obtained was managed as per the General Data Protection Regulation (GDPR) (EU, 2016). The survey targeted respondents' biodata, major cropping activities, and patterns, acreage, choice of finger millet variety, yield, criteria for seed selection and storage, utilization and marketing, production constraints, farmers' knowledge and perceptions regarding finger millet blast disease occurrence, causes, transmission, and management principle, and source of technical expertise.

3.3 Data analysis

The data obtained was secured and assembled on a server in real-time and quality checks were concurrently performed to maintain data integrity. At the end of the survey, data were downloaded and exported for further analysis to the SPSS statistical software version 25. Measures of variability were done by calculating percentage frequencies, means, and standard errors. Comparative statistical tools such as t-tests and chi-square were employed to assess the disparities present among the farm characteristics, socio-demographics and the farmers' understanding, their

attitudes and preferences of the blast disease management practices. Quantitative variables having normal distribution and homogenous variances were evaluated using analysis of variance (ANOVA). A factor analysis model was further utilized to determine the farmers' knowledge and factors linked to finger millet productivity to establish their impact on finger millet yield. The means were separated using Tukey's Honest Significant Difference (HSD) at a significance level of $p \leq 0.05$.

3.4 Results

3.4.1 Household socio-economic status

Nearly all the finger millet growing households surveyed were headed by adults (90%) where female heads were highest in Kisii (77%), followed by Busia (65%), Bungoma (61.5%) and both Machakos and Makueni 50%. The level of education was significantly low among the household heads of which only 25% had gone beyond the secondary level in Kisii, 13% in Busia, 16% in Makueni, and 10% in Machakos compared with 37% in Bungoma. The main source of livelihood among the respondents from Kisii (93%), Busia (70%), Bungoma (72%), Makueni (85%) and Machakos (80%) was food crop farming (Table 3.1).

Table 3.1: Demographic characteristics of sampled finger millet-producing household heads in Busia, Kisii, Bungoma, Machakos and Makueni Counties, Kenya

Family head attributes	Variable	Respondents (%)					Chi square	degrees of freedom
		Busia	Bungoma	Kisii	Machakos	Makueni		
Age	≥ 35 years	25	19.6	35.5	10	23	7.36	4
Gender	Female	65	61.5	77.4	50	50	11.2*	4
Education	Secondary and above	13	37	25	10	16	26.7*	5
Livelihood	Farming	70	72	93	80	85	17.8*	4

Key *Statistically significant correlation at $p \geq 0.05$

3.4.2 Farming attributes

Maize was the most treasured crop produced by 60.1% of the interviewees followed by finger millet at 46.1% in all the counties (Table 3.2). In Bungoma and Busia counties, other crops sown to augment the two were sweet potatoes and beans while in Kisii county they were tea, bananas and beans, in Machakos and Makueni were green grams, cowpeas, beans and fruit trees such as mangoes and oranges. All in all, among the respondents, cultivation of finger millet had been done for a longer time in Kisii (16 years), Busia (13 years) and Bungoma (12 years) counties compared to, Makueni (8 years) and Machakos (3.5 years) among the households surveyed. Overall, finger millet generated more than 25% of the household proceeds for 63.5% of the respondents and above 50% for only 32.1% of the respondents in all the surveyed counties.

3.4.3 Seed sources and finger millet varieties cultivated

A majority of the respondents were unaware of the names of the different finger millet varieties they cultivated but were able to distinguish between the local and hybrid varieties. This was based on the nature of the finger millet head (open or closed) and the colour of the spikes and the grain. Time to maturity and taste were also other attributes considered. There were a few respondents that mixed both the local and hybrid varieties. However, in Bungoma and Kisii, 59% of the respondents preferred cultivating hybrid varieties while in Busia it was 48%, Machakos at 60% while Makueni had the least at 20% (Figure 3.1). Early maturity period and good taste, were the key reasons cited (62%) by the respondents for cultivating local varieties. Those preferring hybrid varieties indicated high yield (63%), early maturity (62%), and good for milling/ grinding (58%) as their reason for preference (Figure 3.1). Pest and disease resistance was not a priority factor in selecting the variety preferred by the respondents (Figure 3.1).

Table 3.2: Farm characteristics of sampled households in Kisii, Busia, Makueni, Machakos and Bungoma Counties, Kenya

Farm characteristics	Busia	Bungoma	Kisii	Makueni	Machakos	Overall
Land owned (Ha)	0.43±0.02	0.45±0.042	0.15±0.04	0.68±0.035	0.75±0.14	0.45±0.3
Land tenure Owner with title	75±0	70±2.8	83±2.8	74±2.8	71±1.4	76.1
Experience with finger millet farming (years)	16*±5.6	6±2.8	13±2.8	6.5*±2.1	3.5*±0.71	10.5
Priority crop:						
Maize	68±0	65±1.4	62±1.4	50±0	48±2.8	60.1
Finger millet	45±2.1	40± 0	55±0	43±1.4	40± 1.4	46.1
>25% income from finger millet	58±2.8	70±1.4	77±0	55±0	50± 2.8	63.5
>50% income from finger millet	35±1.4	26±1.4	22±1.4	35±1.4	35±0	32.1

± standard deviation; * =means statistically sigficant at $p \leq 0.05$

Seed sources were mainly carried over from the previous seasons with 45% for the local variety and 33% for the hybrid variety (Figure 3.2). KALRO extension officers had previously provided the hybrid varieties. Other seed sources included borrowing from neighbours, buying from the market or agricultural research institutions or agro-dealers which was rather rare. Farmers in Busia county were the most likely ones to utilize the hybrid variety because of the proximity to Kenya Agricultural Livestock Research Organization (KALRO), Alupe compared to finger millet farmers from other counties. In addition, a pilot finger millet research programme was being carried out in Machakos thus the farmers there were also growing hybrid finger millet varieties (Figure 3.2).

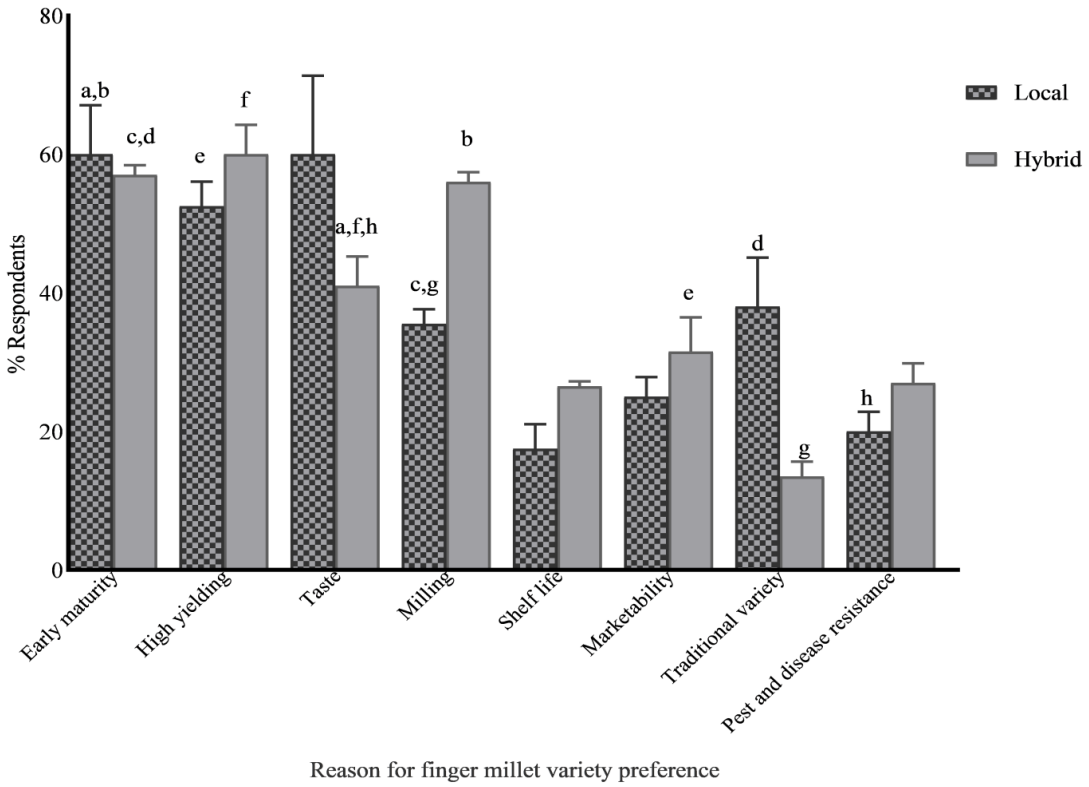

Figure 3.1: Reasons for variety preference among finger millet farmers in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya.

Key: Bars having similar alphabetical letters have a statistically significant difference at $p \leq 0.05$.

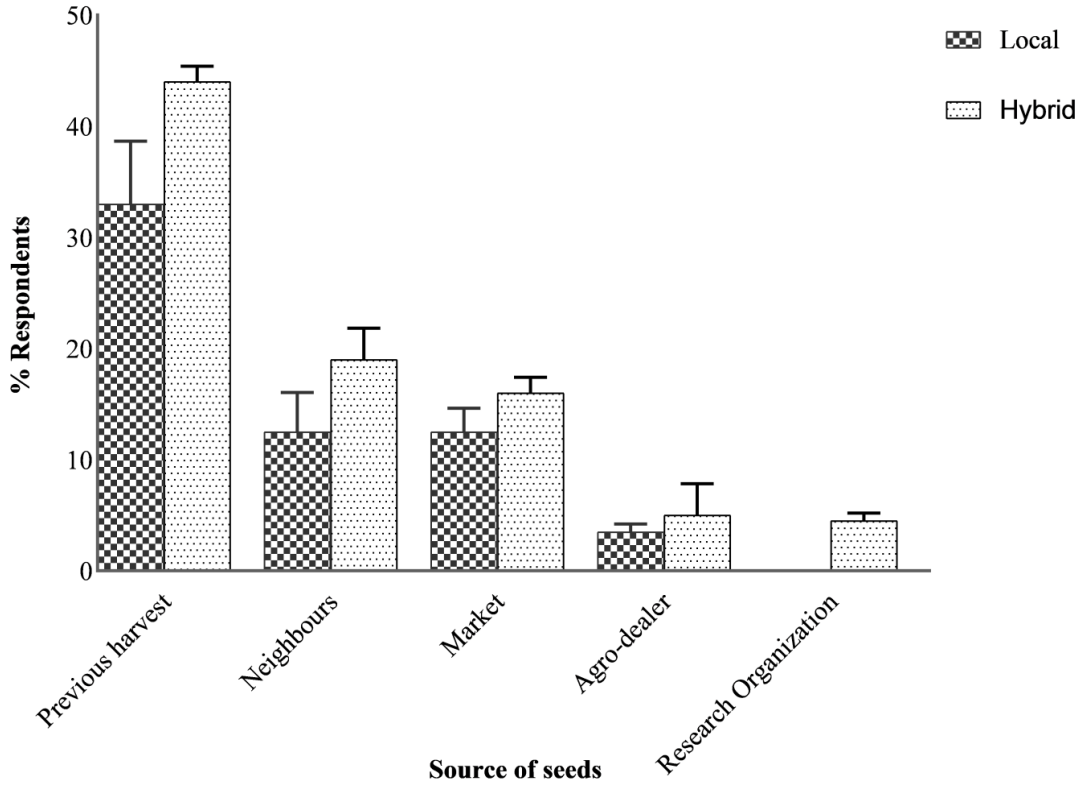

Figure 3.2: Source of finger millet seed sown during the survey in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya.

3.4.4 Finger millet production and land acreage

On average, finger millet production according to the respondents occurred at 0.75 ha in the survey counties where 0.04 ha was the minimum while 1.21 ha was the maximum (Figure 3.3). There was no significant difference in the yield obtained from the five counties surveyed in the year 2018. Bungoma county produced 826.2 kg/ha, Busia 637 kg/ha, Kisii 503.9 kg/ha, Makueni 450 kg/ha while Machakos 325 kg/ha (Figure 3.3). Finger millet returns also varied significantly with higher prices obtained in Kisii at USD 1.9 per kg compared to USD 1.5 per kg in Bungoma, Busia, Machakos and Makueni counties (Figure 3.3). In all the counties, seventy per cent of the farmers were the sole determinants of the selling price of the grain. About 83% of the respondents sold their crop at their local market with Kisii county having the highest (94%) and Machakos the least at 61% (Figure 3.3). The majority of the

farmers from the counties surveyed were able to sell all of their products except for 28% of the respondents from Bungoma County (Figure 3.3).

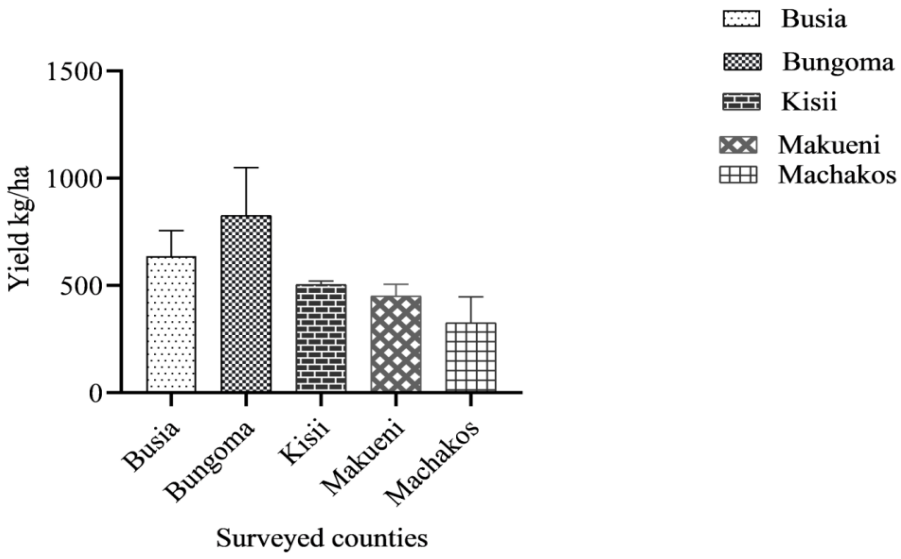

Figure 3.3: Finger millet yield kg/ha obtained in 2018 of the preferred finger millet variety in the surveyed Counties.

3.4.5 Finger millet production constraint

The finger millet farmers interviewed identified several constraints to finger millet production where blast disease was perceived as the greatest constraint by 90% of the respondents. Other factors included high labour requirements, especially during land preparation and weeding (60%), bird damage (43.3%), and lack of extension services and or training (16.8%). There was a significant difference at $p \leq 0.05$ in the number of respondents who agreed that laborious activities, birds and heavy rains were a serious threat to finger millet cultivation in Makueni and Busia, Machakos and Busia, and Makueni and Kisii respectively (Figure 3.4).

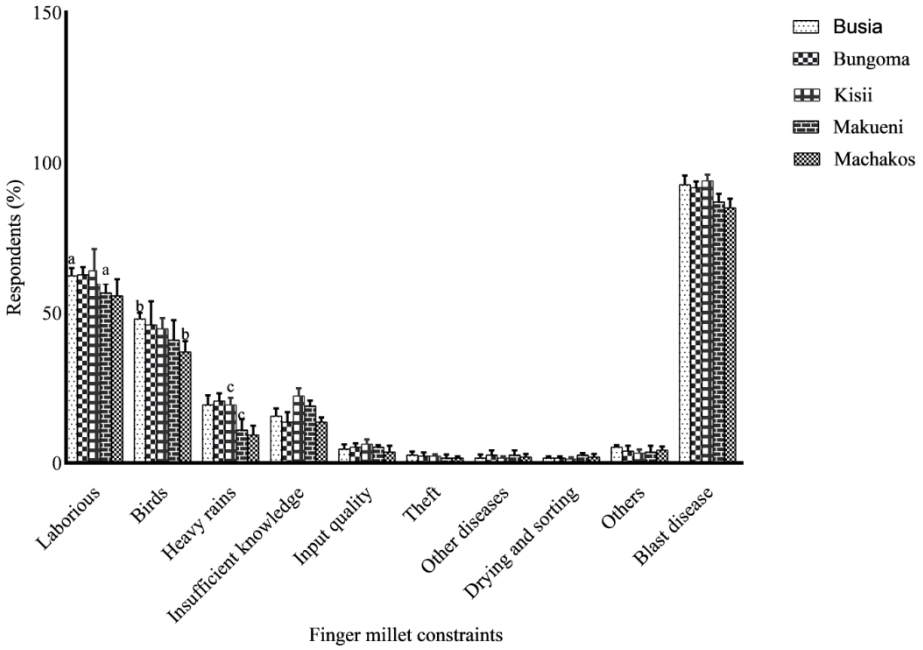

Figure 3.4: Constraints to finger millet production in Kisii, Busia, Bungoma, Machakos and Makueni Counties in Kenya.

Key: Bars having similar alphabetical letters have a statistically significant difference at $p \leq 0.05$.

3.4.6 Occurrence and perception of finger millet blast

The survey results established that 100% of the farmers had finger millet blast disease in their farms but with varying disease intensity. The infection symptoms were described as white to grey-green lesions or spots on leaves, initially showing dark green borders that later transform into elliptical or spindle-shape with whitish to grey centres with reddish-brown/necrotic borders. This description is typical of that of finger millet blast disease. Only 51% of the farmers were aware of the blast disease and knew its name while others could recognize it once the symptoms were described (Table 3.3). The farmers were conversant with the disease on their crops at different developmental stages. In Kisii and Busia counties, 32% and 45% respectively could diagnose the disease at the vegetative stage (leaf blast) whereas, in Bungoma, Makueni and Machakos, 93%, 63% and 95% respectively of the farmers

recognized the disease at the maturity stage (spike/head blast) as displayed in Table 3.3.

Table 3.3: Finger millet blast disease awareness and occurrence in Farms in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya

Finger millet blast	Busia (%)	Bungoma (%)	Kisii (%)	Makueni (%)	Machakos (%)	Overall	Chi square
>1 Finger millet crop per year	72	30	76	25	8	48.7	63.5***
Blast awareness	65	26	63	25	15	45.3	15.2***
Blast occurrence	100	100	100	100	100	100	ns
Blast at vegetative stage	35	18	32	8	2	19	9.1***
Blast at reproductive stage	90	68	93	55	45	70.2	ns

*** Significant means at $p \leq 0.001$, ns= means not significant at $p \leq 0.001$

3.4.7 Seasonality and symptoms of finger millet blast disease

Most of the farmers describe the blast symptoms as damage to the spike/head (91%), neck (61.5%) and leaves (56.1%) of the finger millet crop when the crop is affected. They also noted that the disease occurred all year round posing a great challenge to its control and management as mentioned by 46% of farmers in Bungoma, 65% in Busia, 42% in Kisii and 38% in Makueni and Machakos counties (Figure 3.5). A statistically significant difference at $p < 0.05$ in the type of plant part affected by the blast was obtained according to the farmers' responses in all the counties surveyed (Figure 3.5). In terms of disease seasonality, about 13% of the respondents from all counties revealed that it was a seasonal disease, whereas 10% of the farmers mentioned that the disease was sporadically occurring in pockets of some areas and only during specific periods (Figure 3.4). Another 60% of the respondents were able to expose the epidemic nature of the disease by mentioning incidences of sudden serious outbreaks (Figure 3.5).

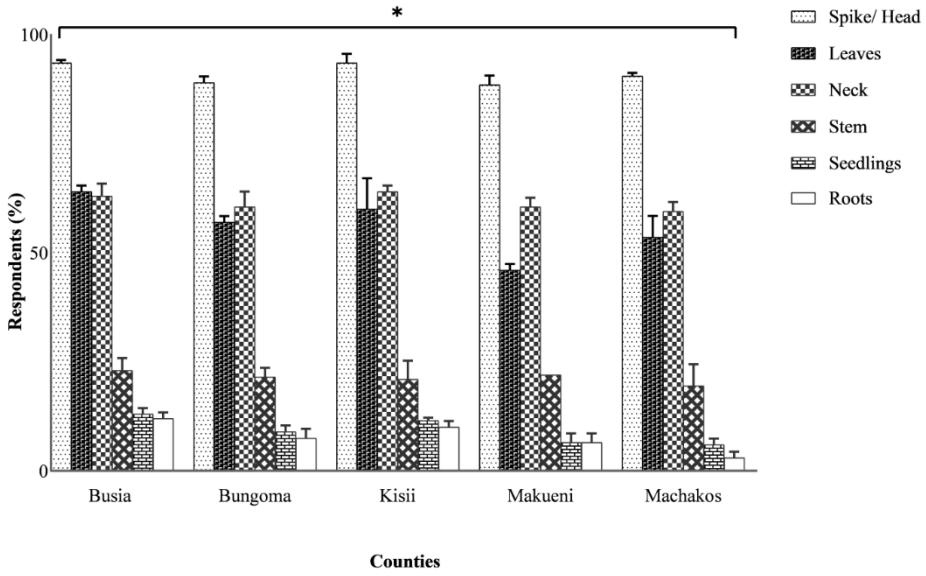

Figure 3.5: Farmers' response to the plant parts affected by finger millet blast disease in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya.

Key: * means that there was significance difference at $p \leq 0.05$ across the counties.

Disease seasonality was further broken down to determine its intensity either as low, moderate or high (Figure 3.6). There was no significant difference in the number of respondents who identified the disease as either seasonal, sporadic or persistent with intensity factor ratings being low, moderate or high (Figure 3.6). Busia county had the highest number of respondents (8%) while Machakos county had the least 3% who agreed that the disease was endemic, seasonal with low intensity (Figure 3.6).

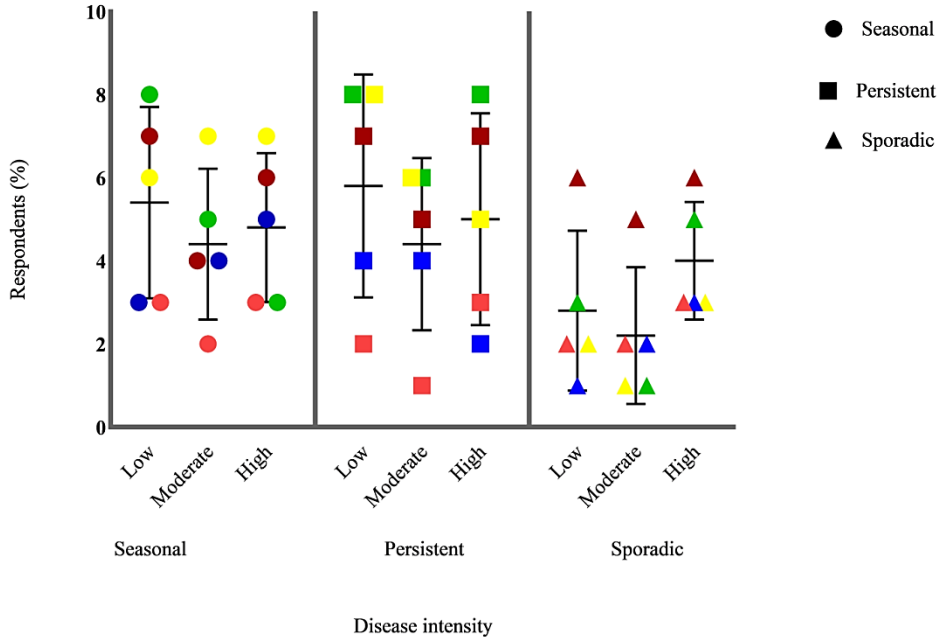

Figure 3.6: Finger millet blast disease seasonality (endemic) in Kisii, Busia, Bungoma, Makueni and Machakos counties.

Key: The red colour is for Machakos, Blue for Makueni, Green for Busia, Maroon for Bungoma and Yellow for Kisii.

Most of the respondents agreed that the disease was a seasonal epidemic rather than endemic with Busia county having the highest number of respondents at 23 % while Makueni had the least at 11% (Figure 3.7). Others explained that the disease occurred routinely in moderate proportions but was confined to a few specific areas where again Busia (10%) had the highest number of respondents while Makueni had the least at 1.3% (Figure 3.7). Overall, 51% of the farmers observed that the disease was more severe during the dry season while 32% of them agreed that high blast severity was experienced during the wet season. Moreover, 71% of respondents from Kisii pointed out that disease severity solely relied on the season (chi-square = 3.9**) especially during the second season or when higher rainfall was experienced (Figure 3.7). Several farmers in Makueni (68%), Bungoma (65%), and Machakos (59%) cultivated the crop only once a year while those from Kisii (76%) and Busia (75%) cultivated finger millet twice a year (Figure 3.7).

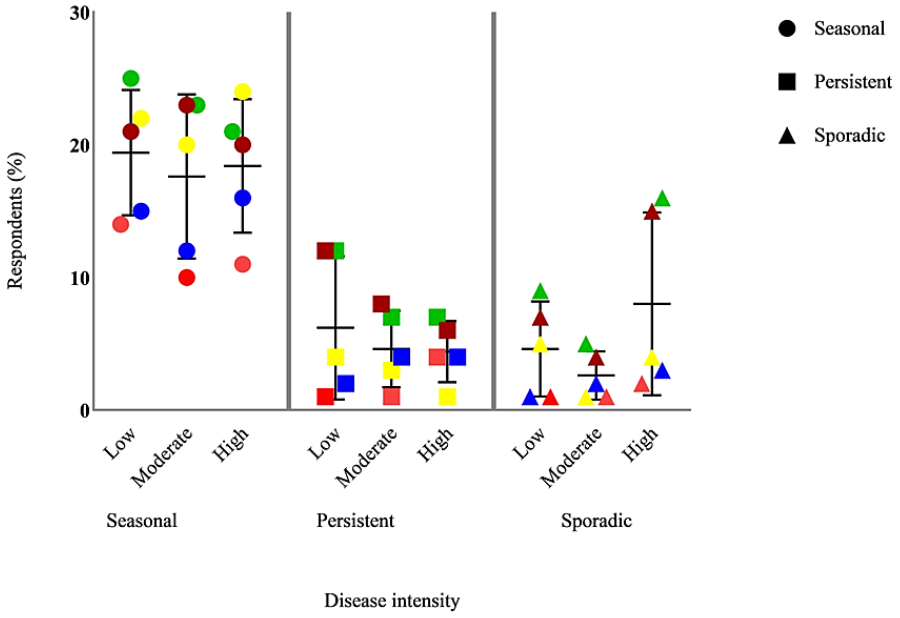

Figure 3.7: Finger millet blast disease seasonality (epidemic) in Kisii, Busia, Bungoma, Makueni and Machakos counties.

Key: The red color is for Machakos, Blue for Makueni, Green for Busia, Maroon for Bungoma and Yellow for Kisii.

3.4.8 Methods of blast disease spread

The perceived methods of finger millet blast disease spread varied between the surveyed counties. In Busia, Bungoma and Makueni counties, about 67%, 65% and 38% of the farmers ascribed the disease spread to seed or variety is grown (Figure 3.8). In Kisii County, only 10% of respondents correlated the disease spread with the crop variety or seed; a majority of them (35%) associated the disease with weather patterns such as heavy rain, wind and erratic weather as the main elevators of finger millet blast disease. In Machakos county, 45% of the respondents did not have an idea of the possible cause of finger millet blast disease (Figure 3.8).

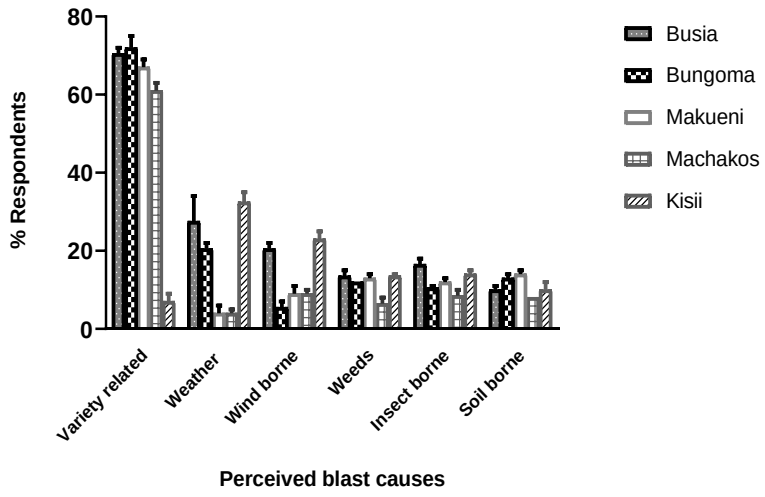

Figure 3.8: Farmers' perceptions of the factors associated with finger millet blast disease in Bungoma and Kisii counties, Kenya.

Key: Bars having similar kinds of asterix are significantly different ($p \leq 0.05$).

3.4.9 Constraints to finger millet blast disease

All the respondents in both counties did not have a properly laid out plan on how to control finger millet blast disease upon symptom emergence in the field (Table 3.4). The perceived method of control was hand weeding earlier identified as a very laborious task. Other management practices of blast in the field included uprooting diseased plants, ash application, and use of herbicides utilized by 60% of the farmers. The farmers indicated that they only used the management practice once within the growing season. Others (65%) reported leaving the diseased strover on the farm to wither and die whereas 30% of them fed the diseased strovers to livestock. Burning of contaminated strovers was done by 17% of farmers in Bungoma, 25% in Busia, 34% in Makueni and 25% in Machakos compared to only 1% of the respondents in Kisii.

Table 3.4: Perceived effectiveness of finger millet blast management practices among farmers in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya

Perceived effectiveness	Number of respondents (%)				
	No action	Hand weeding	Uprooting infected plants	Ash Dusting	Others
0-20	25	39	16	11	0
21-40	2	21	9	5	0
41-60	0	25	2	5	5
61-80	0	11	0	0	3
81-100	0	3	0	0	0

3.4.10 Yield losses as a result of finger millet blast disease

The presence of finger millet blast disease portends a serious decline in yield and grain quality. A yield loss of 80 kg/ha was disclosed at the outbreak of finger millet blast disease in the farms. There was a yield decline ranging between 623.77 kg/ha and 441.27 kg/ha at the start and end of the disease outbreak respectively. The respondents further revealed that infected grains varied in color (71%) and taste (45%) compared with the non-infected grain (Figure 3.9). Twenty two percent of the respondents who contradicted the change in the quality of the grain due to blast infection but agreed with the decline in yield (Figure 3.9).

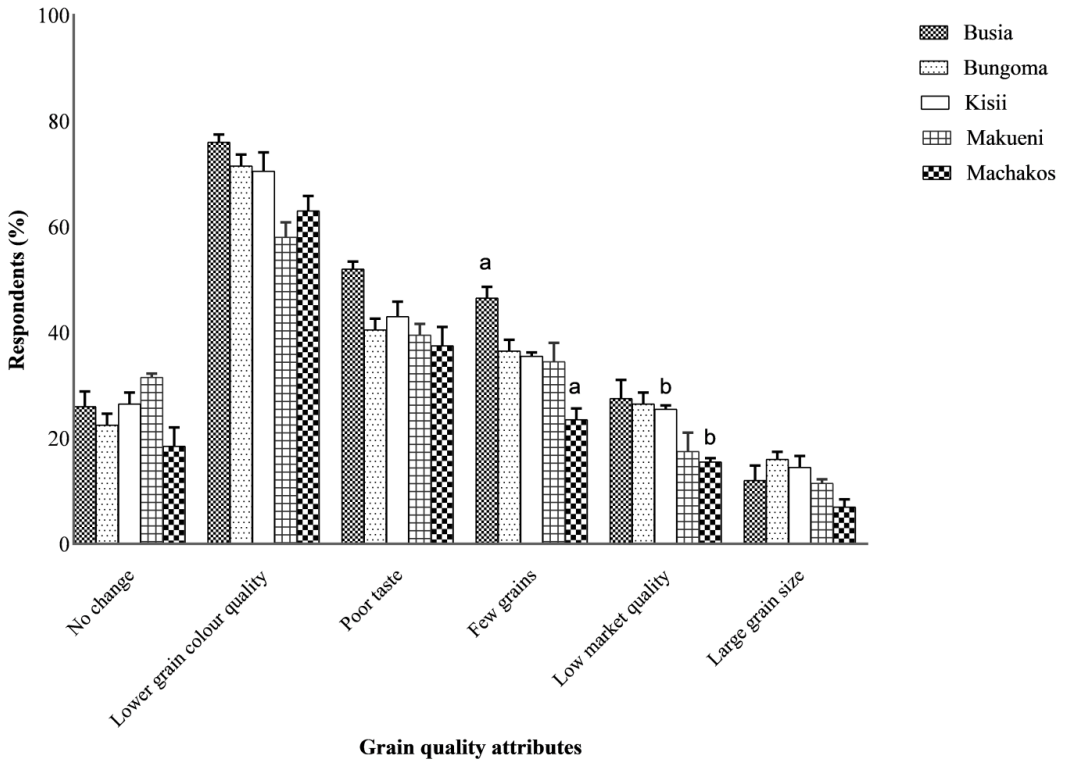

Figure 3.9: Effect of finger millet blast on grain quality in Busia, Bungoma, Kisii, Makueni and Machakos Counties, Kenya.

Key: Bars with similar letters represent significantly different means at $p \leq 0.05$.

3.5 Discussion

Global food security especially in developing countries is under constant threat due to damage to crops caused by fungal diseases. Safeguarding important crops against plant diseases in small-scale agriculture is important to maintain a healthy ecosystem and at the same time reduce rural poverty. Previous studies have revealed that blast disease is the greatest constraint to finger millet disease worldwide (Ramakrishnan *et al.*, 2016, Gupta *et al.*, 2017, Manyasa *et al.*, 2019). This study evaluated farmers' knowledge and perceptions of finger millet blast disease and its management practices in Western Kenya. It revealed the highly diverse nature and structure of small-scale farm households in rural areas including, varying numbers of family members, heads of family, land area, different types of livestock, cropping patterns and farm management approaches. Deciphering the diversity and dynamics of rural

households is essential because global and regional challenges like population growth, climate change and food demand are either directly or indirectly associated with plant pests and diseases (van Wijk *et al.*, 2020).

This study revealed that the average size of the family unit in the surveyed counties ranged from 6.9 (Kisii) to 4.8 (Machakos) persons which are greater than Kenya's average household size of 3.9 persons (KNBS, 2019). Persons over 18 years of age are considered adults and thus have the express decision-making power to select the type and quantity of crop variety, size of land to cultivate and the choice of farm management methods. These factors have an influence therefore on finger millet production (Yaméogo *et al.*, 2018).

From this study, larger family sizes played a key role in influencing the type of farming system chosen especially those requiring high labour holding promise to sustainable finger millet production. For instance, households having at least four members and above were more efficient in finger millet farming as opposed to those with fewer numbers who had to contract extra labour or neglected cultivating the crop together. The study by Mrema *et al.*, (2017) demonstrated that sorghum cultivation in Tanzania was often carried out by large family units having 7.1 individuals. The cost of outsourcing labour is greatly minimized for families with larger family members. It is imperative to note that both sorghum and finger millet crops are laborious crops and this explains why a parallel can be drawn between the two.

Many of the households surveyed were headed by women compared to men. The role of women in agriculture is very significant and it accounts for 42 to 65% of agricultural labour needs (Diiro *et al.*, 2018). This study affirmed that the decision to plant finger millet in the surveyed farms was more or less influenced by women rather than men. The surveyed counties revealed a low level of education among the household heads, implying that the majority of the finger millet farmers had a very basic understanding of primary farming techniques. This would have an impact on the kind of management practices the farmer would employ to mitigate finger millet

blast. A study in Ethiopia on Enset (*Ensete ventricosum* (Welw.) Cheesman)- an important root crop among the Ethiopians, demonstrated that proper management of *Xanthomonas* wilt was greatly influenced by the farmers' level of education (Yemataw *et al.,* 2017). In addition, the indiscriminate cultivation of *enset* was minimized with increased levels of education and community-based participatory training. Agricultural extension services and researchers play a crucial role as agents of change on the nature and importance of new finger millet technology and agricultural inputs yet their presence in these communities is hardly felt. Information on land sizes owned by the respondents was not forthcoming due to its sensitive nature. Literature search revealed that a typical household in the surveyed regions was is 1.83 ha (Berazneva *et al.,* 2018).

It was noticeable during the survey that some finger millet farmers practised biodiversity cultivation. This technique has proven useful in the control of blast disease through reduced disease occurrence and variability, thus sustaining high yields (Han *et al.,* 2016). However, great care is needed when choosing the kinds of crops for intercropping and biodiversity cultivation to avoid including alternative hosts. This is because blast pathogen infects more than 50 species of cultivated and wild monocots. In this study, both mono-cropping and intercropping farming systems were carried out.

There was a serious decline in the production and productivity of finger millet in the surveyed farms due to high labour requirements and a negative reputation for the crop. Low mechanization, inadequate technological level coupled with minimal use of improved seeds, fertilizers and agrochemicals also played a role in production and productivity decline. The study findings are similar to those of Handschuch and Wollni, (2016) and Onyango, (2016) who also revealed low-level mechanization and technology among finger millet farmers in Kenya compared to other competitive crops like maize. Such crops have elaborate research, technology, extension services and a strong research-extension linkage thus enhancing the proper dissemination of information to the farmers.

The respondents in the surveyed farms were aware of blast disease and that it was the most devastating disease to finger millet production. They believed that the disease could completely destroy a crop or harvest and that no agrochemicals or disease management techniques could be used to control it.This suggests that the farmers' perspective of blast-infected finger millet plants does not compare to the scientific diagnosis. The scientific model of the disease is very distant from the mindset of the smallholder finger millet farmers suggesting a weak link between the farmer and agricultural extension officers. The observation was evidenced by poor cultivation and disease management approaches the farmers employed. Weed management is one of the most laborious activities in finger millet farming.

Households with more family members have an advantage since they can help (Lenne *et al.*, 2007). The weeds themselves are also alternative hosts of the fungus thus timely weeding limits the spread of propagules. There is a need to innovate alternative weeding methods to replace the hand weeding techniques that are the norm in finger millet cultivation to reduce labour cost implications and control of blast as part of an integrated blast control and management plan.

As a result of favourable weather, various dispersal methods and proximity to several alternative hosts, that allow pathogen inoculum to remain viable for many seasons, there was a substantial high occurrence and severe finger millet blast in the farms surveyed in Kenya. This disease greatly ravages the crop causing shrivelled, poorly filled or blasted grains and in some cases failed grain formation(Takan *et al.*, 2012). The majority of the farmers in this study could easily explain the disease progression, implying that either they had monitored it for a long period or they were enlightened about it. However, they failed to correlate finger millet blast disease occurrence with the different finger millet varieties they cultivated. The landraces and other varieties the farmers cultivated were susceptible to blast and the farmers preserved them for use from one season to another.

The disease management practices which farmers employed included crop rotation, good agronomic husbandry and in some cases use of agrochemicals. Unfortunately, in the study areas utilization of fungicides was untenable due to genetic variability of the pathogen, effects on the environment and high-cost implications for the smallholder farmers (Mbinda & Masaki, 2021). The farmers were also not aware of the existence of resistant varieties even though, such varieties face the challenge of resistance breakdown with time (Manyasa *et al.,* 2019). A more integrated approach that incorporates all possible blast control methods will be more effective rather than a single control measure.

Many different types of finger millet varieties were cultivated in the study areas according to the farmers' preferred traits including adaptability to abiotic and biotic stresses, low input requirements, yield, taste and good cooking quality, high market value and seed availability. Low yields were obtained in this study compared to the average yields (2500 kg/ha) achieved under research conditions (Owere *et al.,* 2014). There was not much difference in the yields produced by local and hybrid varieties in the study areas which could be attributed to the low technological level characteristic of the smallholders in traditional cereal production systems. Studies have revealed that yields from on-station research and field trials can be increased substantially when modern agronomic practices and varieties are used (Handschuch & Wollni, 2016; Gimode *et al.,* 2016). Improvement of finger millet varieties that are resistant to blast, better adapted to perform in harsh environments, and with the traits preferred by farmers will be more easily adopted as it is not currently the case in many parts of Africa (Nkongolo *et al.,* 2009). A top-down approach to the improvement of cultivars and seed production in many sub-Saharan African countries produced varieties that either did not meet farmers' preferences or are not well adapted to the local climatic or socio-economic conditions. Consequently, only a few of these improved varieties have been adopted by farmers so there is no major improvement in finger millet yields and blast disease management in practice.

This study revealed that farmers sold their finger millet grains in the local market thus earning lower prices compared to when the grains are sold in supermarkets and or major towns. Minimal marketing strategies for finger millet were also noted due to limited use among consumers compared to other major crops like maize, wheat and rice. These major cereals are marketed through government agents that are charged with the procurement, management, and distribution of these crops. Further, the governmental programs include regional disease management strategies, besides the boundaries of individual farms compelling all actors in these crop value chains to act synergistically and assist one another. This study revealed an unstructured nature for effective blast disease management and the attraction of large-scale investment. A well-coordinated blast management approach would work efficiently if the smallholder farmers (who form the bulk of finger millet producers) are targeted to build neighbourhood–based partnerships. A good start would be to implement a blast disease reporting and management system that integrates several finger millet farmers. This would demand formalized structures to assure consistency in data collection and reporting, assessing management conduct and imposing sanctions. In addition, advancing the preference for finger millet consumption would create more opportunities for improving crop productivity and marketability.

The development of improved varieties is usually a concerted effort by government agencies, but this is lacking for finger millet landraces and local varieties where seeds are freely exchanged between farmers or sold in the local markets. In this study, 59% of those interviewed revealed that the hybrid seeds which they planted were either sourced from their neighbors or chosen from their own farms' previous season. At present, finger millet sources are from neighbours, local markets, agricultural input stores and research institutions. The information on public sector engagement in finger millet seed production is limited. On the other hand, the crops demand threshold is too low to boost the interest of the private sector to produce improved seeds that are resistant to finger millet blast varieties (Lenne *et al.*, 2007). Deliberate attempts should therefore be made to boost interactions among researchers, agro-dealers and producers. Another approach would be a farm seed production system to increase access to improved seeds without compromising the

seed quality. This would meet local demand on time and at a fair price. However, finger millet is a self-pollinating plant and this attribute greatly impedes the chances of cross-pollination (Sharma *et al.*, 2018).

CHAPTER FOUR

OCCURRENCE, DISTRIBUTION AND SEVERITY OF FINGER MILLET BLAST

4.1 Introduction

Finger millet (*Eleusine coracana*) is emerging as one of the potential cereal crops critical for sustainable food and nutritional security, climate-resilient farming and agricultural diversification. It plays an essential role in the dietary, cultural habits, and economy of subsistence farmers in Asia and East Africa. It is cultivated in regions with minimal rainfall and medium-sized agricultural zones in developing economies (Dida *et al.,* 2021). Taxonomically, this tetraploid monocotyledonous, self-pollinating plant is found in the Poaceae family, Chloridoideae subfamily of Tribe Eragrostideae, and genus *Eleusine* (Schoch *et al.,* 2020). Worldwide, it is placed fourth in significance to other millets after sorghum, pearl millet, and foxtail millet. This crop can resist harsh weather conditions compared to most cereal crops, as such it favourably adapts to a wide range of environments (Gupta *et al.,* 2017).

The nutritive value of finger millet seeds cannot be overemphasized. The minuscule seed has a protein content ranging from 7-14% that is peculiarly rich in amino acids methionine and tryptophan, iron, and calcium. The grain is gluten-free and has a low glycemic index with plenty of phytochemicals and exquisite malting characteristics (Wambi *et al.,* 2021). Moreover, millets utilize local agricultural biodiversity to give nutritious and sustainable diets. Cultivation of millets also minimizes problems associated with monocultures such as degradation, and low water usage which saves on groundwater sources and minimizes the overuse of chemical inputs that may in turn lead to soil and water pollution and reduces susceptibility to pests and disease (ICRISAT, 2021b).

Despite these positive attributes, this cereal crop has been underutilized in crop improvement research (ICRISAT Explore it, 2021). Oftentimes, it has been considered an orphan crop because of limited global efforts to improve its quality or

productivity and an inadequate focus on the global value chain. Some common attributes of orphan crops include minimal or lack of research and development, limited agricultural extension services, weak and underdeveloped value chains, poor perception of the crop and lack of knowledge concerning its nutritive value (ICRISAT, 2021b).

Although finger millet is highly resilient to erratic climatic conditions, cumulative evidence indicates that its growth and productivity are slowly gaining susceptibility to some climate-generated biotic and abiotic stresses. Such stresses include but are not limited to pests and diseases, solar radiation, drought, temperature rises, and increased salinity (Wambi *et al.,* 2021). These stress collectively negatively impact the production, productivity, and geographical distribution of finger millet thus promoting serious economic losses and threatening global food security (Gupta *et al.,* 2017). Blast disease caused by *Pyricularia oryzae* (anamorph *Magnaporthe oryzae*) is an arduous plague that has caused yield losses of up to 100% in endemic seasons (Mbinda & Masaki, 2021). Other than finger millet, *P. oryzae* exploits a wide host range causing infections among other members of the Poaceae family such as rice, wheat, barley, and their wild relatives. Despite this, pathogenicity studies have shown that *P. oryzae* strains are host specific although cross specificity has been identified on a limited range of hosts (Klaubauf *et al.,* 2014; Longya *et al.,* 2020).

In East Africa, the disease occurs throughout the year due to favourable weather conditions and the presence of alternative host plants. This disease attacks finger millet at all growth stages although neck and finger infections are the most pronounced both of which cause a reduction in biomass and total grain yield loss of up to 100% per year (Dida *et al.,* 2021). Initial finger millet blast symptoms are characterized by small grey or brownish dots on the leaves that later coalesce to form diamond-shaped lesions with a white or greyish center 2-3 days post-infection. Blast disease incidences have intensified due to the ability of *P. oryzae* to cross infect other graminaceous species, climate change, and an open free trade market (Langner *et al.,* 2018). In addition, pathogenic genetic groups have been sighted among finger millet

blast populations posing a serious problem in the management of the pathogen complex (Dida *et al.,* 2021).

At present, finger millet blast disease management involves an integrated approach including the planting of resistant cultivars, chemical control, crop residue destruction as well as biological control, but with limited success (Imam *et al.,* 2015). This is a consequence of pathogen resistance breakdown a while later brought about by rapid and frequent genetic variation of the pathogen to escape plant immunity. Also, the use of chemical control is too costly for the already resource-poor farmers (Yadav *et al.,* 2019). Insights into the structure of the finger millet blast fungus population and genetic diversity are essential for the development of durable resistant cultivars that are regionally acclimatized. This will significantly contribute to the control and management of the disease (Gladieux, *et al.,* 2018).

4.2 Materials and methods

4.2.1 Study sites

Samples were obtained from some of the main finger millet growing areas. These sites included Machakos and Makueni representing the Eastern agro-ecological zone and Busia, Bungoma, and Kisii representing the Western agro-ecological zones. Machakos County spans from latitudes $0^{o}45'$south to $1^{o}31'$South and longitudes $36^{o}45'$East to $37^{o}45'$ East (Figure 4.1). Its altitude ranges between 1000-1600 meters above sea level. Makueni County is found between latitude $1^{o}35'$ and 32^{o} 00 ′ South and Longitude $37^{o}10'$ and 38^{o} 30′ East (Figure 4.1). Both are found in the former Eastern Province of Kenya. Kisii County spans between latitude 0030 ′ and 100 South and longitude 340 38 ′and 350 East. It is in the former Nyanza Province in southwestern Kenya. Busia County lies between coordinates 00^{o} 27 ′48.0 ″N, 34^{o}06 ′19.0 ″ (Figure 4.1). Its average elevation is 1227 meters above sea level. Bungoma County is found between latitude 00 28′ and latitude 10 30′ North of the Equator and longitude 340 20′ East and 350 15′ East of the Greenwich Meridian (Figure 4.1). The altitude ranges from 1200m to over 4,321m (Mt. Elgon) above sea level. Both Busia and Bungoma are found in the former Western province of Kenya.

The western ecological zone experience two rainy seasons, long rains from March to May and short rains from October to December. Thus, it is characterized by a humid lower midland climate with altitudes spanning 900 to 1800m above sea level. These conditions are quite suitable for blast fungi to thrive. The Eastern zone on the other hand is semi-arid and thus receives reduced amounts of rainfall annually and is less humid (Odeph *et al.*, 2020).

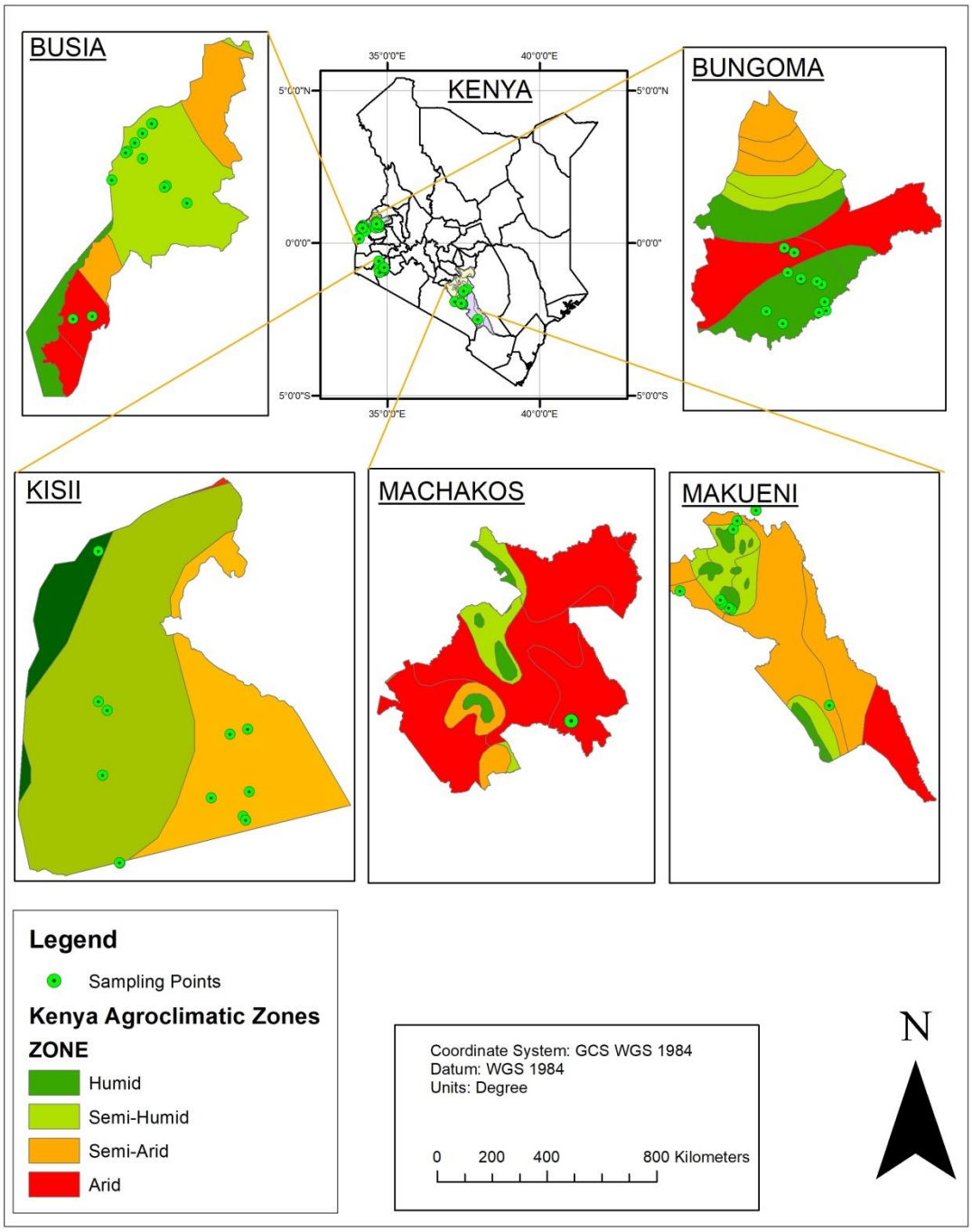

Figure 4.1: Finger millet blast sampling sites in Kenya.

Key: The green spots represent sampled areas with varying blast disease severity. The surveyed areas represent the main finger millet growing areas.

4.2.2 Sampling and survey methodology

In order to locate the finger millet farms, guided purposive sampling was used, with the assistance of extension officers, where symptomatic plants were picked. The plants were checked physically for typical diamond-shaped lesions on the leaves and neck, while a rusty brown appearance was chosen for the finger. The finger millet specimen collected included leaf, finger, and neck and was placed in brown sugar bags to avoid contamination. Six severely infected plants were randomly collected per farm. The number of farms sampled per county depended on the availability of finger millet in the farm. The extent of disease infection was classified based on occurrence, distribution, and severity. Disease occurrence was based on the presence or absence of disease on the surveyed farms. Disease severity was based on blast symptoms on the plant parts (finger, leaf, and neck) along with disease symptoms spread on the whole farm. Disease severity data were recorded and scored as less severe (0-25%) = 1, moderately severe (30-60%) = 2 and very severe (70% and above) = 3 (Karangwa et al., 2016). The identity of the fungal isolates was determined using molecular techniques. GPS (global positioning system) data were used to determine farm locations and disease density per county.

4.2.3 Sample collection

Finger millet crops were physically examined for the presence of diamond-shaped lesions on the leaves and neck, while a rusty brown appearance was observed on the finger. The samples (leaf, finger, and neck tissues) were cut from the mother plant using a sterile scalpel and placed in sterile paper bags to avoid contamination before labeling. Six severely plants were randomly collected per farm. The number of farms sampled per County depended on the availability of finger millet in the farm. The samples were thereafter transported to the Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Juja and stored at -80 °C for subsequent experiments.

4.3 Statistical analysis

The Pearson's chi-squared test of association was used to investigate the existence of statistically significant associations between severity on plant parts and counties; severity on whole plant and counties using SPSS version 25 software (IBM Corp, NY, USA).

A spatial distribution map was designed using ArcGIS Software 10 to show the extent of disease spread within the different counties surveyed to portray disease distribution within the counties (Figure 4.1).

4.4 Results

4.4.1 Blast disease occurrence and distribution

Finger millet blast disease caused by *P. oryzae* occurred in all the farms visited in Busia, Machakos, Makueni, Bungoma, and Kisii. This was equated to a 100% disease occurrence on all the farms visited (Table 4.2). Sampling was done from two main agro-ecological zones (Western and Eastern) represented by Bungoma, Busia, Kisii, Machakos, and Makueni counties as shown in the spatial analytical map (Figure 4.1). Similar to blast disease occurrence, blast disease was distributed across all the counties surveyed. However, there was heterogeneity in the density of the disease distribution in all the counties visited.

Within Busia and Bungoma counties, the farms were closely dispersed as such disease distribution followed the same pattern. In Machakos and Makueni the farms were far apart and so disease distribution followed the same pattern. In contrast, the farms surveyed in Kisii County were sparsely distributed, but disease density within each farm was higher. The distribution of diseased plants did not deviate much from those observed in each County. In farms having large fruit trees, there were more blast symptoms compared to those without. Additionally, farms that were on the leeward side had fewer blast symptoms compared with those on the windward side. Disease distribution based on plant parts infected revealed that the fingers were most infected compared to the leaves and neck. The distribution of finger millet growing farms per county surveyed varied. Busia county had the highest number of farms sampled (60) since it was used as a benchmark, this was then followed by Bungoma (13), Kisii (13), Makueni (10), and Machakos (5) in that sequence.

Table 4.1: Finger millet blast disease occurrence in sampled Kenyan Counties in 2018 and 2019.

Agro-ecological zone	County	Number of farms surveyed	Blast occurrence
Western	Busia	60	+
	Bungoma	13	+
	Kisii	13	+
Eastern	Machakos	5	+
	Makueni	10	+

(+) a sign indicates the presence of blast disease.

4.4.2 Blast severity

Based on the symptoms observed, Busia (82%) county had the highest finger millet blast symptoms severity, then Bungoma and Machakos (67 and 66% in sequence) with Makueni having the least at 61% (Table 4.2). Equally, a higher number of infected fingers were observed in Busia with 74.2%, neck at 37.7%, and leaf at 17.1% (Table 4.2). A statistically significant correlation test using Pearson's chi squared correlation for whole-farm finger millet blast disease symptoms between the counties surveyed was done (Table 4.3). These results showed that there was a

significant association ($p<0.01$) existing between blast symptom severity on the whole farm and the severity of neck and leaf infected in the different counties surveyed. This was significantly higher in Busia than in the other counties. The intensity of disease infection was highest in Busia at 82.3%, and least in Makueni with 61% (Table 4.3) in the whole farm. The test further revealed that blast was significantly associated with plant parts infected ($p\leq0.05$) (Table 4.3). Symptoms of the disease on fingers decreased sequentially from Busia (74.2%), Makueni 59%, Kisii (58.3%), Bungoma (57.1%), and Machakos (56%).

Table 4.2: Finger millet blast symptom severity and plant part infected in sampled farms Kenyan sub-counties in 2018-2019.

County	Latitude	Longitude	Subcounty	Severity scale 1-3	Whoel farm % blast severity	Main part of plant part infected	% blast severity per plant par			Level of severity
Busia	$0^0$27'26.8'' N	$34^0$07'07.6'' E	Teso south	3	**90**	f, n, l	**80**	**60**	**30**	**very severe**
	$0^0$33'41.0'' N	$34^0$11'12.3'' E	Teso south	3	**90**	f, n, l	**80**	**60**	**20**	**very severe**
	$0^0$34'59.9'' N	$34^0$12'32.3'' E	Teso South	2	**80**	f & n	**70**	**50**	**10**	**moderately severe**
	$0^0$34'60.0'' N	$34^0$12'26.1'' E	Teso south	3	**90**	f, n, l	**80**	**50**	**25**	**very sever**
	$0^0$32'26.3'' N	$34^0$10'11.0'' E	Teso south	2	**90**	f & l	**80**	**10**	**40**	**very severe**
	$0^0$31'17.9'' N	$34^0$09'13.4'' E	Teso south	2	**80**	f & n	**80**	**35**	**10**	**very severe**
	$0^0$31'03.9'' N	$34^0$08'57.1'' E	Teso south	2	**80**	f& n	**70**	**15**	**40**	**very severe**
	$0^0$26'40.7'' N	$34^0$14'23.1'' E	Nambale	2	**60**	f, n	**70**	**30**	**10**	**moderately severe**
	$0^0$26'26.7'' N	$34^0$14'07.7'' E	Nambale	2	**70**	f, n	**60**	**35**	**5**	**very severe**
	$0^0$24'20.6'' N	$34^0$17'11.6'' E	Nambale	2	**70**	f, n	**70**	**30**	**5**	**very severe**
	$0^0$30'19.2'' N	$34^0$11'11.9'' E	Teso south	3	**90**	f, n, l	**70**	**40**	**20**	**very severe**
	$0^0$08'50.2'' N	$34^0$01'56.4'' E	Bunyala	2	**90**	f & n	**80**	**40**	**5**	**very severe**
	$0^0$09'10.1'' N	$34^0$04'30.4'' E	Bunyala	2	**90**	f& n	**75**	**35**	**5**	**very severe**
Mean					**82.31±2.8**		**74.2±1.8**	**37.7±4.2**	**17.1±3.6**	

Bungoma	0°31'49.6" N	34°34'44.9" E	Kanduyi	2	**70**	f & n	**70**	**35**	**5**	**very severe**
	0°31'40.1" N	34°34'44.8" E	Kanduyi	2	**70**	f& n	**70**	**25**	**5**	**very severe**
	0°37'02.3" N	34°40'29.3" E	Kanduyi	1	**80**	f	**60**	**10**	**5**	**very severe**
	0°37'07.0" N	34°40'29.3" E	Kanduyi	2	**70**	f & l	**70**	**5**	**30**	**very severe**
	0°29'39.5" N	34°37'29.3" E	Kanduyi	2	**70**	f & l	**60**	**5**	**30**	**very severe**
	0°33'16.6" N	34°44'20.8" E	Webuye west	1	**60**	f	**50**	**5**	**5**	**moderately severe**
	0°31'29.6" N	34°43'29.6" E	Webuye west	1	**70**	f	**40**	**5**	**5**	**very severe**
	0°31'29.6" N	34°43'28.6" E	Webuye west	3	**70**	f,n&l	**70**	**30**	**10**	**very severe**
	0°36'10.4" N	34°43'58.4" E	Webuye west	1	**50**	f	**40**	**5**	**5**	**less severe**
	0°36'40.7" N	34°43'09.5" E	Webuye west	1	**50**	l	**20**	**5**	**30**	**less severe**
	0°42'10.4" N	34°37'41.9" E	Kabuchai	2	**70**	f&l	**70**	**5**	**30**	**very severe**
	0°42'14.8" N	34°37'45.8" E	Kabuchai	2	**80**	f& n	**70**	**20**	**5**	**very severe**
	0°41'28.7" N	34°39'25.9" E	Kabuchai	1	**50**	l	**40**	**5**	**30**	**moderately severe**
	0°38'09.8" N	34°38'17.9" E	Kabuchai	3	**80**	f & n	**70**	**30**	**5**	**very severe**
Mean					**67.1±2.3**		**57.1±4.5**	**13.6±3.1**	**14.3±3.2**	
Kisii	0°54'09.4" S	34°53'08.2" E	Bobasi	1	**40**	f	**40**	**5**	**2**	**moderately severe**

	0°54'27.0" S	34°53'18.6" E	Bobasi	3	**80**	f,n &l	**70**	**40**	**20**	**very severe**
	0°52'26.0" S	34°53'33.7" E	Bobasi	1	**60**	**f**	**50**	**10**	**5**	**moderately severe**
	0°52'51.2" S	34°50'51.0" E	Bobasi	2	**60**	**f & n**	**50**	**20**	**2**	**moderately severe**
	0°46'37.6" S	34°43'27.5" E	Bomachoge chache	2	**70**	**f & n**	**70**	**20**	**5**	**very severe**
	0°46'00.5" S	34°42'50.8" E	Bomachoge chache	1	**60**	**f**	**60**	**5**	**5**	**moderately severe**
	0°51'14.8" S	34°43'09.1" E	Bomachoge chache	1	**60**	**f**	**50**	**5**	**5**	**moderately severe**
	0°57'29.2" S	34°44'22.2" E	Bomachoge chache	2	**70**	**f & n**	**60**	**20**	**5**	**very severe**
	0°57'28.8" S	34°44'20.8" E	Bomachoge chache	1	**50**	**f**	**50**	**5**	**5**	**very severe**
	0°35'19.7" S	34°42'50.4" E	Nyaribari chache	1	**80**	**f**	**60**	**5**	**5**	**very severe**
	0°48'19.4" S	34°52'12.0" E	Nyaribari chache	2	**70**	**f &n**	**70**	**30**	**5**	**very severe**
	0°47'57.8" S	34°53'27.2" E	Nyaribari chache	1	**50**	**f**	**70**	**10**	**5**	**very severe**
Mean					**62.3±3.1**		**58.3±3**	**14.6±1.3**	**5.8±3.3**	
Machakos	$1^0$30’40.7’’S	$37^0$36’18.2’’E	Mwala	1	**60**	**f**	**50**	**10**	**5**	**moderately severe**
	$1^0$30’40.7’’S	$37^0$36’18.2’’E	Mwala	1	**60**	f	**60**	**10**	**5**	**moderately severe**
	$1^0$30’40.7’’S	$37^0$36’18.2’’E	Mwala	1	**70**	l	**30**	**5**	**50**	**very severe**
	$1^0$30’40.7’’S	$37^0$36’18.2’’E	Mwala	1	**70**	f	**70**	**15**	**5**	**very severe**

	$1^0$30’40.7’’S	$37^0$36’18.2’’E	Mwala	1	**70**	f	**70**	**20**	**10**	**very severe**
Mean					**66±2.4**		**56±7.5**	**12±2.6**	**15±8.8**	
Makueni	$1^0$55’09.5”S	$37^0$13’10.5’’E	Kilome	1	**50**	f	**50**	**10**	**5**	**moderately severe**
	$2^0$00’48.0”S	$37^0$28’51.3”E	Nzaui	3	**70**	f,n&l	**60**	**40**	**20**	**very severe**
	$2^0$00’48.0”S	$37^0$28’51.3”E	Nzaui	1	**50**	f	**50**	**2**	**30**	**moderately severe**
	$2^0$00’19.4”S	$37^0$28’05.8”E	Kibwezi west	1	**60**	f	**50**	**10**	**10**	**moderately severe**
	$1^0$59’09.6”S	$37^0$25’51.3”E	Makueni	1	**50**	f	**60**	**4**	**11**	**moderately severe**
	$1^0$58’35.0”S	$37^0$26’05.7”E	Makueni	2	**60**	f& l	**50**	**5**	**5**	**moderately severe**
	$1^0$57’50.9”S	$37^0$25’28.7”E	Makueni	2	**60**	f &l	**60**	**5**	**10**	**moderately severe**
	$2^0$29’56.9”S	$37^0$58’38.8”E	Kibwezi East	3	**70**	f, n&l	**65**	**15**	**11**	**very severe**
	$1^0$36’26.5”S	$37^0$29’27.2”E	Mbooni East	2	**60**	f &l	**55**	**10**	**5**	**moderately severe**
	$1^0$33’53.1”S	$37^0$30’35.0”E	Mbooni East	3	**80**	f, n&l	**90**	**50**	**70**	**very severe**
Mean					**61±3.1**		**59±3.9**	**15.1±5.2**	**17.7±6.3**	

Key: F=finger, L=leaf and N=Neck; S.E= standard error

4.4.3 Comparison of blast symptom severity in various counties in Kenya

Blast symptom severity per county showed that within Busia County, for instance, Teso South had a higher number of farms with plants possessing blast symptoms on fingers at 80%, Bunyala at 77.5%, and Nambale at 66.7% (Table 4.2). In Bungoma, Kanduyi sub-county had the highest number of plants with blast symptoms on fingers (72%), Kabuchai (70%), and Webuye West (60%). In Kisii County, Nyaribari chache sub-county led with the most number of plants showing blast symptoms on fingers with 66.7% followed by Bomachoge chache at 62% then Bobasi at 60%.

In Makueni county, both Kibwezi East and Mbooni East sub-Counties showed the highest percentage of plants with blast symptoms on fingers at 70%. Kibwezi west and Nzaui on the other hand, had 60%, Makueni 56.7%, and Kilome with 50% (Table 4.2). In Machakos county, only one sub-county had finger millet growing on the farm at the time of the survey namely Mwala sub-county which showed 66% of its plants having blast symptoms on fingers. All in all, these results revealed a positive correlation between blast symptom severities on the whole plant in the whole farm and those on the individual fingers at a particular farm. This implied that the extent of disease severity on a particular farm was reflected by the number of plants showing symptoms on the fingers rather than the neck or leaf. At the same time a positive correlation was observed ($p \leq 0.05$) between blast symptoms on fingers with those at the neck using the Pearson correlation test (Table 4.3). On the contrary, there was no correlation between the severity of the finger and the severity of the leaf.

4.4.4 Neck blast symptom severity

A significant difference ($p<0.01$) was observed in neck blast severity across the different counties surveyed (Table 4.3). There was a decrease in neck blast symptom severity across the counties as follows Busia (37.7%), Makueni (15%), Kisii (14.6%), Bungoma (13.6%), and Machakos (12%) (Table 4.2). At the sub-County level, the extent of blast disease symptoms severity varied. Teso south had the

highest severity at 40%, Bunyala at 37.7%, and Nambale at 31.7% within Busia County (Table 4.2).

In Bungoma, the Kabuchai sub-county had the highest at 18.3%, Kanduyi at 16%, and Webuye west at 11%. In Kisii, the Nyaribari chache sub-county had the highest neck severity at 15%, Bomachoge chache at 11%, and Bobasi at 9% (Table 4.2). Mwala sub-county in Machakos had a severity of 12%.In Makueni; Mbooni east posted the highest neck blast symptom severity at 50%, Nzaui at 21%, Kibwezi east at 15%, Kibwezi west, and Kilome at 10% each, and lastly Makueni at 4.7% (Table 4.2). There was no significant correlation found between neck and leaf blast symptom severity (Table 4.2).

Table 4.3: Finger millet blast severity in 5 Counties in Kenya

County	Whole plant mean severity	Finger severity	Neck severity	Leaf severity
Machakos	66.0± 2.4 [a]	56.0± 7.5 [a]	12.0± 2.6 [b]	15.0± 8.8 [b]
Makueni	61.0± 3.14 [a]	59.0 ± 3.9 [a]	15.1± 5.2 [b]	17.7± 6.3 [b]
Busia	82.3± 2.8 [a]	74.2 ±1.8 [a]	37.7± 4.2 [b]	17.1± 3.6 [b]
Bungoma	67.1± 2.3 [a]	57.1± 4.5 [a]	13.6± 3.1[b]	14.3± 3.2 [b]
Kisii	62.3± 3.1[a]	58.3 ± 3.0 [a]	14.6± 1.3 [b]	5.8± 3.3 [b]

[a]Means with similar letters within the same column are not significantly different at $p \leq 0.05$.

The correlation analysis between blast severity on different plant parts and counties is as shown in Fig. 4.2. There were two functional clades: one clustered Busia county and another clustered the other four counties together according to their severities. This showed that Busia county had the highest severity in all plant parts except the leaf indicated by the red colour dominating its column in Fig 4.1. Machakos county recorded relatively low severity levels for all the plant parts with the lowest being on

the leaf compared to other counties evaluated (Fig. 4.2). This is shown by the white colour in its column (Fig. 4.2).

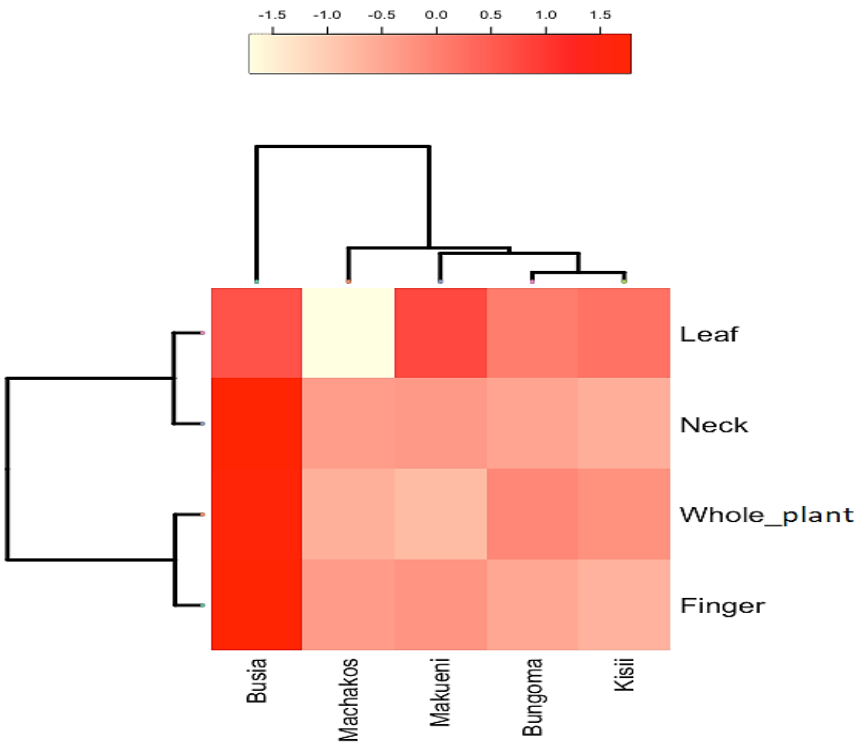

Figure 4. 2: Hierarchical clustergram showing blast severity in different finger plant parts and whole plants across the five counties.

Key: Hierarchical clustergram generated using means of blast severity from different plant parts and whole plant from the different counties. It shows the relationship between blast severity and the different parts infected.The coloured scale bar indicates the significant strength of the severity. The red and white colours indicates the highest and least recorded significant mean values respectively at $p \leq 0.05$.

4.5 Discussion

Finger millet blast disease symptom from all the farms surveyed indicates high blast disease prevalence in the main finger millet growing areas in Kenya. This position is coherent with similar studies done by Oduori (2008) and Gashaw *et al.,* (2014). Their study revealed high blast incidence in Busia, Teso, and Kisii counties causing yield losses of 10-80% in both Kenya, Uganda and Ethiopia. Earlier on, Owere (2013) had also reported that finger millet blast was endemic in Uganda but disease severity varied depending on prevailing weather conditions. Zhang *et al.,* (2016) equally observed the high blast prevalence in areas cultivated with host plants worldwide. Global blast incidence especially on rice and finger millet, the key hosts for *P. oryzae*, has affected more than 85 countries. Despite this fact, farmers still have limited knowledge about finger millet blast thus affecting the management of the disease.

The distribution of finger millet blast symptoms varied from farm to farm and from county to county. The results of this study show that blast symptom distribution was grouped into two categories, namely dense and sparse. In the Western agro-ecological zone, the disease was generally more densely distributed compared the Eastern zone made up of Makueni and Machakos where it was sparse. This could be explained by the sparse distribution of farms, various farm management techniques, type of finger millet varieties and/or the agro-climatic conditions present on the farms and/or counties. It has been reported that sufficient well-distributed rainfall influences the blast disease profile (Onyango, 2016). According to this study, there were regional differences in the blast disease severity, with the Western agro-ecological zone being more severely affected than the Eastern agro-ecological zone. In the Western region particularly in Kisii county, there were many bananas and blue gum trees planted on the farms where finger millet was also cultivated. Such conditions reduce light intensity and increase humidity that favors the distribution of the pathogen. A similar pattern was observed in Makueni County, where finger millet farms that were intercropped with numerous fruit trees experienced higher

blast incidence than those farms without fruit trees.These results are consistent with (Gashaw *et al.*, 2014).

It is notable that environmental conditions especially relative humidity plays a key role in stimulating the sporulation, release and germination of blast conidia (Wekesa *et al.,* 2019). Machakos and Makueni counties are semi-arid regions. They receive depressed rainfall amounts of 830 and 834 mm respectively coupled with low humidity. Busia, Bungoma, and Kisii receive much higher rainfall of amounts of 1691mm, 1628mm, and 1922 mm respectively. High levels of precipitation lead to high levels of humidity, which favor the growth of the blast pathogen (*Pyricularia oryzae*) (Wilson, 2009b). This explains why the Western agro-ecological zones had more blast disease symptoms than the Eastern agro-ecological zones. Another feasible theory could be related to the origin of the finger millet pathogen. Previous studies have shown for instance that the *P.oryzae* population that overwhelms finger millet and rice within sub-Saharan Africa exhibit unique patterns of genetic diversity, mating-type distribution, fertility status, and host compatibility. This pattern mimics the different histories and patterns of agronomy within East Africa (Takan *et al.,* 2012). They also confirmed that the frequency of occurrence of *P.oryzae* isolates having transposable DNA elements such as *grh* element was influenced by differences in lineage.

The study further revealed that the existence of these elements among rice blast isolates was a result of gene flow between rice and other *P. oryzae* hosts (Shittu, 2018). This knowledge has exposed unique insight into the population dynamic model of *P.oryzae* from different hosts. Earlier studies on the genetics of finger millet blast fungus confirmed the presence of the *grh* element only in *P. oryzae* populations from Asia (Japan, Nepal, and India) and some West African countries (Viji *et al.,* 2000; Tanaka *et al.,* 2007). PCR results showed that some indigenous blast populations did not have the element and were therefore different from the Asian blast population. This confirmed that the *P. oryzae* isolates possessing the *grh* repeat element were imported while most of the pathogenic East African counterparts lacked it (Shittu, 2018; Takan *et al.,* 2012). Probably, the blast pathogen was

introduced through the western route precisely through cross-border transfer of the finger millet seeds. This is because Busia is a border town shared by Kenya and Uganda; Uganda is one of the places from which finger millet originated. The presence of some haplotypes in certain areas in Kenya and Uganda validates the point that pathogen movement occurred (Lenne *et al.,* 2007). Kenya–Uganda border market proximity could have also easily influenced the purchase and /or exchange of seeds from one country to the other (Onziga, 2015).

Both blast disease symptoms and severities followed the same pattern where most farms in the western agro-ecological zones had severe infections in comparison to the Eastern zone. Busia county is found between altitudes 1000-1300m above sea level and a mean temperature of 22^0C. It is a humid lower midland zone with properly drained agricultural soils thus greatly plagued by *P.oryzae* infection. Both Bungoma (1200-1400m above sea level) and Kisii (1500-1800m above sea level) share similar agro-climatic conditions with Busia county. Because of the proximity of Busia and Bungoma counties, farmers in both counties can exchange finger millet seeds. Kisii county is uniquely characterized by cloudy skies with frequent rains and drizzles. This situation favours long periods of dew accumulation on leaf surfaces increasing blast incidence thus predisposing the plant to blast infection. Also, the tropical climate favours the build-up of airborne blast spores all through the year which favours extended disease development (Onyango, 2016).

Both Machakos (1000-1600 m above sea level) and Makueni (1200-1600m above sea level) are semi-arid regions with low rainfall and humidity. In contrast to expectations, the findings of this study showed higher blast disease symptoms. The huge fruit trees that are typical of most of the finger millet fields surveyed may have influenced disease progression. These trees generate a microclimate that minimizes the necessary light intensity, increasing the relative humidity within the farms. These conditions therefore favor the spread of the dreadful blast infection (Gashaw *et al*., 2014). Furthermore, the majority of the farms were on the windward side; wind plays an important role in the transmission of conidia, promoting the spread of the pathogen and exposing more plants to disease infection (Shahriar et al., 2020). A

well-managed farm paves way for a bumper harvest and minimizes disease infection rates (Meena *et al.,* 2017). Some of the surveyed farms were properly managed where good agronomic practices such as timely weeding, timely planting, crop rotation with non-alternative hosts among others were employed. In such farms, there was lower disease severity at the whole farm level compared to those that were poorly managed. It is thus evident that there is a need to train finger millet producers on the best finger millet agronomic practices to minimize disease severity and obtain higher yields.

CHAPTER FIVE

CHARACTERIZATION OF THE GENETIC DIVERSITY OF THE FUNGAL COMMUNITY CO-EXISTING WITH *Pyricularia oryzae* USING ITS AND 28S rDNA MOLECULAR MARKERS

5.1 Introduction

Successful control and management of finger millet blast disease require that one understands the structure and genetic diversity of the fungus coupled with its associated fungal community. This was elucidated by Gladieux *et al.,* (2018) who noted that to develop durable, resistant cultivars that can easily adapt to a specific region it is essential to properly establish the structure and genetic diversity of finger millet blast populations (clonal lineage). Finger millet like any other plant is colonized by various microbes whose infectivity may either be enhanced or suppressed by the presence of other microbes. Such microbial communities seem to be ubiquitous among species in the Plant Kingdom interacting closely with the plant either as symbionts or commensals. These microbial communities have played significant roles in enhancing plant fitness leading to increased tolerance to stress and competition with pathogens (Rojas *et al.,* 2019). Thus, it is imperative to understand the relationship and behaviour of these microbial communities incongruent with the pathogen during disease outbreaks. This is because they share the same habitat and may have similar characteristics as the pathogenic microbe in question. Ascertaining the type of microbial species present, how and when they manifest coupled with the distinct roles they play amid these interactions is very important (Busby *et al.,* 2015). Taking this proposition into consideration for purposes of plant disease management and control then in-depth understanding of the pathogen-microbe behaviour when the pathogen appears and when disease occurs is of essence (Latz *et al.,* 2018). This synergism provides the much-needed integrated strategy to plant disease control and would establish novel methods for combating plant diseases. Furthermore, a broad understanding of the roles of these fungi would

yield significant progress towards the deployment of improved management strategies for plant diseases such as finger millet blast.

Morphological characterization is one of the basic units used in fungal species identification. However, in more recent times, the discovery of molecular tools in concert with morphology has elevated fungal taxonomy to a higher level concerning fungal identification. These tools are relatively abundant, providing specified information with high reproducibility. A greater number of fungal species have greatly similar morphological characteristics which cause misidentification. Precise identification of fungal species is critical, especially for crop disease management (Kusai *et al.,* 2015). The internal transcribed spacers (ITS) and 28SrDNA regions are usually used as phylogenetic markers for fungal taxonomic analysis. At present, there is limited information on the status of fungal species affiliated with finger millet blast pathogens in Kenya. Thus, this study used both morphological and molecular approaches to evaluate the fungal microflora co-existing with *P. oryzae* in plants having finger millet blast disease.

5.2 Materials and methods

5.2.1 Isolation of fungi from finger millet plants

Plant tissue samples were obtained during the planting season from May to June 2018 and in February and June 2019 from five finger millet growing counties of Kenya (Machakos, Makueni, Kisii, Busia, and Bungoma). A purposive sampling technique was used to single out finger millet farms. At least six plant tissues with typical blast symptoms were randomly sampled from each of the surveyed farms per county. A total of 290 finger millet plant tissues were thus collected and placed in clean sample collection bags and transported to the Institute for Biotechnology Research Molecular Biology laboratory for succeeding experiments.

Approximately 4-mm segments of each plant tissue were aseptically excised, rinsed with tap water to lessen surface contaminants, and then sterilized with 3 % sodium hypochlorite followed by three consecutive rinses with sterile distilled water to remove any other contaminants. The samples were then dried on sterile blotting paper followed by culturing on oatmeal agar (OMA) media supplemented with 50mg/L of chloramphenicol at 27 °C for 7-10 days. Emerging colonies were streaked on a 4% water agar with a sterile 10µl loop. After 48h, the streaks were observed under a stereomicroscope until the most extended and germinated single conidium was noticed. A single germinated conidium was then removed using a scalpel blade by cutting the agar closely around the conidium. The agar piece containing the spore was lited and transferred onto the OMA supplemented with chloramphenicol and incubated with a 12/12 h light /dark photoperiod at 27 °C to induce growth and sporulation. All of the resultant pure cultures were maintained in 30% glycerol containing half-strength potato dextrose broth at -20 °C.

5.2.2 Morphological identification

The fungal isolates' macro- and micro-morphological characteristics were examined. Each pure fungal isolate was cultured on OMA amended with 50mg/L chloramphenicol and incubated at 27 °C for five days. The mycelial characteristics including colony colour, margination, and elevation were noted down. Conidia production was induced by scraping the surface of the fungal mycelium with a sterile spreader. The isolates were then incubated in the dark for another 5 days. Conidia characteristics including shape and hyphae were observed under a Leica ICC 50 E compound microscope at X100 magnification.

5.2.3 Molecular characterization based on ITS and 28SrDNA

Fungal genomic DNA was extracted using a modified CTAB method (Panda *et al.,* 2017). PCR amplification was carried out using two primer sets, ITS1/ITS4 (ITS1: 5´-TCC GTAGGT GAA CCT GCG G-3´ and ITS4: 5´- TCC TCC GCT TATTGA TAT GC-3´) and NL1/4(NL1: 5' GCA TAT CAA TAAGCG GAG GAA AAG -3' and NL4: 5' GGT CCG TGT TTCAAG ACG G 3'), for the fungal ITS and 28S

rDNA regions, respectively (White *et al.,* 1990). PCR conditions were set as follows: initial denaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation, annealing and extension at 95 °C for 30 seconds; 55 °C for 15 seconds (for ITS1) or 53 °C for 40 seconds (for 28S rDNA); 72 °C for 30 seconds (for ITS1) or 72 °C for 1 minute (for 28S rDNA); and a final extension at 72 °C for 5 seconds (for ITS1) or 7 minutes (for 28S rDNA). The PCR products were resolved by 1% agarose gel electrophoresis. ITS amplicons were sequenced by Macrogen Europe B.V. using an Applied Biosystems 3730xl DNA Analyzer and 28SrDNA amplicons by Inqaba Biotec using an ABI 3500XL Genetic Analyzer platform. Both the forward and reverse ITS and 28S rDNA sequence data were assembled and manually edited using BioEdit sequence alignment editor software. The taxonomic relationships between the isolates were ascertained with standard nucleotide BLAST searches against the nucleotide database in the UNITE fungal database (http:// www.unite. ee), NCBI RefSeq (http:// www. ncbi. nlm. gov/ refseq/targe tedlo ci/) and GenBank (http:// www. ncbi. nih. gov/ blast). The fungal ITS and 28S rDNA gene sequences obtained in this study were deposited in GenBank under accession numbers MW151763-MW151817 and MW644907-MW644961, respectively.

5.2.4 Community structure analysis

The sequences obtained from the ITS and 28S rDNA primers were analyzed both separately and in the concatenated form to confirm their phylogenetic placement, using *Methanoculleus thermophillus* as the outgroup. Two phylogenetic trees were constructed using a Neighbor-joining method with Molecular Evolutionary Genetic Analysis (MEGA X) software, while another tree was constructed using concatenated sequences for both ITS and 28SrDNA with MAFFT alignment software version 7.407. To evaluate the best DNA model substitution pattern, a Bayesian information criterion score (BIC) was considered, which revealed a K2 + G + 1 model as the best fit (Kumar *et al.,* 2018). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

The evolutionary history was inferred using the Maximum Likelihood method. The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site(Tamura *et al.,* 2004). The differences in composition bias among sequences were considered in evolutionary comparisons(Tamura & Kumar, 2002). The concatenated sequences were analyzed using MAFFT alignment v.7.407(Katoh & Standley, 2013); alignment curation was performed via the Block Mapping and Gathering with Entropy (BMGE) package v.1.12 (Criscuolo & Gribaldo, 2010); the IQ-Tree v2.03(Nguyen *et al.,* 2015) algorithm was used to infer the tree by maximum likelihood and visualized in Figtree v.1.4.4.

5.3 Data analysis

SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used to determine the percentages for the variables of interest based on the descriptive data. The chi-squared test (α = 0.05) was used to compare the proportion of fungal compositions from different plant tissues, namely leaf, finger and neck. Phylogenetic analysis was performed using MEGA X. and MAFFT alignment v.7.407.

5.4 Results

5.4.1 Isolation of fungi from finger millet plants

The regions from which the samples were collected are shown in the Distribution Map (Figure 3.1**).** A total of 55 pure fungal isolates were recovered from 290 plant tissues sampled from Machakos (5), Makueni (11), Kisii (13), Busia (16) and Bungoma (10) counties, consisting of different blast-diseased tissues (finger, leaf and neck) of finger millet (Table 5.1). The percentages of fungal isolates obtained from the different host tissues were 45.4%, 38.2% and 14.4% for the finger, leaf and neck tissues, respectively, showing typical blast symptoms (Figure 5.1). The macroscopic characteristics (colony colour, margin and elevation) and microscopic characteristics

(conidia shape) of the 55 fungal isolates associated with finger millet blast disease were examined (Fig. 5.1;Table 5.1).

A correlation analysis between morphological descriptors and fungal isolates revealed three main functional clades (Fig. 5.1). Mycelia colour(top)was the most significant characteristic used to cluster the isolates while plant part from which the fungi was isolated from was the least. There was no specific clustering of isolates based on their counties; the morphological characteristics cut across the different counties surveyed.

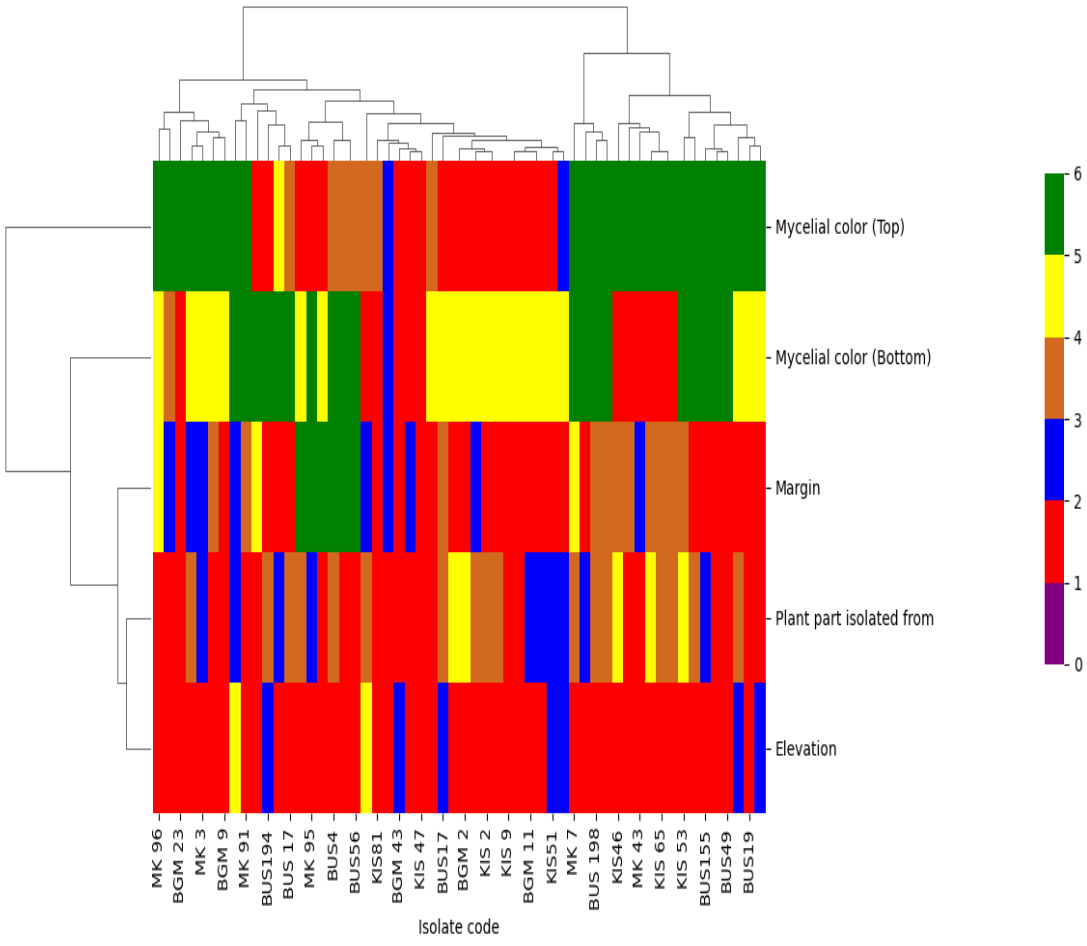

Figure 5.1: Hierarchical dendrogram of fungal isolates from the five counties based on their colony morphology and plant part isolated.

Key: This dendrogram was generated using morphological characteristics of the isolated fungi. The heatmap was based on Manhattan metric revealed the relationship between the fungal isolates from different counties and their morphological characteristics. Green colour in the color bar indicated the most significant descriptor while purple and red wer the least descriptors used in clustering the isolates.

5.4.2 Morphological identification

The strains were purified based on their distinct morphology on OMA and PDA media and differentiated based on their macroscopic and microscopic characteristics. At the macroscopic scale, the isolates cultured on OMA media showed mycelia with varied features in terms of appearance and colour. Five major mycelial colours were observed, grey (27), white (6), pinkish/purplish (8), greyish white (7) and black/greyish black (5), while 2 were brownish (Table 5.1, Figure 5.2).

Table 5. 1: List of all isolates, locality, species and GenBank accession numbers for ITS and 28SrDNA sequences

Isolate code	County[(a)]	Year	Plant part affected [(b)]	Mycelial features [(c)]	`Conidia Patterns (d)	Closest match in BLAST	Size (bp)	Accession numbers(e)	Similarity (%)	Affiliated to[(f)]
F11-1	Machakos	2019	F	Dark grey, filiform, raised	G	*Cochliobolus sp.*	548	MW151763	99	KT199720.1
F17-2	Machakos	2019	F	Black, filiform, raised	G	*Exserohilum rostratum*	596	MW151764	100	KT933711.1
F16-4	Machakos	2019	L	White, filiform, raised	G	*Fungal sp. strain*	568	MW151765	99	KT923234.1
F1-M5	Machakos	2019	L	Whitish grey, undulate flat	R	*Curvularia petersonii*	542	MW151816	99	MH414905.1
F5-8	Machakos	2019	N	White with concentric rings, entire, raised	G	*Sarocladium sp.*	581	MW151766	99	MF784844.1
MK22-10	Makueni	2019	N	Greyish brown, filiform, umbonate	G	*Curvularia lunata*	550	MW151767	99	MN173127.1
MK13-13	Makueni	2019	L	Grey with white spots, undulate, raised	E	*Bipolaris sp*	570	MW151768	100	KX219610.1
MK91-18	Makueni	2019	F	Grey with white margin,undulate,raised	C	*Fusarium solani*	548	MW151769	100	MH612967.1
MK95-19	Makueni	2019	N	Grey,entire,raised	G	*Alternaria alternata*	548	MW151770	99	KT192393.1
MK49-20	Makueni	2019	F	pinkish, entire, raised	G	*Fusarium annulatum*	535	MW151771	100	MN548436.1
MK74-21	Makueni	2019	F	Greyish brown, filiform, raised	G	*Curvularia lunata*	580	MW151772	99	MH183196.1
MK7-22	Makueni	2019	L	Pinkish grey concentric rings, ciliate, raised	G	*Curvularia hominis*	574	MW151773	100	HG779006.1

MK43-68	Makueni	2019	F	Greyish brown, undulate, raised	I	*Curvularia lunata*	571	MW151804	99	MN213745.1
MK98-69	Makueni	2019	L	Grey, entire, raised	G	*Fusarium sp.*	520	MW151805	100	KX982211.1
MK3-71	Makueni	2019	L	Grey, filiform, raised	E	*Cochliobolus akaiiensis*	579	MW151806	99	LT631342.1
MK96-72	Makueni	2019	L	Greyish brown, undulate, raised	R	*Curvularia lunata*	542	MW151807	100	MN173127.1
KIS2-M24	Kisii	2019	L	Grey,entire, raised	C	*Exserohilum rostratum*	574	MW151817	100	MN326698.1
KIS30-25	Kisii	2019	F	Whitish grey, undulate, raised	I	*Curvularia lunata*	548	MW151774	100	KR012913.1
KIS53-27	Kisii	2019	W	Pinkish, undulate, raised	R	*Sarocladium sp*	571	MW151775	99	MF784844.1
KIS62-28	Kisii	2019	L	Pinkish, undulate, raised	C	*Fusarium chlamydosporum*	521	MW151776	100	MK729132.1
KIS65-29	Kisii	2019	L	Pinkish, rhizoid, raised	I	*Epicoccum sp.*	507	MW151777	100	MK809031.1
KIS51-30	Kisii	2019	N	Grey, entire, raised	R	*Curvularia mebaldsii*	540	MW151778	100	MH414903.1
KIS47-32	Kisii	2019	F	Grey, entire, raised	I	*Curvularia clavata*	561	MW151779	100	MK736276.1
KIS9-33	Kisii	2019	F	Grey, entire, raised	I	*Cochliobolus bicolor*	486	MW151780	99	KJ909762.1
KIS58-34	Kisii	2019	L	Purplish, entire, raised	R	*Epicoccum sorghinum*	534	MW151781	99	MN555348.1
KIS88-35	Kisii	2019	F	White, entire, raised	R	*Fungi*	562	MW151782	99	JN897397.1
KIS8-74	Kisii	2019	L	Grey, entire, raised	I	*Curvularia trifolii*	581	MW151808	100	MH855614.1
KIS46-75	Kisii	2019	F	Greyish brown, entire, raised	I	*Curvularia lunata*	541	MW151809	100	MF101868.1

KIS37-78	Kisii	2019	L	Black, entire, raised	E	*Bipolaris simmondsii*	562	MW151810	99	KX452454.1
BU229-36	Busia	2018	W	Greyish brown, entire, raised	G	*Curvularia lunata*	546	MW151783	100	MN173127.1
BU188-37	Busia	2018	F	Black, entire, raised	R	*Penicillium citrinum*	485	MW151784	100	MT186193.1
BU155-40	Busia	2018	N	Whitish grey, entire, raised	C	*Epicoccum sp.*	524	MW151785	100	LT592927.1
BU180-41	Busia	2018	F	Pinkish grey, entire, raised	R	*Epicoccum sorghinum*	511	MW151786	99	KT989557.1
BU2-42	Busia	2018	F	Whitish, rhizoid, raised	C	*Fusarium oxysporum*	522	MW151787	100	MK429839.1
BU4-44	Busia	2018	L	Grey, entire, raised	E	*Gibberella intricans*	522	MW151788	95	MK780235.1
BU56-45a	Busia	2018	F	Whitish, rhizoid, raised	R	*Fusarium incarnatum*	520	MW151789	100	MN534779.1
BU29-46	Busia	2018	L	Pinkish, undulate, raised	C	*Epicoccum sorghinum*	515	MW151791	99	KT989557.1
BU85-47	Busia	2018	L	Greyish black, entire, flat	C	*Exserohilum rostratum*	579	MW151792	100	MN599590.1
BU19-49	Busia	2018	F	Whitish grey, entire, raised	C	*Cochliobolus bicolor*	508	MW151793	99	KJ909762.1
BU91-52	Busia	2018	L	Greyish black, entire, flat	C	*Exserohilum rostratum*	574	MW151794	100	MT075801.1
BU49-53	Busia	2018	F	Whitish grey, entire, raised	C	*Epicoccum sp.*	507	MW151795	100	MH824376.1
BU17-A	Busia	2018	F	Whitish grey, entire, raised	C	*Curvularia lunata*	573	MW151812	99	MN213745.1
BU49-CA	Busia	2018	F	Whitish grey, entire, raised	C	*Curvularia lunata*	557	MW151813	99	KR012913.1
BU198-D	Busia	2018	L	Greyish brown, entire, raised	C	*Curvularia lunata*	550	MW151814	100	LC317566.1

BU229-G	Busia	2018	N	Greyish brown, entire, raised	G	*Curvularia lunata*	548	MW151815	100	MN173127.1
BG1-54	Bungoma	2019	L	Greyish, filiform, raised	G	*Curvularia clavata*	561	MW151796	100	MK736276.1
BG2-55	Bungoma	2019	W	Grey, entire, raised	I	*Curvularia lunata*	553	MW151797	100	KRO12913.1
BG9-56	Bungoma	2019	L	Greyish black, entire raised	E	*Curvularia trifolii*	577	MW151798	100	MK370649.1
BG11-57	Bungoma	2019	N	Greyish black, entire, raised	E	*Curvularia trifolii*	582	MW151799	100	MG664782.1
BG17-58	Bungoma	2019	N	Greyish black, entire, raised	E	*Curvularia mebaldsii*	551	MW151800	100	MH414902.1
BG29-60	Bungoma	2019	F	Grey black, entire, flat	E	*Bipolaris cynodontis*	556	MW151801	100	MH856862.1
BG43-61	Bungoma	2019	F	Greyish,entire, raised	E	*Curvularia trifolii*	578	MW151802	99	MG664782.1
BG10-66	Bungoma	2019	N	Black, entire, flat	E	*Curvularia panici*	601	MW151803	96	AB164703.1
BG26-45b	Bungoma	2019	L	Grey, entire, raised	I	*Bipolaris cynodontis*	557	MW151790	100	MT032464.1
BG69-81	Bungoma	2019	F	White, entire, raised	C	*Epicoccum sorghinum*	510	MW151811	100	MN420978.1

Key (a) Finger millet blast sampling site. (b) Plant part from which fungi was isolated; F: finger, L: leaf, N: neck and W: weed. (c) Fungal mycelia characteristics on oat meal agar (OMA), including colony color, margin and elevation. (d) Conidial structure under the light microscope. G: globose, E: elliptical, C: cylindrical, R: round and I: irregular (e) GenBank accession number for sequences generated in this study for ITS gene;(f) GenBank accession numbers for reference relatives; (g) Genbank accession numbers for 28SrDNA sequences generated in this study. (h) Genbank accession numbers for reference relatives using 28SrDNA gene.

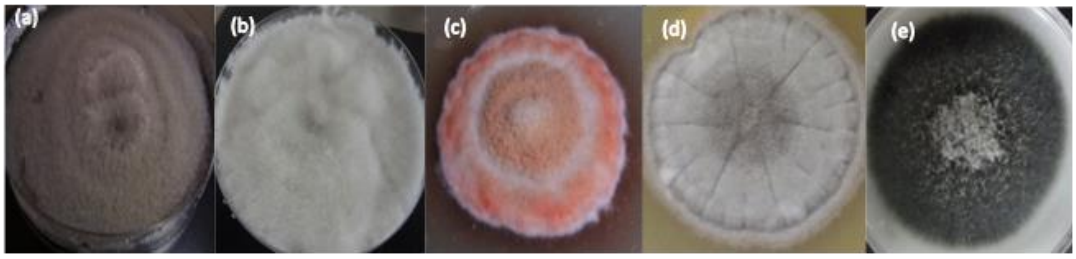

Figure 5.2: Morphological variation in selected fungal mycelia on OMA medium, showing differences in colours and mycelial growth patterns.

Key: (a): grey, entire, raised; (b). white, rhizoid, raised; (c): pinkish, undulate, raised; (d): greyish white, undulate, flat; (e): black, entire, raised.(a: is isolate BU229-G, b:BU2-42 , c: BU29-46, d: F1-M5, e: BG17-58).

The number of mycelial growth patterns, characterized by colony colour, elevation and margin, also varied across the blast-infected plant tissues. The highest number of mycelial growth pattern combinations was observed in the finger tissues (46%), followed by the leaf (38%) and neck tissues (16%), as shown in Figure 5.3.

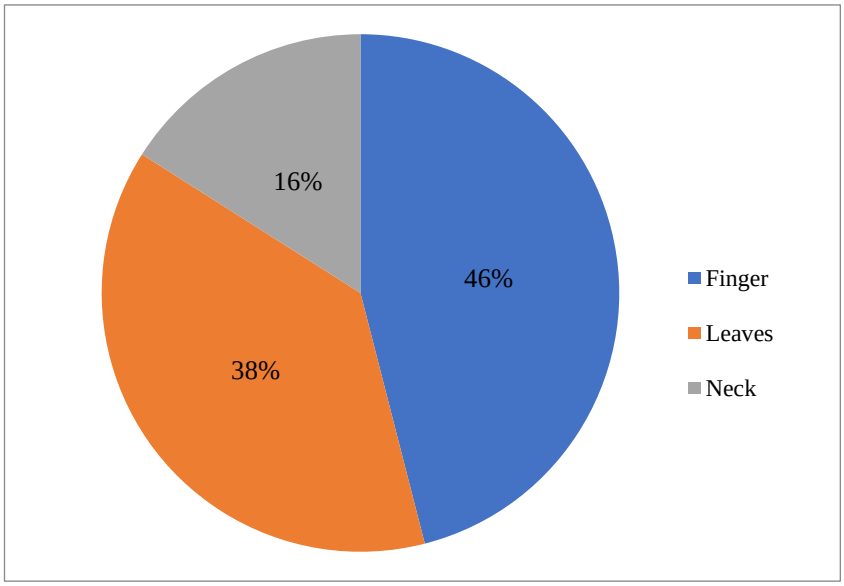

Figure 5.3: The mycelial growth patterns on different plant parts.

The proportion of mycelial growth pattern combinations exhibited by different fungal genera isolated from different plant parts sampled from five finger millet growing counties in Kenya.

Hyphal characteristics were differentiated as either septate or non-septate. Forty fungal isolates had septate hyphae while 15 were aseptate. None of the fungal isolates showed a pseudo-hyphae type of septation in their hyphal structures. The conidial shapes observed under the microscope were differentiated into four main patterns: globose, elliptical, irregular and cylindrical (Figure 5.4).

Most of the conidia shapes were either cylindrical (25.5%) or globose (23.5%), while 18.2% had an irregular pattern, 16.4% had an elliptical shape and 16.4% had a round shape (Table 5.1 & Figure 5.4).

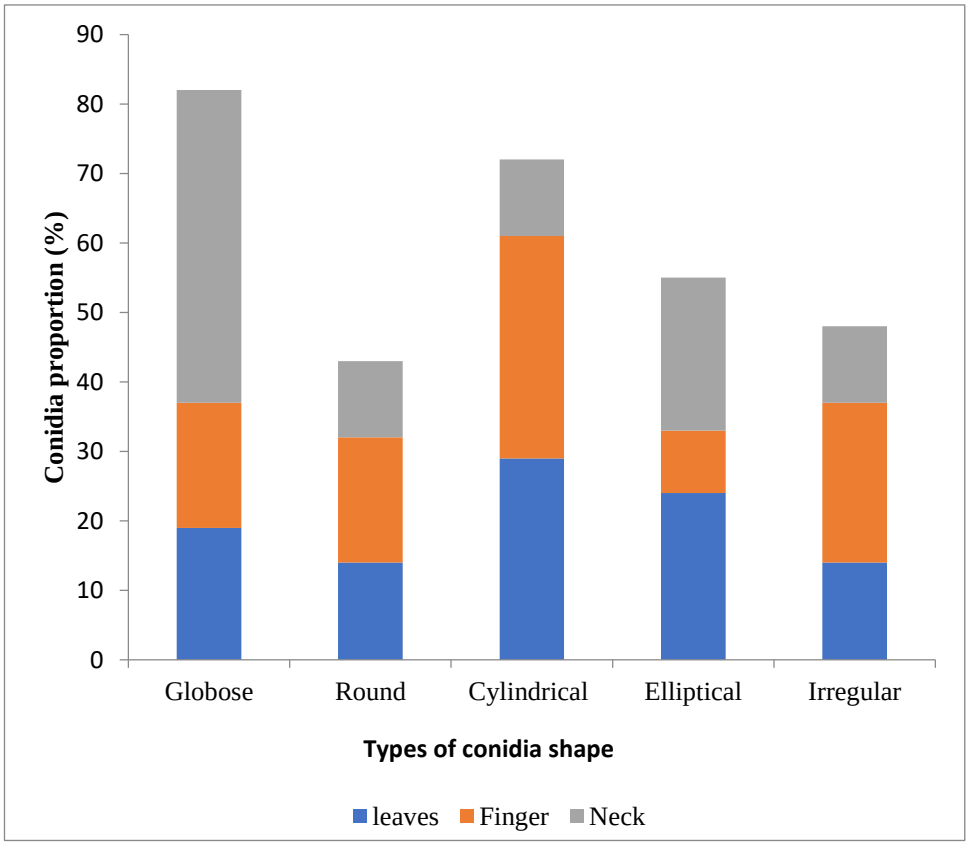

Figure 5.4: Conidia shapes per plant part infected and different types of conidia shapes observed from fungal isolates obtained from finger millet plant tissues.

All of the conidia patterns lacked observable appendages and had smooth surfaces. The conidia colour ranged from pale yellow to deep pigmentation, but none were hyaline (Figure 5.4). Some of the conidia were either simple (lacking septa; Figure 5.5a, e) or septate (having cross walls; Figure 5.5b, c, f). These cross walls were either transverse (Figure 5.5b), pseudo septate (Figure 5.5c, d) or longitudinal (Figure 5.5f). Isolates having similar conidial features and morphology were grouped in the same genus. However, due to the similarity of fungal morphological characteristics and the risk of misidentification, molecular identification was pursued confirmation of fungal identities.

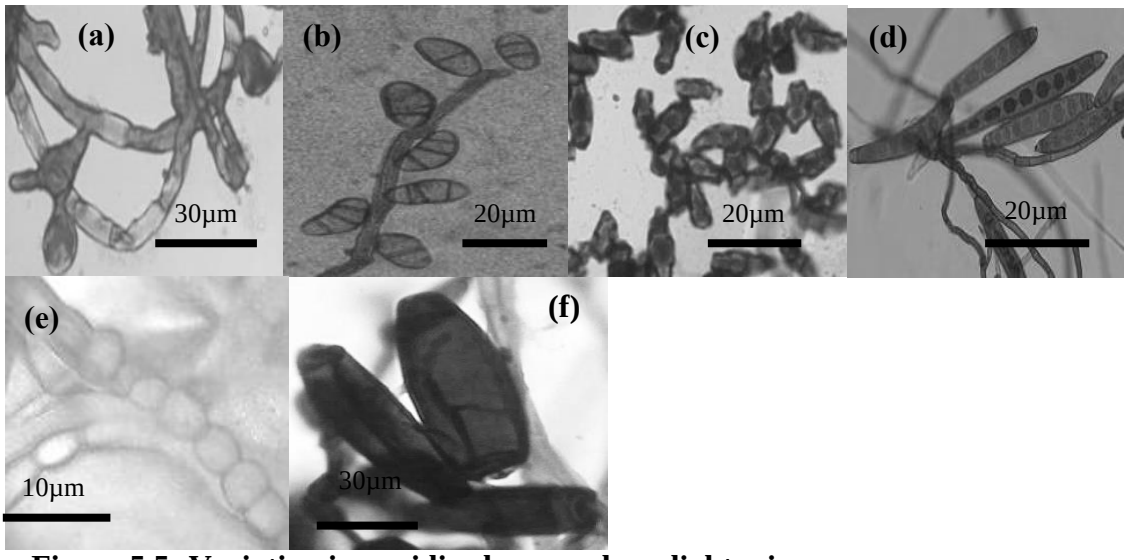

Figure 5.5: Variation in conidia shape under a light microscope.

Key: (a), globose; (b), elliptical; (c), irregular; (d), cylindrical; (e), Round and (f), Cylindrical with longitudinal septate. (a: is isolate F17-2 b: isolate MK49-20; c: isolate KIS47-32; d: isolate BU85-47; e: isolate KIS53-27; f : isolate KIS2-M24.

5.4.3 Molecular characterization based on ITS and 28S sequencing

Molecular analysis of the fifty-five fungal isolates was determined by sequencing PCR products of the ITS and 28SrDNA genes. In this case, genomic DNA was

extracted from the 55 samples of fungal strains cultured from blast-infected finger millet tissues for molecular analysis. The DNA was successfully amplified and sequenced. The sizes of the 28S rDNA and ITS regions ranged from 557–616 and 484–601 bp, respectively, after sequence editing (Table 5.1).

After a nucleotide BLAST search, the ITS and 28S rDNA sequences exhibited high percentage similarities between 99 and 100% with the UNITE and GenBank databases. The identities of the isolates based on the ITS and 28S rDNA sequences are shown in (Table 5.1).

The isolates were assigned to three classes of Phylum Ascomycota, namely Eurotiomycetes, Dothiodeomycetes and Sordariomycetes, and further separated into 10 genera and two unnamed fungi. For the ITS sequences, the isolates are related to *Exserohilum rostratum* (3), *Sarocladium* sp. (2), *Fusarium equiseti* (1), , *Fusarium* sp. (1), *Fusarium annulatum* (1), *Fusarium oxysporum* (1), *Fusarium incarnatum (1), Fusarium chlamydosporum* (1), *Epicoccum* sp. (3), *Epicoccum sorghinum* (4), *Cochliobolus* sp. (1), *Curvularia lunata* (12), *Curvularia mebaldsii* (2), *Curvularia panici* (1), *Curvularia trifolii* (4), *Curvularia clavata* (2), *Curvularia petersonii* (1), *Curvularia hominis* (1), *Curvularia akaiiensiis* (1), *Bipolaris bicolor* (2), *Bipolaris cynodontis* (2), *Bipolaris simmondsii* (1), *Bipolaris* sp. (1), *Penicillium citrinum* (1), *Setosphaeria rostrata* (1), *Alternaria alternata* (1), and *Alternaria* sp. (1) as shown in Figure 5.4. The 28S rRNA sequences are related to *Exserohilum rostratum* (3), *Sarocladium kiliense* (2), *Sarocladium* sp. (1), *Fusarium equiseti* (1), *Fusarium pseudocircinatum* (1), *Fusarium annulatum* (1), *Fusarium oxysporum* (1), *Fusarium chlamydosporum* (1), *Epicoccum nigrum* (2), *Epicoccum proteae* (1), *Epicoccum sorghinum* (4), *Cochliobolus kusanoi* (1), *Cochliobolus hawaiiensis* (1), *Curvularia lunata* (16), *Curvularia akaiiensis* (1), *Curvularia hawaiiensis* (1), *Curvularia trifolii* (2), *Curvularia geniculata* (2), *Curvularia coatesiae* (2), *Bipolaris woodii* (4), *Bipolaris cynodontis* (2), *Phoma* (1), *Penicillium citrinum* (1), *Setosphaeria rostrata* (1), *Alternaria alternata* (1), and *Alternaria alstroemeriae* (1) as shown in Figure 5.6. The fungal composition in terms of plant parts from which they were isolated

per fungal genera are as shown in Figure 5.7. Most *Curvularia* species were isolated from the neck while finger and leaves had the same number of isolates. The unassigned fungi, *Setosphaera*, *Penicillium*, *Phoma* and *Sarocladium* were only isolated from the fingers while *Alternaria* was obtained from the neck (Figure 5.7).

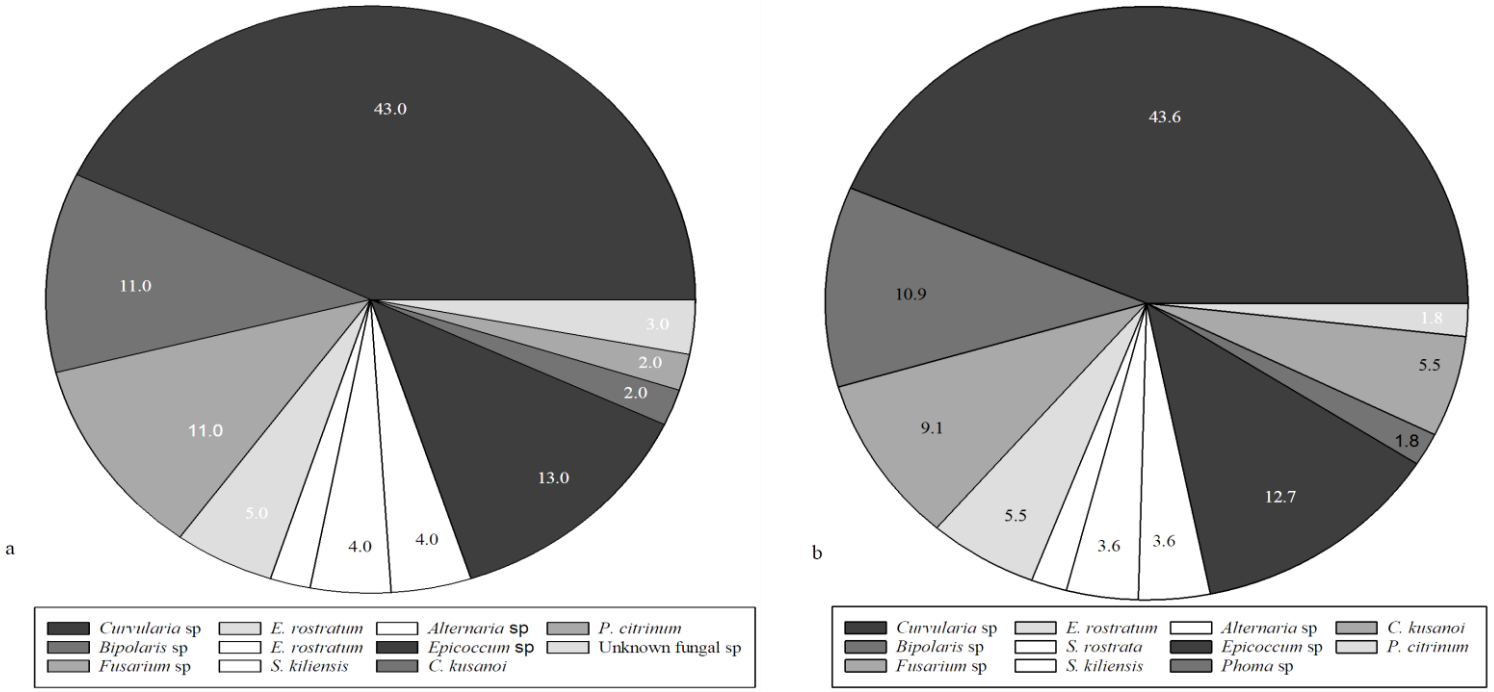

Figure 5.6: Proportional distribution of different fungal species obtained.

Key: (a) Fungal isolates identified using ITS primers (b) fungal species identified using 28S rDNA primers

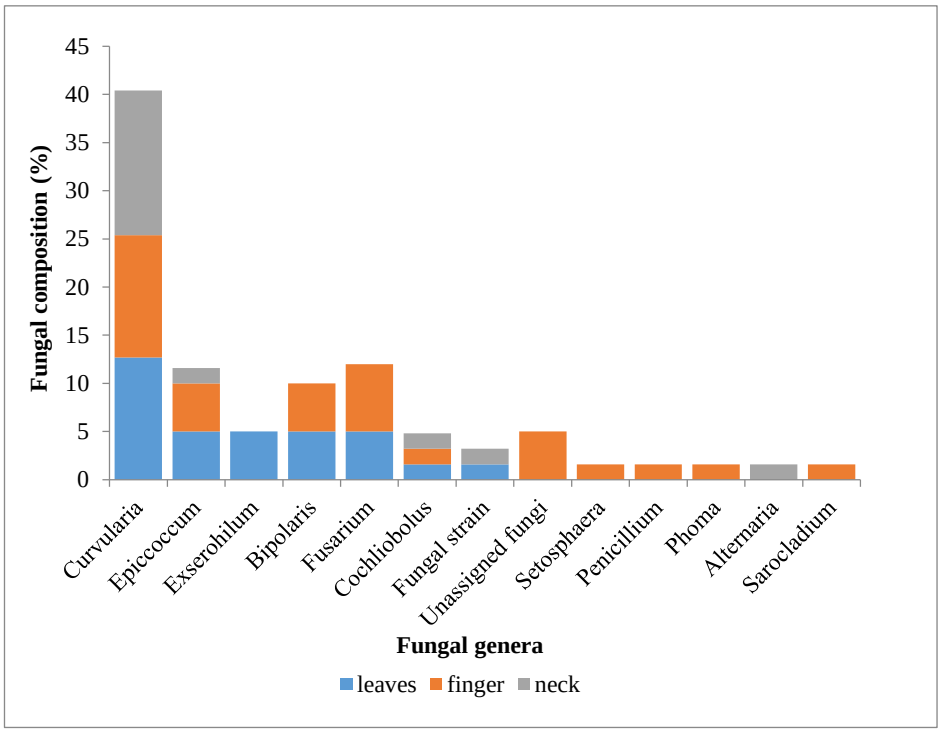

Figure 5.7: Number of fungal genera per finger millet plant part infected by the blast.

5.4.4 Community structure analysis

Three phylogenetic trees were constructed and the relationships between all isolates were evaluated for ITS and 28SrDNA sequences, as shown in (Figures: 5.8, 5.9 and 5.10) respectively. Each dataset consisted of 55 fungal isolates recovered after culture. A further 33 and 19 sequences for ITS and 28S rDNA, respectively, including the outgroup species, were retrieved from GenBank. Several clustering methods including Neighbor-joining, maximum-evolution, and unweighted pair group method with arithmetic mean (UPGMA) generated nearly identical topologies in MEGA.

The optimal trees generated by the individual ITS and 28S rDNA sequences as well as their concatenated sequences together with their relatives yielded similar topologies. The trees were divided into two major groups based on the three classes

of Phylum Ascomycota. The first category consisted of *Fusarium* species, *Sarocladium* and *Penicillium* of classes Sordariomycetes and Eurotiomycetes. Notably, isolate BU188-37 was the only isolate in the three trees that clustered with *Penicilium citrinum*, a member of the Aspergillaceae family, which is known for secondary metabolite production. The second category was assigned to members belonging to class Dothidiomycetes including *Curvularia* species, *Exserohilum, Bipolaris, Cochliobolus, Alternaria, Epicoccum* and *Dothidiomycete* sp. (Figures 5.8 and 5.9). *Setosphaeria* (synonym for *Exserohilum*) and *Phoma* formed an ambiguous branch and were therefore removed from the tree. All trees displayed a high species richness of genus *Curvularia,* since a majority of the isolates clustered with a number of its species. *Methanoculleus thermophilus* was used as an outgroup to root the tree. BLASTN and phylogenetic analyses both indicated that a total of 11 genera for 28SrDNA and 10 together with two unnamed fungi for ITS were generated from this study. The phylogeny of the concatenated sequences (Figure 5.10) similarly divided the tree into two major categories of three classes of Phylum Ascomycota namely Sordariomycetes, Eurotiomycetes and Dothidiomycetes. This validates the phylogenetic placement of all the fungal species isolated in this study. It further validates the identity of the fungal isolates obtained in this study.

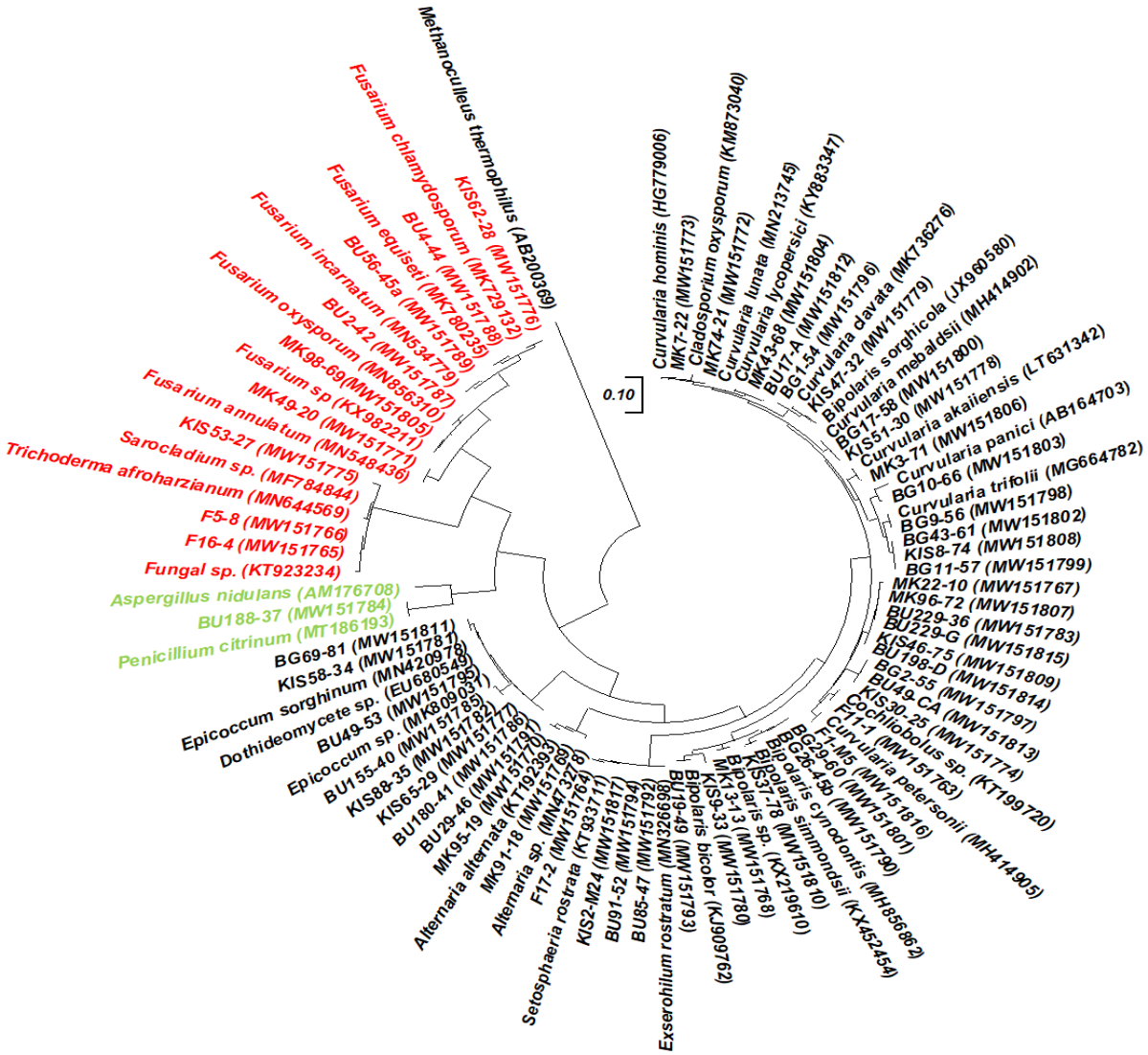

Figure 5.8: Phylogeny is based on the ITS sequences of 55 fungal genera

Key: *Methanoculleus thermophilus* is the out group. Evolutionary analysis was inferred using the Neighbor Joining method at 1000 bootstrap. Red colour represent Class Sordariomycetes, green represents Class Eurotiomycetes and black represents Class Dothidiomycetes all of the Phylum Ascomycota.

Although the total numbers of genera found were the same for both genes (Figures 5.5 & 5.6), the total species count was slightly higher for 28S rDNA than for ITS (Table 5.1). The fungal species identified varied among the different blast-infected finger millet tissues as shown in (Figure 5.7).

The genus *Curvularia* made up the highest proportion of the species obtained from all of the sampled plant tissues compared to the other genera, representing 44% of the composition of fungal isolates followed by *Epicoccum* (13%) and *Fusarium* (13%). Notably, genera *Curvularia* and *Epicoccum* were represented by species in all of the sampled blast-infected finger millet tissues (finger, leaf and neck). The genera *Sarocladium, Bipolaris,* unassigned fungal strain and *Fusarium* each had at least one species obtained from either finger and neck or finger and leaf but not from all three blast infected plant tissues (Table 5.1). The rest of the genera, namely *Setosphaeria*, *Cochliobolus*, *Exserohilum*, *Penicillium* and *Alternaria* each had only one species from the sampled blast-infected plant tissues (Table 5.1). The fungal communities recovered from the five sampling sites did not show a distinct distribution, and the fungal taxa were randomly distributed across the different sites. Fungal richness was mainly observed in the western agro-ecological zone made up of Busia, Bungoma and Kisii, where a total of 39 fungal species were recovered, compared to the eastern agro-ecological zone (Machakos and Makueni) with only 16 fungal species.

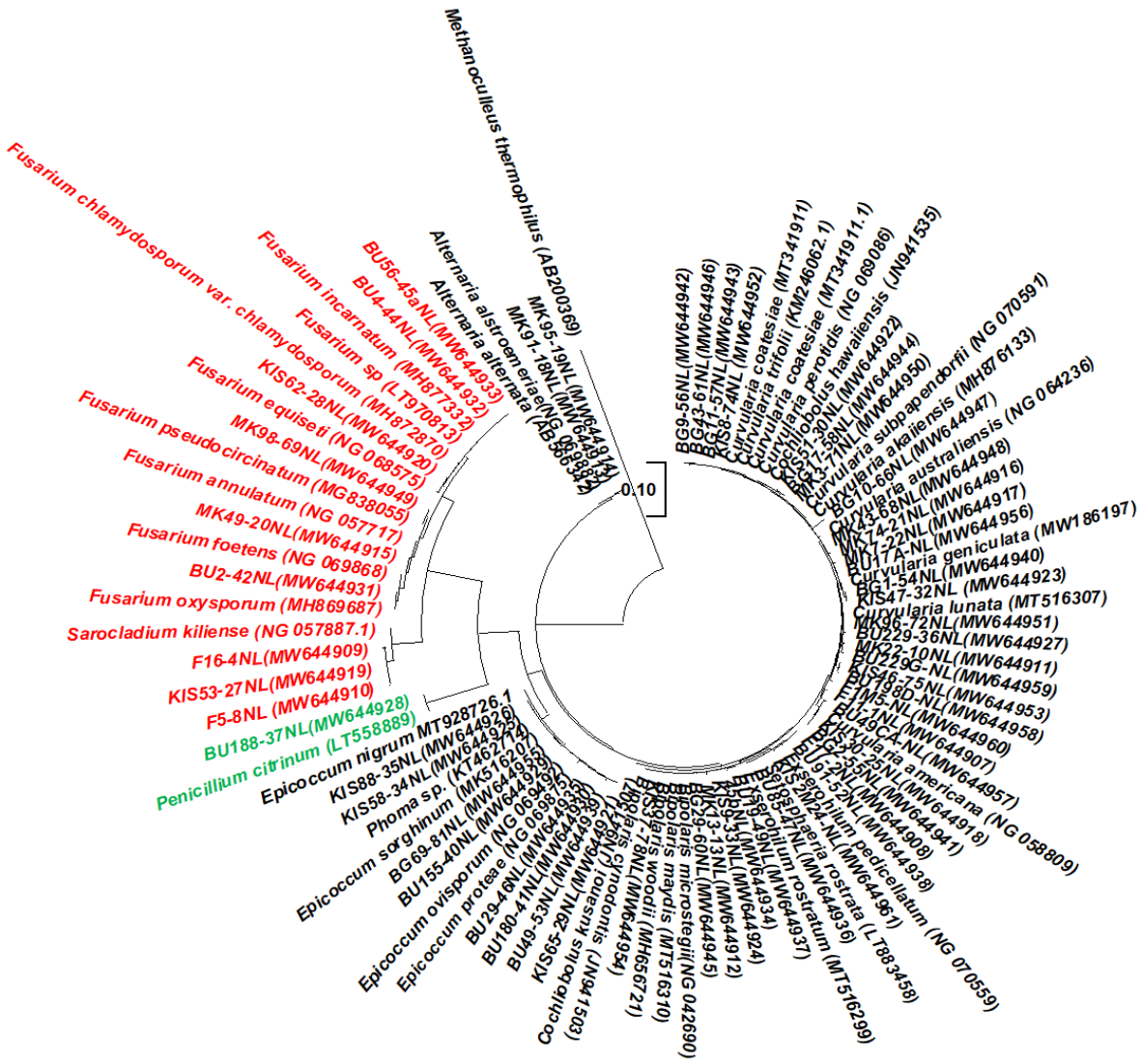

Figure 5.9: Phylogeny is based on the 28S rDNA sequences of 55 fungal genera

Key: *Methanoculleus thermophilus* is the out-group. Evolutionary analysis was inferred using the Neighbor Joining method at 1000 bootstrap. Red colour represent Class Sordariomycetes, green represents Class Eurotiomycetes and black represents Class Dothidiomycetes all of the Phylum Ascomycota.

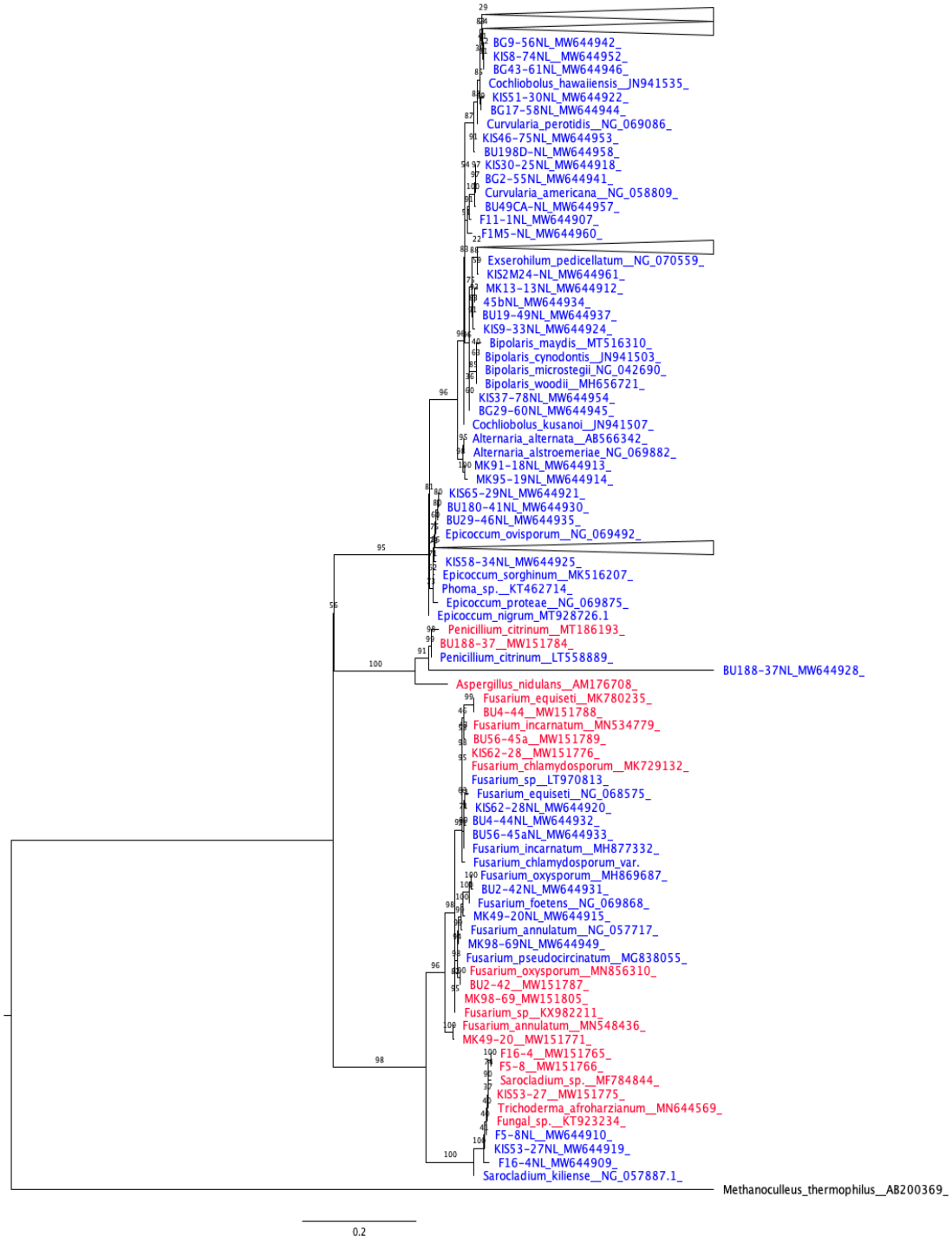

Figure 5.10: Phylogenetic tree for the concatenated sequences of ITS and 28S rDNA regions.

Key**:** Red colour represent ITS sequences while blue colour represent 28SrDNA sequences

5.5 Discussion

This is the first report of fungal species associated with the finger millet blast disease pathogen P. oryzae that combines multigene sequence phylogenetic analysis with a morphological approach. Our study identified twenty-six species based on the 28SrRNA gene and twenty-eight species plus two unnamed fungal species with the ITS gene as fungi coexisting with *P. oryzae* during finger millet blast infection.The genera identified included *Cochliobolus* (*C. kusanoi, C.hawaiiensis, Cochliobolus* sp.); *Setosphaeria* (S. *rostrata*);*Sarocladium* (*S. kiliensis, Sarocladium* sp.); *Curvularia*(*C. hawaiiensis, C.geniculatus, C. lunata, C. coatesiae, C.hominis, C. petersonii, C. panici, C. clavata,* C. *trifolii, C.mebaldsii*); *Bipolaris* (*B. woodii, B. cynodontis, B. bicolor,B. simmondsii, Bipolaris* sp.); *Fusarium* (*F. annulatum, F. pseudocircinatum, F. chlamydosporum,* F. *oxysporum, F. equiseti, F. incarnatum, Fusarium* sp.); *Alternaria* (*A.alstroemeriae,* A. *alternate, Alternaria* sp.); *Epicoccum* (*E.nigrum, E. proteae, E. sorghinum*); *Exserohilum* (*E. rostratum*);*Penicillium* (*P. citrinum*) and *Phoma*. Overall, thesefungal species were grouped into three classes of Phylum Ascomycota-Eurotiomycetes, Dothiodeomycetes and Sordariomycetes—for both genes.The results from this work show that *Curvularia* species were the most frequently isolated in all the blast-infected tissues. *P. oryzae* is known to be a weak saprophyte (Rajashekara *et al.,* 2016). Growth of the blast fungus on media was likely eclipsed by the genus, which is why most of the fungal isolates were from the genus *Curvularia* at the expense of *P. oryzae*.

Accurate identification of *P. oryzae* causing finger millet blast can therefore easily be clouded by the necrotic spots exhibited by *Curvularia* species on blast-infected finger millet samples. However, in culture, the visual characteristics of the two can be easily differentiated.The mycelium for *Curvularia* tends to have a greyish black appearance on oatmeal agar media with a black underside (Kusai *et al.,* 2015), while that of blast fungus displays features ranging from white to greyish/black (Gowrisri *et al.,* 2019 ; Longya *et al.,* 2020).

Saprophytic fungi such as *Alternaria* and *Curvularia* species are important because they primarily infect necrotic lesion centres from behind the advancing front of parasitic mycelium(Rajashekara *et al.,* 2016). These findings further identified *Curvularia lunata* as the most common compared to other *Curvularia* species. This genus is made up of more than 40 species, which include saprophytes, endophytes and pathogens. This pathogenic group has been implicated in heavy losses among cereals and vegetables because they form necrotic spots on the leaves of these plants (Kusai *et al.,* 2015; Kee *et al.,* 2020; Khemmuk *et al.,* 2016 ;Schoch *et al.,* 2020). From these findings, the role played by the identified fungal isolates in *P. oryzae* pathogenesis was not clear. Perhaps some of the microbes could be enhancing the aggressiveness of *P. oryzae* during colonization. For example, *Epicoccum nigrum* has been identified as a beneficial co-inhabiting fungus in sugarcane due to its secretion of cell-wall-degrading enzymes that facilitate the successful invasion of plant cell walls and ultimately cause disease, especially during the saprophytic phase of the fungal cycle (Kubicek *et al.,* 2014). A comprehensive study is therefore recommended to understand the impacts of some of the identified species on the severity of the blast disease and the likely endophytic co-inhabitants in finger millet.

A. alternata has a wide host range and is known to be prevalent on the surface of finger millet seeds as well as a causative agent for leaf spots and other diseases in several plants (Jain 2020). This fungus has been identified as seed-borne mycoflora in sorghum and foxtail millet together with *Curvularia lunata*, *Aspergillus flavus*, and *Phoma* sp. in Korea (Yago *et al.,* 2011). *A. alstroemeriae* has also been implicated as the causative agent for black spot on Alstroemeria, a perennial ornamental plant(Yamagishi *et al.,* 2009).This is the first report of its presence in the finger millet plant, a finding that requires further investigation.

Fusarium is a widely distributed genus of pathogens that affects several plants. Finger millet and other millets including proso, pearl and foxtail millets have been identified as hosts to various species of this pathogen. *F. equiseti* is known to cause ear rot in foxtail millet, while *F. oxysporum* is very destructive to most millet varieties (Chala *et al.,* 2019). Other than causing diseases, *Fusarium* species are also

known to produce mycotoxins that contaminate the millet, making it unfit for human consumption (Akanmu *et al.,* 2013). *F. chlamydosporum* has been implicated in mycotoxin contamination of millet, although it tends to be host-selective and region-specific. *F. solani* has previously been obtained from African millet (Choi *et al.,* 2021). Several other *Fusarium* species not isolated in this study have also been implicated as pathogens associated with millets. Some of these include *F. nygamai, F. compactum, F. fujikuroi*, and *F. semitectum* (Choi *et al.,* 2021).

Bipolaris species have also been recorded as causative agents for many diseases in cereals and grasses. *B.australiensis,* for example, is pathogenic in *Chloris* and *Pennisetum* species in Australia, India and Kenya. This species has also been implicated as the causative agent of brown leaf spot in *Cynodon* spp. and pearl millet as well as leaf spot in betel vine (*Piper betle*) and date palm from China, India, Pakistan and Iraq (Fang *et al.,* 2007). More recently, it was also established as a pathogen in foxtail millet in Iran (Mirzaee *et al.,* 2020). Other species such as *B. oryzae, B. cynodontis*, *B. sorghicola* and *B. victoriae* have been involved as causal agents of rice brown spot disease in Iran (Nazari *et al.,* 2015). In finger millet, *B. setariae* has been reported as a pathogen (Jain 2020) but not *B. bicolor*, *B. cynodontis, B. woodii* or *B.simmondsii*. In Kodo millet, *Sarocladium oryzae* has been implicated as the causal agent for sheath rot, which causes grain discoloration, thus lowering grain quality (Nagaraja *et al.,* 2016). This fungus has also been detected in rice residues and several weed species present in rice fields in India (Yadav and Thrimurty 2006).

Cochliobolus lunatus has been detected as the causal agent of rice sheath rot, which causes black kernel disease in India and Bangladesh (Bigirimana *et al.,* 2015). In this study, *C. kusanoi, C. hawaiiensis*, and *Cochliobolus* sp. were identified in finger millet. *Exserohilum rostratum* is reported in finger millet for the first time in this study.

Some *Exserohilum* species have been reported as pathogenic among the small millets, including *E. oryzicola*, which has been identified as the causal agent of leaf spot in barnyard grass in Japan. This species has also been pathogenic in rice, barley, bread wheat and durum wheat, with milder but nonpathogenic foliar symptoms in soybean (Tomioka *et al.,* 2021). *E. turcicum* is the causal agent for maize northern leaf blight (NLB) and has been reported as a serious pathogen in maize in East Africa and elsewhere (Nwanosike *et al.,* 2015).

Isolate BU188-37 clustered with *Penicillium citrinum*. Although this fungus causes mold in maize and rice (Gowrisri *et al.,* 2019), the genus *Penicillium* is largely known for producing commercially valuable secondary metabolites such as alkaloids, antibiotics, hormones and mycotoxins (Shahid *et al.,* 2020). The results therefore suggest that isolate BU188-37 could possess valuable secondary metabolites whose potential needs to be exploited.

Although the distribution of isolated fungi varied across the agro-ecological zones, *Curvularia*, *Epicoccum*, *Bipolaris* and *Fusarium* were the most dominant genera in all four of the agro-ecological zones under study. Moreover, the samples collected from Kisii County showed higher fungal community richness than those from Machakos. The observed variation in fungal composition could be associated with the environmental characteristics of the different agro-ecological zones where the samples were collected. For example, certain areas in eastern Kenya experience a dryer environment as opposed to the wet and humid conditions in western Kenya. In previous study on the occurrence, distribution and severity of finger millet blast disease in Kenya (Chapter 4 of the study), it was reported that blast disease varied according to ecological characteristics (Odeph *et al.,* 2020). The high humidity in some agro-ecological zones could facilitate the relatively high percentage of finger, leaf and neck infections. A similar observation was reported for *Colletotrichum* species associated with anthracnose in avocados (Sharma *et al.,* 2017).

The findings further revealed that of the three sampled tissues infected with finger millet blast, the percentage of fungi isolated from fingers was significantly higher (46%) compared to leaves (38%) and neck tissues (16%). *P. oryzae* is a hemibiotrophic pathogen, and it combines both biotrophic and necrotrophic characteristics. The pathogen attacks all plant tissues by sustaining both lifestyles concurrently (Marcel *et al.,* 2010). The mechanism of pathogenesis is hypothesized to be the same in all plant tissues. However, it is now clear that the fungus has tissue invasion preferences. The ability of *P. oryzae* to use either hyphopodia or appressoria allows the pathogen to modify its invasion mechanisms to the physiological and nutritional characteristics of the target organ (Tucker *et al.,* 2010). This raises questions as to the degree of similarity between finger, leaf and neck infection approaches. The fungus, therefore, combines the common and organ-specific components described in the *P. oryzae* genetic toolbox to achieve its initial infiltration. From our findings, we could not specifically deduce why more fungi species were isolated from finger tissues compared to the leaf and neck.

CHAPTER SIX

SCREENING FOR HOST TOLERANCE OF THE BLAST PATHOGEN IN SELECTED KENYAN FINGER MILLET VARIETIES

6.1 Introduction

Finger millet is a low-value crop mainly grown under rainfed conditions by resource-poor farmers living in arid and semi-arid regions of sub-Saharan Africa and Asia. A majority of finger millet farmers do not employ chemical control techniques to mitigate the effects of blast disease owing to their limited resources. The most practical and promising approach to combat finger millet blast disease among smallholder farmers is to employ genetic defence through the development of varieties with genetic resistance (Babu *et al.,* 2013). To ensure that this programme is feasible and prospers well, stable resistance sources must be expeditiously identified and subsequently utilized in breeding. Since the germplasm is the raw material, a broad genetic source is needed now and in the future.

A good reservoir for unique genes that can be included in crop improvement programs is wild accessions and landraces of finger millet (Dida *et al.,* 2021). For instance, host plant resistant lines have been exploited in rice through the introgression of genes from landraces and wild relatives (Yadav *et al.,* 2019). This can also be achieved for finger millet because wild finger millet relatives and landraces are in abundance in eastern Africa. Moreover, these finger millet sources exhibit high diversity and have coevolved with the pathogen under non-exhaustive cultivation mechanisms (Dida *et al.,* 2021). Host plant resistance employs two mechanisms namely tolerance and resistance. Tolerance is the crop's capability to produce under the high manifestation of the fungal pathogen while resistance is the capacity of the crop to inhibit infection of the fungal pathogen (Makani, 2019). So far, complete resistance to blast has not been documented thus the development of blast tolerant finger millet varieties seems to be a good alternative that can be exploited in the control and management of finger millet blast disease.

6.2 Materials and methods

6.2.1 Experimental design

A pot experiment was performed in the greenhouse at the Jomo Kenyatta University of Agriculture and Technology. The experiment was arranged in a complete randomized block design with three replications. The experimental units were the six finger millet varieties namely Okhale, Engundi, GBK 043050(42), GBK 043124(21), GBK043137(17), and Black Eastern. The factors were the two types of treatments using the *Curvularia lunata* and *Pyricularia oryzae* spores as inoculum and disease infection levels. In control treatments, each finger millet variety was treated with sterile distilled water only.

6.2.2 Finger millet seeds pre-germination

At least 30 finger millet seeds of each variety were surface sterilized in 1% sodium hypochlorite for 1 minute and rinsed three times with double distilled water for 5 minutes. This was followed by 70% ethanol sterilization and four rinses of 5 minutes each with double distilled water. The seeds were pre-germinated in 2% water agar media in 9 cm diameter Petri-dishes and incubated at 28°C for 48 hours (Fagundes *et al.,* 2020). The germination rate was determined by seedling emergence and was calculated using the following formula:

$$\text{Germination \%} = \frac{\text{Number of seeds that germinated}}{\text{Total number of seeds}} x100$$

Only the seeds that germinated were transplanted onto pots for subsequent experiments.

6.2.3 Finger millet germination on pots

The seedlings were planted in pots measuring approximately 10 cm x 7 cm x10 cm representing the length, width and height of the container respectively. The pots were filled with three-quarters (120 grams) of sieved forest soil mixed with farm yard manure at a ratio of 3:1 and sterilized by autoclaving for 60 minutes at 121°C four

times. Once cool, 2 g/l of inorganic fertilizer (NPK; 17: 17:17) was added to the soil before transplanting five pre-germinated finger millet seedlings of each variety onto the pots; one at each corner and one at the centre of the pot. The seedlings were then sprayed with 1 g/l of magnesium sulphate once a week early in the morning to boost foliage growth and photosynthesis and 100 ml of sterile distilled water every day. Thinning was done two weeks after planting leaving three to four seedlings per pot. The seedlings were allowed to grow for 4-5 weeks before inoculating them with the fungal pathogen (Tembo *et al.,* 2020).

6.2.4 Fungal pathogen inoculum preparation

Fresh conidia were harvested from a 10-14 day old cultured plate of *Curvularia lunata*; the dominant fungal isolate that coexists with *Pyricularia oryzae* in blast infected tissues and was isolated in this study, by flooding the plate with 5 ml ice-cold sterilized water (for rapid growth of spores) and mixed properly with a spreader. The plates were then kept at 4°C for 2-4 hours to release the spores. The spores were harvested by filtering the suspension from each plate through two layers of cheesecloth to remove mycelia and then diluted in 20 ml of ice-cold sterilized water. The spores were counted using a haemocytometer under a microscope and adjusted to a concentration of $5x10^5$ spores per millilitre of water (Karki and Halterman, 2021b).

6.2.4 Detached leaf infection assay

Healthy full-grown leaves were obtained from 5-8 week old plants ensuring that old (those starting to show yellowing signs) or very young leaves (not –fully developed) were not picked. Leaf sections of approximately 5 cm^2 from 5-week-old finger millet plants were plated on media containing 1% of plant tissue agar, 1.5 ml/l lactic acid, 45 µg/ml of BAP (6-benzyl amino purine) (Aregbesola *et al.,* 2020). The abaxial side of the leaf sections was inoculated with a drop of 10 µl of spore suspensions (5 $x10^5$spore/ml) amended with a surfactant; 1% Tween 20 to maintain universal spore dispersal, using a pipette. The plates were then incubated for 7 days 12-hour photoperiod, 25 °C in a complete randomized design with 3 replicates per treatment.

Leaf sections inoculated with 10 µl sterile distilled water in which 1% Tween 20 was added served as the negative control.

Disease severity was visually observed and assessed as the percentage of leaf area affected. Initial symptoms were observed 3 days after inoculation as black or brown lesions, sporulation and a water-soaked area at the point of pathogen inoculation. In the case of highly susceptible genotypes, subsequent symptoms enlarge and cover the whole leaf after 5 days. The leaves were visually assessed for the appearance of symptoms and disease severity assessed on a scale of 1-9 scale on the fourteenth day after inoculation (Babu, 2011; Karki *et al.*, 2021a). Using the scale disease rating of 1 = the most resistant and 9= the most susceptible(Babu, 2011). A detailed description of the disease rating scale, the severity of symptoms, and disease reactions are presented in Table 6.1. Disease incidence (DI) percentage was calculated using the following formula: D I% =number of infected leaves/the total number of leaves inoculated in each replication x 100% (Babu, 2011).

Table 6.1: Finger millet disease rating scale, symptom description and disease reaction

Disease* rating scale	The severity of symptoms*	Disease reaction*
1	No lesions to small brown specks of pinhead size (0.1-1mm), less than 1% leaf area infected	Highly resistant
2	Typical blast lesions covering 1-5% of leaf area covered with lesions	Resistant
3	6-10% leaf area covered by lesions	Resistant
4.	11-20% leaf area covered by lesions	Moderately resistant
5	21-30% leaf area covered by lesions	Moderately susceptible
6	31-40% of leaf area covered by lesions	Susceptible
7	41-50% leaf area covered by lesions	Highly susceptible
8	51-75% leaf area covered by lesions and many leaves dead	Highly susceptible
9	No lesions to small brown specks of pinhead size (0.1-1mm), less than 1% leaf area infected	Highly susceptible

Key:*Disease rating scale, symptom description and disease reaction adopted from (Babu *et al*., 2013).

6.2.5 Potted plant infection assay

Fifty millilitres of the spore suspension for the selected fungus was poured into a 100 ml sprayer with an adjusted nozzle to produce a uniform film of water droplets. The plants were slightly wounded and then sprayed with fungal inoculum ($5x10^5$ spores/ml). Three other plants per finger millet variety were inoculated with sterile distilled water to act as a control. After 15 minutes post-inoculation, the pots were covered with plastic bags to generate an environment with maximal relative humidity. The plants were allowed to grow in the greenhouse with a 16-hour day/8hour night cycle, a temperature of 28 °C±3 and relative humidity of 60%. Forty-eight hours post inoculation; the plastic bags were removed. The plants were watered regularly and relative humidity was maintained at 60-80%. Visual inspection of inoculated plants for disease symptoms commenced 3 days post inoculation (Babu, 2011; Fagundes *et al.,* 2020; Tembo *et al.,* 2020). Disease incidence assessments and disease severity ratings were carried out as mentioned above using the same scale.

6.2.6 Data collection

6.2.6.1 Agronomic traits

The agronomic traits considered in this study were apparent leaf area index and apparent plant height. The plant height was measured from the base of the plant to the longest fully expanded leaf after every three days. Leaf area index was measured from the base of the longest fully grown leaf to the tip. Leaf width was measured as the distance from side to side of the leaf. Both leaf length and leaf width were used to compute the leaf area index by multiplying the two. The unit of measurement for all these values was in centimetres using a meter ruler.

6.2.6.2 Chlorophyll content determination

Approximately 0.1 g of finger millet plant leaves were cut into smaller pieces and placed in falcon tubes containing 10ml of dimethyl sulphoxide (DMSO) solvent

(Manolopoulou *et al.,* 2016; Wellburn, 1994). The tubes were incubated in a water bath at 60-65°C for one hour and then allowed to cool at room temperature for 30 minutes, filtered and absorption measured at 665nm and 649nm for chlorophyll a and b respectively. Blank determination was performed with DMSO while absorption measurement was done with a spectrophotometer using a VersaMax microplate reader/molecular devices of wavelength bandwidth ≤2nm. Chlorophyll concentration was expressed as µg/ml fresh weight and determined by the following formulae (Wellburn, 1994).

$$\text{Chlorophyll } a \text{ (µg/ml)} = (12.19\ \text{A665} - 3.45\ \text{A649})$$

$$\text{Chlorophyll } b \text{ (µg/ml)} = (21.99\ \text{A665} - 5.32\ \text{A649})$$

Total carotenoids (µg/ml) were calculated using the formula:

$$\text{C x + c} = \frac{(1000\text{A480} - 2.14\text{Ca} - 70.16\text{Cb})}{220}$$

Where A_{665} is absorption value at 665nm, A_{649}= absorption value at 649nm, A_{480} is absorption value at 480nm.

6.3 Data analysis

Data were analysed using Graph pad Prism software version 8.0.2.263 and means separated using Tukeys LSD at a 5% significance level; Analysis of variance (ANOVA) was done for repeated measures of apparent plant height, apparent leaf area index, chlorophyll and carotenoid contents.

6.4 Results

6.4.1 Finger millet seeds pre-germination

All six finger millet varieties seeds responded positively after 48 hours of pre-germination on 20% water agar media (Figure 6.1 a-f). There was no significant difference in the germination rate of all the six finger millet varieties pre-

germination. A germination rate of 100% was achieved (Figure 6.1: a-f) implying that seeds from all the varieties used in this study were viable.

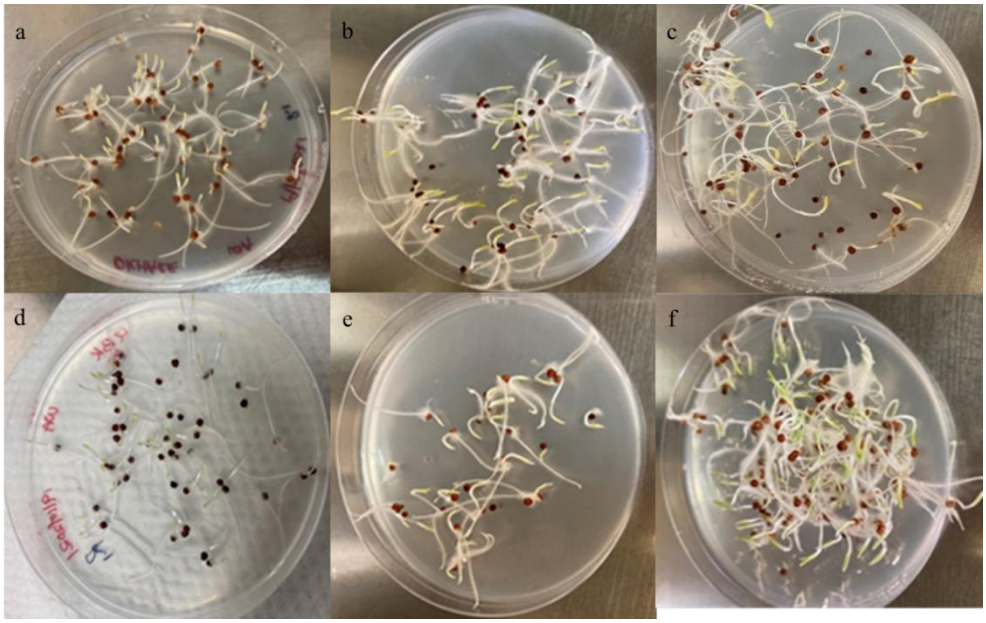

Figure 6.1: Pre-germinated seeds of the six-finger millet genotypes on 20% water agar media.

Key:(a) Okhale seedlings; (b) Black E seedlings (c) Engundi seedlings (d) GBK 42 seedlings; (e) GBK 21 seedlings (f) GBK 17 seedlings.

6.4.2 Detached leaf assay

All the finger millet leaves for all the six varieties tested were visibly diseased after seven days and severely diseased 15 days after inoculation. Figures 6.2 and 6.3 show the detached leaf assays in presence of *C. lunata* and *P. oryzae* respectively. Disease symptom is displayed by the appearance of black lesions on the detached leaves. Disease symptoms developed as time progressed as evidenced by the enlargement of the lesion 7 and 15 days post-inoculation. The control plates are placed side by side with the treated plates.

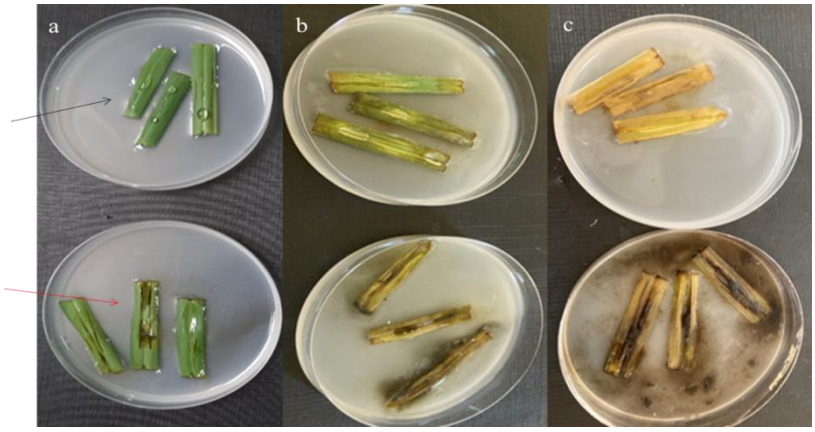

Figure 6.2: Detached leaves obtained from Okhale leaves in presence of sterile distilled water as the control treatment (black arrows) and *Curvularia lunata* spore inoculum treatment (red arrow).

Key:(a) 3 days post inoculation; (b) 7 days post inoculation; (c) 15 days post-inoculation.

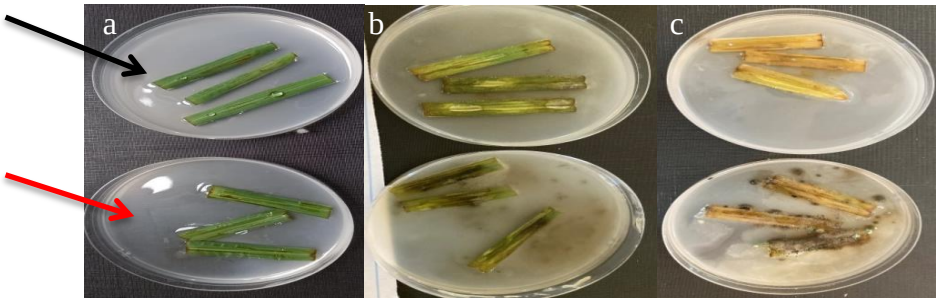

Figure 6.3: Detached leaves obtained from Okhale leaves in presence of sterile distilled water as the control treatment (black arrows) and *Pyricularia oryzae* spore inoculum treatment (red arrow).

Key:(a) 3 days post inoculation; (b) 7 days post inoculation; (c) 15 days post-inoculation.

6.4.3. Potted plants infection assay

All the finger millet plant genotypes exhibited disease symptoms for both treatments with *P. oryzae* and *C. lunata* inoculum in the greenhouse as well. The disease incidence for the potted plants ranged from 65-85% and averaged 74.5% in both *C. lunata* and *P. oryzae* treated plants. However, disease symptoms were more severe in

finger millet varieties treated with *C. lunata* compared with those of *P. oryzae*. Symptoms were visible and widespread in the treated plants as shown in Figure 6.4 (b). Finger millet variety *Engundi* was one of the highly susceptible based on the results of this experiment when treated with spores of both *C. lunata* and *P. oryzae* (Figure 6.5). There was some variation in the levels of disease reaction among the varieties tested giving rise to two levels of disease reactions namely susceptible and moderately susceptible in the potted plant experiments.

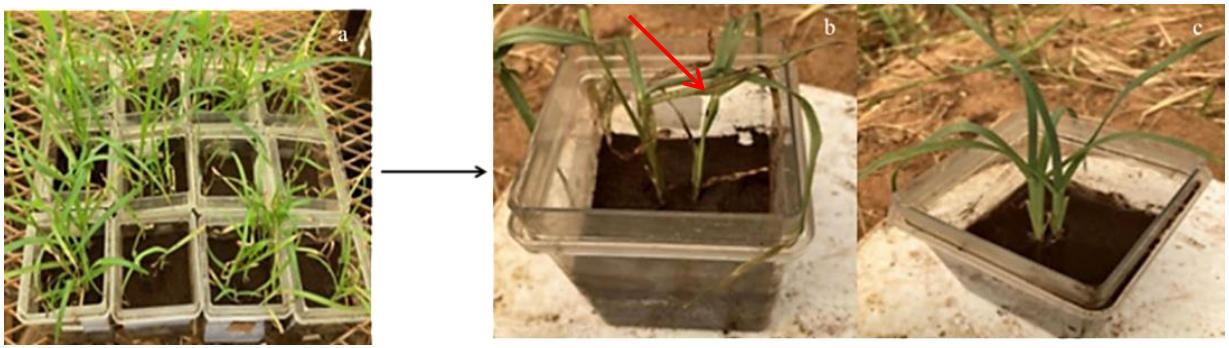

Figure 6.4: Potted finger millet plant with *Engundi* variety treated with *Curvularia lunata*.

Key: (a) 4 weeks old seedlings 7 days post inoculation. (b) Engundi seedlings treated with *C. lunata* spore inoculum 14 days post inoculation; (c) the same finger millet variety treated with sterile distilled water. The red arrow shows the disease symptoms following treatment with *C. lunata* spore inoculum.

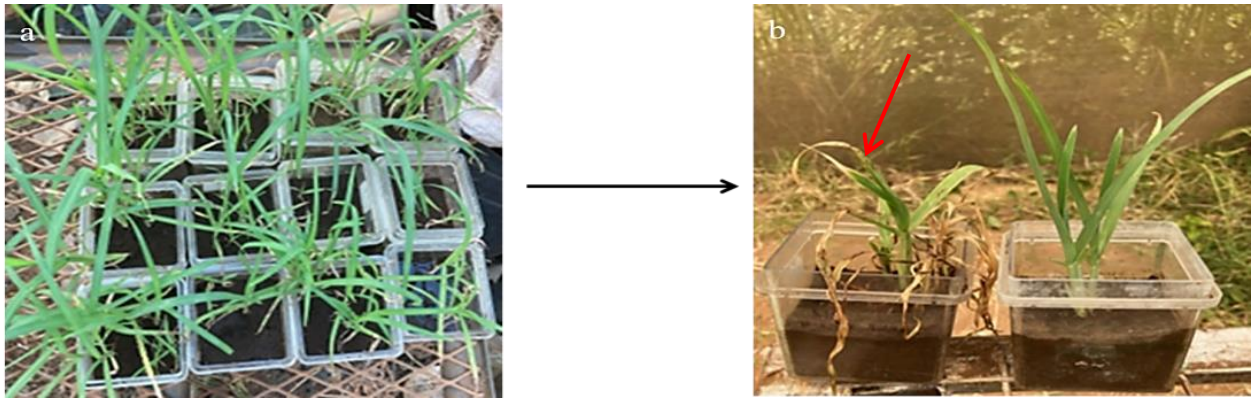

Figure 6.5: Potted finger millet plant with *Engundi* variety treated with *Pyricularia oryzae*.

Key:(a) 4 weeks old seedlings treated with *P. oryzae* spore inoculum 7 days post inoculation, (b) the same finger millet variety treated with *P. oryzae* 14 days post inoculation (c) Engundi seedling treated with sterile distilled water. The red arrow shows the disease symptoms following treatment with *P. oryzae* spore inoculum.

6.4.4 Plant height and leaf area index

There was a progressive increase in both the plant height and leaf area index 14 days post-inoculation, albeit minimal. These results show a significant difference in the plant height among the different finger millet genotypes as shown in Tables 6.2 for plants treated with *C. lunata* and *P. oryzae* respectively. Finger millet variety Okhale had the highest plant height at 5.57cm while GBK 21 had the least (1.90cm) in the presence of *C. lunata* spores (Table 6.2). In the presence of *P. oryzae*, Okhale had the least height (3.70cm) and GBK 21 had the highest (6.96cm) as in Table 6.2. The leaf area index of GBK 17 was the highest (2.12 cm) while GBK 21 was the least (0.78 cm). There was a significant difference in both the plant height and leaf area index among the six genotypes and between the two pathogens as indicated in Table 6.2 at $p \leq 0.05$.

Table 6 2: Plant height and leaf area index of the different finger millet varieties 14 days post inoculation with the *Curvularia lunata* and *Pyricularia oryzae* spores

Finger millet varieties	*C. lunata-induced*		*P. oryzae- induced*	
	Plant height (cm)	Leaf area index (cm^2)	Plant height (cm)	Leaf area index (cm^2)
Okhale	5.57±0.16[a]	1.22±0.23[a]	3.70±0.60	1.11±0.02
GBK 21	1.90±0.21[ab]	0.78±0.16[a]	6.96±0.37[a]	0.65±0.18[b]
GBK 17	2.07±0.32	1.10±0.94	4.73±0.10	2.12±0.56
GBK 42	3.00±0.29[c]	0.36±0.08 [bc]	6.20±0.40[ab]	1.46±0.23 [a]
Engundi	4.13±0.49	1.24±0.18[a]	4.93±0.10	0.69±0.18[a]
Black E	4.00±0.38[b]	0.83±0.35[ab]	4.40±0.45[a]	1.35±0.12

Mean ± standard error of mean. Means with similar letter within columns are not significantly different at $p \leq 0.05$.

6.4.5 Chlorophyll and carotenoids content

The photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids are reliable biomarkers of environmental stress such as disease infection. There were higher chlorophyll content (2.53 $\mu g.ml^{-1}$) and carotenoids (1.69 $\mu g.ml^{-1}$) among finger millet varieties infected with *P.oryzae* spores compared to *C. lunata* infected

varieties. Though a higher chlorophyll-*b* content was observed in *C. lunata* (1.07 µg.ml^{-1}) infected finger millet varieties. There was a significant difference at ($p \leq 0.05$) in the carotenoids, and chlorophyll *a* and *b* content of the six finger millet varieties induced by *C. lunata* and *P. oryzae* as shown in Table 6.3. The finger millet variety GBK 42 exhibited the highest chlorophyll *a* (0.69 µg.ml^{-1}) and carotenoid (0.44 µg.ml^{-1}) content in the presence of *C. lunata* spores and *P. oryzae* (0.64 µg.ml^{-1} and 0.40 µg.ml^{-1}) respectively compared to the other finger millet varieties in this study. Finger millet varieties, GBK 42 and Engundi, on the other hand, were not statistically significant (Table 6.3), indicating that their levels of tolerance were identical. Finger millet variety GBK17 had the lowest chlorophyll-a (0.24 µg.ml^{-1}) when infected with *C. lunata* spores while Okhale (0.13 µg.ml^{-1}) had the least when induced with *P. oryzae* spores. A statistically significant difference in the content of the photosynthetic pigment was observed among the finger millet varieties as well as between the two pathogens at $p \leq 0.05$ as shown in Table 6.3.

Table 6.3: Analysis of chlorophyll a,b and carotenoids content of finger millet varieties inoculated with *Curvularia lunata* and *Pyricularia oryzae* under green house conditions 14 days post in days

Finger millet varieties	***Curvularia-infected***			***P.oryzae- infected***		
	Chlorophyll-a (µg.ml^{-1})	**Chlorophyll-b (µg.ml^{-1})**	**Carotenoids (µg.ml^{-1})**	**Chlorophyll-a (µg.ml^{-1})**	**Chlorophyll-b (µg.ml^{-1})**	**Carotenoids (µg.ml^{-1})**
Okhale	0.25±0.03^{b}	0.15±0.02^{ab}	0.19±0.01^{b}	0.31±0.05^{b}	0.13±0.01^{b}	0.27±0.01^{ab}
GBK 21	0.29±0.02^{b}	0.22±0.05^{a}	0.18±0.02^{b}	0.47±0.05^{ab}	0.20±0.02^{ab}	0.27±0.03^{ab}
GBK 17	0.24±0.01^{b}	0.09±0.01^{b}	0.17±0.01^{b}	0.45±0.04^{ab}	0.17±0.02^{ab}	0.32±0.02^{ab}
GBK 42	0.69±0.04^{a}	0.23±0.03^{a}	0.44±0.03^{a}	0.64±0.07^{a}	0.24±0.02^{a}	0.40±0.04^{a}
Engundi	0.55±0.04^{a}	0.24±0.02^{a}	0.39±0.03^{a}	0.35±0.05^{b}	0.14±0.03^{b}	0.22±0.04^{b}
Black E	0.31±0.06^{b}	0.14±0.01^{ab}	0.20±0.03^{b}	0.31±0.06^{b}	0.14±0.01^{b}	0.21±0.03^{b}

Mean ± standard error of means. Means with similar letters within a column are not significantly different at $p \leq 0.05$.

6.4.6 Disease severity of detached leaf

Inoculation of detached finger millet leaves for all the varieties tested with spores of *C. lunata* and *P. oryzae* resulted in clearly defined necrosis (Figures 6.2 and 6.3 respectively) which rapidly spread across the leaves within a few days. Necrosis was observed in all leaves inoculated with the two pathogens that were also shown to be pathogenic in whole plant inoculations. The symptom development began appearing as early as two days post inoculation except for the ones used in control treatments. The severity scores for the detached leaf assays induced with both pathogens are shown in Table 6.4. There was no difference in the disease intensity for all the finger millet varieties in used in the detached leaf assay.

Table 6.4: Severity score for detached leaf assays for six finger millet varieties inoculated with *Curvularia lunata* and *Pyricularia oryzae* 7 days post infection.

Finger millet variety	*C. lunata*		*P. oryzae*	
	Severity score	Disease reaction	Severity score	Disease reaction
Okhale	7	Highly susceptible	7	Highly susceptible
GBK 21	8	Highly susceptible	8	Highly susceptible
GBK 17	9	Highly susceptible	8	Highly susceptible
GBK 42	9	Highly susceptible	9	Highly susceptible
Engundi	8	Highly susceptible	8	Highly susceptible
Black E	9	Highly susceptible	8	Highly susceptible

6.4.7 Disease severity of potted plants in greenhouse

Severity score was carried out on the treated and control plants on the fourteenth-day post inoculation using a scale of 1-9 (Babu, 2011). Disease severity among the treated finger millet varieties did not vary much. The symptoms started developing in most of the varieties before disease ratings commenced on the 14^{th} day. At the end of the evaluation, four of the finger millet varieties tested displayed disease symptoms that were equivalent to a disease reaction of either susceptible or moderately

susceptible except for variety GBK 42 and Engundi (Table 6.5). These two varieties were able to withstand the effect of both *P. oryzae* and *C. lunata* infection as is shown by statistical analysis at $p \leq 0.05$ that they are similar (Table 6.5). Thus, these both Engundi and GBK 42 can be considered to be fairly tolerant to both pathogens compared to all the other varieties tested in this study.

There was no evidence of resistance to *C. lunata* and *P. oryzae* fungal pathogens among all the varieties tested but necrosis rapidly spread especially in the detached leaf assay experiment. Thus, the results clearly show that all the varieties tested were somewhat tolerant to both pathogens tested and none was resistant.

Table 6.5: Severity score for potted plants for finger millet varieties inoculated with *Curvularia lunata* and *Pyricularia oryzae* at 14 days post inoculation

Finger millet variety	***Curvularia lunata***		***Pyricularia oryzae***	
	Severity score	**Disease reaction**	**Severity score**	**Disease reaction**
Okhale	4.7	Moderately susceptible	4.6	Moderately susceptible
GBK 21	5.2	Moderately susceptible	4.2	Moderately susceptible
GBK 17	4.9	Moderately susceptible	4.3	Moderately susceptible
GBK 42	3.6	Moderately resistant	3	Moderately resistant
Engundi	4.9	Moderately susceptible	5.1	susceptible
Black E	5.6	susceptible	5.2	susceptible

6.5 Discussion

Screening germplasm for disease resistance is a laborious and time-consuming procedure. Results from this study cast a new light on the assessment of finger millet blast resistance by inoculating detached finger millet leaves with *C. lunata* and *P. oryzae* without necessarily destroying the whole plant. This reduces the time between inoculation, disease development and assessment. The detached leaf assay presents a

simple, rapid and non-destructive method of identifying finger millet blast disease reliably thus enabling the process of screening to proceed placidly. Moreover, the inoculation studies carried out in the laboratory permit a controlled environment about temperature, humidity and light in a very consistent manner than in a greenhouse; restricting the pathogen to the laboratory and with minimal space (Miller-Butler *et al.,* 2018; Aregbesola *et al.,* 2020).

In this study, the detached leaf assay method was compared with whole plant greenhouse studies. A comparison of the responses of six finger millet varieties under detached leaf assay and greenhouse reveals that reliable resistance/tolerance assessment can be confirmed within seven days which is equivalent to at least 14 days in the greenhouse. This is consistent with studies done on maize and the response of the maize open-pollinated varieties (OPVs) evaluated using detached leaf assays, screen house and field screens confirmed resistance assessment within 16, 33 and 72 days after inoculation respectively. It, therefore, implies that detached leaf assays take the least time to exhibit disease symptoms (Aregbesola *et al.,* 2020). Another advantage of using the detached leaf assay method is that there are lower risks of obtaining obscure results brought about by the co-infection of diverse pathogens, insect damage, or abiotic variables that may occur in greenhouse conditions. The method also utilized little space, low frequency, cost and reduced duration of the experiment. However, the success of the detached leaf assay is dependent upon leaf tissue viability and utilization of phytohormones.

6-Benzylaminopurine was added to the growth medium to help delay finger millet chlorosis efficiently. This plant growth regulator is an anti-senescence hormone and plays a key role in the maintenance of chloroplast structure thus delaying senescence (Siddiqui *et al.,* 2011; Zavaleta-Mancera *et al.,* 2007; Aregbesola *et al.,* 2020). Despite this, detached plant tissue bioassays have one limitation; there is a very high probability of resistance or pathogenicity reactions changes after excision and the likelihood of degradation of parts before successful completion of the experiment. Normally, upon excision of a plant part, the resistance of the plant part is lowered giving the impression that non-pathogenic isolates were pathogenic (Pettitt *et al.,*

2011). There was no occurrence of such as evidenced by the development of necrosis on the treated leaves and as opposed to the control leaves. The presence or absence of necrosis is an indication of pathogenicity, other parameters that can be utilized to evaluate pathogenicity include the rate of necrosis development and the proportion of positive bioassays. Such observations have been utilized to quantify host resistance and/or the virulence of individual pathogen isolates in detached plant tissue assays on other host species (Pettitt & Pegg, 1994). The results of this study displayed positive necrosis response in the detached leaves of all the tested finger millet varieties implying that isolates with low virulence will equally produce low disease incidence in leaf infection and slower rates of necrosis. This observation may however need further verification on several other germplasms of finger millet for authentication.

Screening results on whole plant assays tie well with several previous studies wherein Babu (2011) in his pathogenicity tests showed that *P. oryzae* isolates from finger millet studies caused moderately resistant to highly susceptible reactions on a particular finger millet variety (VR 708). Similarly, two isolates of *P. oryzae* were singled out as the most virulent in infecting all plant tissues. The study further revealed that the two isolates produced low yields as a result of the infection (Lule *et al.*, 2014). The findings of a study by Wekesa *et al.,* (2019) revealed that none of the finger millet varieties he tested for blast severity exhibited complete resistance to blast disease or even bypass blast disease infestation completely. Instead, the different finger millet varieties he used all had varying degrees of either resistance to blast or susceptibility. The study also showed that disease susceptibility was influenced by the physiological maturity of the plant as opposed to plant height. These results are similar to the current study which showed that there was no substantial reduction of apparent plant height and apparent leaf area indices among all the finger millet varieties tested when inoculated with spores for both pathogens.

The interaction between plants and pathogens activates a series of defence mechanisms including biochemical changes associated with stress signalling and thus triggers the plants' defence pathways to repel the pathogens (Kaur *et al.,* 2022). The success of this mechanism solely relies upon their expeditious defence response

commencement which requires an apparent conception of the plant's ability to discern pathogen attack and control the expression of defence mechanisms (Patel *et al.,* 2020). Plants can deal with diverse environmental stresses with the leaves revealing the most noticeable changes (Mukherjee *et al.,* 2019). Whenever a plant encounters a biotic or abiotic stressful situation, the ROS production is enhanced leading to substantial damage to the cellular components of the plant. Consequently, the plants' inherent antioxidants are released to counteract the ROS and thus safeguard the plants' cellular components (Patel *et al.,* 2020).

Studies have shown that plant leaves exposed to high amounts of harmful air pollutants have equally high build-up biochemical compounds such as phenol, proline, malondialdehyde and cellulose with reduced amounts of chlorophyll (Mukherjee *et al.,* 2019). It therefore implies that environmental stresses greatly impact the synthesis of bioactive molecules to circumvent the effect of stress on the plant. Moreover, diverse defence-related genes specifically encoding pathogenesis-related (PR) proteins innate in plants confer resistance to pathogens associated with their potential to keep them off. Thus, the host plant resistance mechanism can be investigated by assessing the changes in defence-related marker enzymes reacting to external stimuli such as biotic and abiotic determinants (Kavino *et al.,* 2009).

Results from this study revealed that plants treated with *P. oryzae* displayed higher chlorophyll *a* and carotenoid pigments content compared to those treated with *C. lunata*. However, higher chlorophyll-b content was observed among finger millet varieties inoculated with *C. lunata* spores than *P. oryzae* (Table 6.3). Chlorophyll plays an essential role as the photosynthetic reservoir that synthesizes light energy into chemical energy. Plants exposed to elevated stressful conditions tend to exhibit a reduced chlorophyll content that is correlated with decreased green colouration in plants which translates to reduced photosynthetic rates and thus stunted growth (Mukherjee *et al.,* 2019). However, in this study, finger millet varieties GBK 42 (0.64mg/ml), GBK 17 (0.45mg/ml) and GBK 21 (0.47 mg/ml) displayed very high chlorophyll *a* content in presence of *P. oryzae* spores compared to their control plants GBK 42 (0.31 mg/ml), GBK 17 (0.26 mg/ml) and GBK 21 (0.26 mg/ml).

These results are in agreement with John *et al.*,(2010) whose findings showed that soybean plants treated with *Pythium arrhenomanes* had a higher chlorophyll-a content than those treated with *Fusariunm oxysporum f.sp adzuki*. In a similar study, chickpea beans treated with *Micrococcus luteus* also displayed high content of photosynthetic pigments namely chlorophyll *a*, chlorophyll *b* and total carotenoids (Patel *et al.,* 2020). Several studies have associated a reduction in chlorophyll content with drought and salinity stresses and that chlorophyll maintenance corresponds with drought and salinity tolerance, however similar works concerning pathogen stress are limited (Mukami *et al.,* 2020; Monteoliva *et al.,* 2021).

Carotenoids are effective antioxidants in the plant defence system. They safeguard the photosynthetic system by employing three main strategies. First, they react with the products of lipid peroxidation to halt the chain reaction, hunt for free radicals, singlet oxygen and heat dissipation, or prevent the oxygen formation by reacting with chlorine and the excited chlorophyll. A study on peanut plants infected by *Rhizoctonia solani* showed that infected peanut plants had higher amounts of carotenoids than healthy ones (Lakshmi *et al.,* 2011). A similar study by Hadi and Kholdebarin, (2018) showed that potato plants infected with *Rhizoctonia solani* and treated with salicylic acid revealed a higher carotenoid content. High levels of carotenoids greatly elevated the potato plant's immune systems as a result of the strong antioxidant property of carotenoids in the plant, leading to a lower disease incidence. Carotenoids which are a group of lipophilic antioxidants have been associated with providing plant defence by detoxifying diverse forms of ROS that are elicited during pathogen infection(Patel *et al.,* 2020). The quantity of photosynthetic biomolecules is an indicator of tolerance level when plants are challenged with either *Curvularia lunata* or *Pyricularia oryzae*. The abscisic acid (ABA) is considered as a stress hormone since various conditions induce its synthesis. The ABA-responsive gene expression is regulated by several transcription factors. Stress related genes can be expressed either through an ABA-dependent or ABA-independent mechanism (Tuteja, 2007). The first extracellular stress signal is detected at the cell membrane by membrane receptors, ion channels, receptor-like kinase or histidine kinase that stimulates a large and complex intracellular signaling pathways that lead to the

production of secondary signal molecules such as Ca^{2+}, inositol phosphates, reactive oxygen species and abscisic acid (Chinnusamy *et al*., 2004). A number of stress sensitive genes are then activated by the stress signal inside the nucleus, and these genes' products ultimately help plants adapt to stress by either directly or indirectly increasing their tolerance to it (Mahajan & Tuteja, 2005). This study, therefore, opens a new chapter that screening for chlorophyll content is a simple and practical strategy to identify pathogen tolerant crops to complement other agronomic traits.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General discussion

The findings of this study revealed that farmers had limited knowledge of causes of blast, identification, detection and coping mechanisms. This could explain why the blast disease is so widespread in Kenya causing low finger millet yields. Farmers' perception of blast disease control was also inconsistent with established practices. These findings are similar to studies by Mgonja *et al.,* (2007) in East Africa and Owere *et al.,* (2014) in Uganda. There were more farmers (> 60 %) in Busia and Kisii compared to those of Bungoma, Machakos and Makueni counties who could identify the blast disease symptoms but didn't know its name. Moreover, reduced production and productivity of finger millet due to negative attitudes which has negatively impacted farmers' livelihoods thus lowering their socioeconomic status. A study by Handschuch & Wollni, (2016) indicated that most farmers in Western Kenya have abandoned finger millet cultivation for crops such as maize and rice due to low returns, poor yield and intensive labour.

This study revealed a serious finger millet blast disease occurrence, severity and distribution on all the farms visited. The study was able to postulate the quantitative measurement of finger millet blast occurrence, distribution and severity. For example, a 100% blast occurrence was observed in all the farms within all surveyed counties with Busia county having the highest severity score at 82% of the crops on the farms. This was and is critical in determining the geographical distribution and disease status throughout the country to prioritize research. The results of this study are similar to that of Gashaw *et al.,* (2014) in Ethiopia which were able to identify finger millet blast disease distribution and severity according to the geographical regions. The blast disease was more severe and densely distributed in the Western agroecological zones especially Busia and Kisii counties than in the Eastern. This could be attributed to the prevailing weather conditions on the farm This position is coherent with earlier studies done by Oduori (2008). Their study revealed high blast

incidence in Busia, Teso, and Kisii counties causing yield losses of 10-80% in both Kenya and Uganda. Studies by Owere (2013) also confirmed that finger millet blast was endemic in Uganda but disease severity varied depending on prevailing weather conditions on the farm

This study also shows that there was little variation in the type and number of fungal species identified by both ITS and 28S rDNA markers from blast-infected tissues. Several studies have utilized both markers to identify pathogenic fungal species with great success (Longya *et al.,* 2020; Fajarningsih, 2016; Xu *et al.,* 2016). The findings revealed fifty-five fungal species that are known plant pathogens including *Curvularia lunata*. Several studies have isolated and identified *P. oryzae* but this study did not manage to (Jia, 2009; Rajashekara *et al.,* 2016; Gupta *et al.,* 2020). Consequently, *Curvularia species* were the more frequently isolated fungus in this study, specifically *Curvularia lunata*. Thus the study sought to evaluate the fungal species that coexist with *P. oryzae* instead. Bungoma county had the highest number of *Curvularia* species (7) while Machakos county had the least (1). The results of this study also suggest that finger millet blast perhaps is caused not only by *Pyricularia oryzae* but rather by a fungal species complex. A total of 10 fungal genera and 2 unnamed fungal species are reported. The diverse genetic structure of fungal communities associated with blast disease attests to the probability of their association with *P. oryzae* during pathogenesis. Studies have revealed that plant tissues are colonized by highly diverse microorganisms whose pathogenesis may be suppressed or aided by the ubiquity of other microbes (Card *et al.,* 2016; Busby *et al.,* 2015; Latz *et al.,* 2018). Although this study did not explore their role, it was able to ascertain the identity, taxonomy and phylogenetic placement.

This study further explored the capability of six-finger millet varieties cultivated by Kenyan farmers to either resist or tolerate the *P. oryzae* or *C. lunata* by employing two methods: detached leaf assay in the laboratory and whole plant experiments in the greenhouse. The detached leaf bioassay has been successfully utilized in many screening studies but is less applied in studies involving finger millet plants (Aregbesola *et al.,* 2020; Pettitt *et al.,* 2011; Miller-Butler *et al.,* 2018). Though, the

method suffers from one or two limitations it gave an accurate prediction of what was to follow in the greenhouse studies. It showed that all the six-finger millet varieties were colonized by the pathogen and produced necrosis on the detached leaf tissues treated with both *C. lunata* and *P.oryzae*. This was also the case in the greenhouse studies which showed that the disease reaction in all the six varieties ranged between highly susceptible, moderately susceptible and susceptible to both pathogens. The finger millet variety GBK 42 proved to be the most tolerant to both pathogens compared to the other five.

7.2 Conclusions

- In all the counties surveyed, it was clear that the knowledge and practices of all the farmers lacked scientific backing and this could be the reason for the rampant spread of finger millet blast
- The greatest constraint to finger millet production identified by farmers from all the counties was blast infection. At least 51% of respondents from Kisii county could identify the blast symptoms at the vegetative stage.
- The farmers were unaware of the availability of blast-resistant varieties suggesting a weak link between the farmer and agricultural extension officers.
- Finger millet blast disease occurred in all the counties visited and was more severe and densely distributed in the Western agroecological zones compared to the Eastern agroecological zone.
- A total of 55 fungal isolates were isolated from blast-infected tissues using ITS and 28S rDNA and were distributed as follows Finger 45.4%, Leaf 38.2% and Neck 14.4%.
- Busia county had the highest number of isolates (16) while Machakos had the least (5).
- The Detached Leaf assay technique provides a reliable and non-destructive method for screening germplasm for resistance or tolerance to pathogens.
- The finger millet variety GBK 42 (GBK 043050) and Engundi were the most tolerant to both *Pyricularia oryzae* and *Curvularia lunata*

7.3 Recommendations

The findings of this study recommend that:

- Great attempts should be made to educate and enhance farmers' approval of the best blast disease management practices including the use of improved/ resistant varieties.
- A deliberate effort to strengthen the weak research-extension linkage for adequate extension service and accurate dissemination of information to the farmers concerning finger millet blast control and management be initiated.
- Identify and recommend resistant finger millet varieties that are well adopted for the different agroecological zones.
- Develop a manual on best agronomic practices for the farmers especially in the Western agroecological zone (Busia County) where the disease is densely distributed.
- A comprehensive study is recommended to understand the impacts of some of the identified species on the severity of the blast disease and the likely endophytic co-inhabitants in finger millet.
- More research on multiple gene markers to characterize and validate identified fungal species.
- Further studies on the qualitative and quantitative traits of all finger millet varieties tested especially, GBK 42 (GBK 043050) and Engundi under field conditions to exploit their ability to tolerate biotic and abiotic stress.
- Use of detached leaf assay as a rapid technique for screening of finger millet varieties.

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APPENDICES

Appendix I: Questionnaire on blast disease caused by *Pyricularia oryzae* affecting finger millet plant.

Part I: GEOGRAPHICAL DESCRIPTION.

a. Questionnaire Number.....

b. County.....Subcounty.....

c. GPS coordinates of sampling sites.....

d. Name of respondent (farmer).....Telephone number of farmer/respondent..... Gender.....

e. Sampling period.....Sample type.....

f. Collection number.....

Part II: Farm Family Head Characteristics

1. Age of Family head a. 20-35 [ ] b. 36-45 [ ] c. 46-60 [ ] d. Above 60 [ ]
2. Farmers gender: Male [ ] Female [ ]
3. Relation of respondent to HH head: self [ ] wife [ ] other (specify).....
4. Level of education for the head of the household

a. None b. Primary c. Secondary d. Tertiary e. University

5. No. of household members?.....
6. What are the main sources of income.....

(1= Formal employment, 2= Farming, 3= casual work, 4= Others (specify).....)

Part III: Production Systems:

7. What is the size of your farm (acres).....
8. Describe the land tenure.....a) Own with title b) Own without title c) Rented in Government land [ ] Community owned [ ]

9. Type of land use practices (a) Crop farming [ ] (b) Mixed farming [ ]

10. What type of cropping system is preferred.

- Monocropping [ ]
- Intercropping [ ]

11. State reasons for growing finger millet.....

(1=Main source of food, 2=Highest source of income, 3=Stable crop in climate variability, 4=Cultural reasons, 5=Others (specify) .....)

12. How many times do you grow finger millet per annum?

- Once [ ].....Short rains [ ] Long rains [ ].

And why.....

- Twice [ ]

13. What makes finger millet the most suited for the season of choice than other crops.....

(1=High yielding, 2=Disease resistant, 3=Drought resistant, 4= Other reasons.....)

14. How long has finger millet been grown in the farm?.....years.

15. What are the uses of finger millet in this area?

- Food [ ]
- Commercial[ ]
- Others.....

	Fingermillet variety	**Local name**	**Reason for variety preference** 1=high yielding, 2= disease resistant, 3=palatability, 4=shelf life, 5=seed availability, other (*specify*)
1.			
2.			

3.			
4			

16. Which finger millet varieties are grown within the farm/region. Indicate local names

17. How long does it take for the different finger millet varieties to be harvested?

Fingermillet variety	**Time of planting** (**Month**)	**Time to maturity (months)**	**Reason for variety preference:** (1=high yielding, 2= disease resistant, 3=palatability, 4=shelf life, 5=seed availability, other (*specify*)

18. Which of these varieties is the most preferred in the market and why?.....
.....

Variety type	Height	Early maturity	Grain yield	Grain colour	Grain size	Head structure	Palatability
1							
2							
3							
4							

19. What soil fertility practices do you apply for finger millet farm.

Fertilizer and other soil nutrient practices applied in the farm			
Type of fertilizer or manure	**Quantity (kg)**	**Application time:1= before planting, 2= at planting,3=top dressing**	**Frequency of application per season**

20. Are there any other products obtained from finger millet farming other than the seeds? If yes which ones.

a) _____

b) _____

c) _____

d) _____

21. What examples of value added products are obtained from finger millet?

a) _____

b) _____

c) _____

d) _____

Farmers' perception on Finger Millet Blast caused by *Magnaporthe oryzae*.

22. Are you aware of blast disease in finger millet? a) Yes [ ] b) No [ ]

23. What is the local nameor vernacular name of blast disease?.....

24. What portion of the farm is affected?.....

(1= whole farm, 2= half way, 3= ¾ way, 4=¼ way)

25. What is the distribution pattern of the diseased plants?.....

(1= uniformly across the area, 2= randomly, 3= localized, 4= Others (specify).....)

26. Which part of the plant is usually affected by the disease?.....

(1= head, 2=Finger, 3= Neck, 4= Leaf, 5= whole plant, 6= Others *(specify)* .....

27. How severe is the disease when it occurs?.....

(**0= no disease symptom, 1= less than 10% plants affected, 2=11-30% of plants affected, 3=31-50% of plants affected, 4= >50% of plants affected)**

28. Which season does it occur most?.....

Short rain [ ] or Long Rain [ ]

29. How often does the disease occurs?.....

(1=Every season, 2= Alternative seasons, 3=Others(*specify*)..........

30. How are you able to identify the symptoms?.....

(**1=Personal examination, 2=Ask a neighbour\friend. 3= Others (specify).....)**

31. In your opinion, are there other disease symptoms that resemble blast disease in finger millet a) Yes [ ] b) No [ ]

32. What are the similarities ..... and differences.....

33. If you have had incidences of finger millet blast on your farm, how do you manage it?.....

1= Do nothing, 2= using inorganic pesticides, 3= using organic pesticides ,

4= using microbe-based-pesticides, 5= abandon finger millet production, 6= Crop rotation, 7= using indigenous technical knowledge, 8= Others *(specify)* .....

34. For the control methods you apply, kindly provide the following information

Disease control (include all chemicals names and other indigenous knowledge)	**Time of application (1=Seed treatment before planting; 2=On farm before planting; 3=Immediately after germination; 4=Before flowering; 5=After flowering; 6=after head formation; 7=when disease symptoms are spotted**	**Effects of control method: Not satisfactory at all; 2= somewhat unsatisfactory; 3=neutral; 4=Somewhat satisfactory; 5=Very satisfactory**

35. How do you manage the diseased plants?.....

1= Feeding to livestock, 2= leave the plant on the farm, 3= transfer finger millet waste from farm to household, 4= Lay the waste in livestock shed 5=Burn the infected plants 6= Others (*please specify)*.....