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EVALUATION OF TOMATO EARLY BLIGHT PATHOGENS' CHARACTERISTICS AND SUSCEPTIBILITY OF LOCALLY GROWN TOMATO VARIETIES TO EARLY BLIGHT INFECTION IN KIRINYAGA COUNTY, KENYA

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill) is a highly consumed and produced vegetable crop with an annual global production of over 185 million tonnes, due to its nutritional and health benefits. However, tomato production is characterized by inconsistent quality and yields partly emanating from biotic constraints attributed to fungal foliar diseases. In Kirinyaga County, where tomato production is a significant source of livelihoods, there exists knowledge gap on the identity of pathogens causing early blight in different Agro-ecological zones (AEZs) and on the susceptibility of locally grown tomato varieties to the infection. The objective of this study was to determine the characteristics of early blight pathogen isolated from tomato leaves collected from different AEZs and assess the susceptibility level of some tomato varieties grown in Kirinyaga County to the early blight pathogens isolates. Pathogen characterization was carried out using morphological and molecular approaches while susceptibility of tomato varieties to fungal pathogens was evaluated by artificial inoculation. Molecular characterization for fungal pathogen involved BLASTIN analysis of DNA amplicon Sanger sequenced nucleotides and use of NCBI databases. The susceptibility assay was carried out in the greenhouse using a completely randomised design (CRD). Data on the fungal conidia lengths (µm) were subjected to Analysis of Variance (ANOVA) and significant means separated by Least Significance Difference (LSD) at $\alpha = 0.05$. Data on the susceptibility (%) of tomato varieties to fungal pathogens isolates was subjected to ANOVA and significance means separated using LSD at $\alpha = 0.05$. Morphologies of early blight pathogen pathogens differed significantly ($p < 0.05$) with conidia lengths from 52.8 and 204.20µm. Molecular characterization of some selected culture implicated *Alternaria solani* and *A. alternate* involvement in causing early blight. Tomato varieties differed in their susceptibility to early blight pathogens ($p < 0.05$). Kilele F_1 was the most susceptible variety (73.84%) while Terminator F_1 was least susceptible (56.48%) to early blight pathogens. It can be concluded that Terminator F_1 was the least susceptible among tomato variety cultivated by farmers in Kirinyaga County and can serve as source for genetic improvement of tomato varieties for Kirinyaga pathosystem.

Keywords: AEZs, Early blight, characterization, tomato susceptibility, Kirinyaga County

INTRODUCTION

Early blight is a destructive and widely spread fungal diseases and may potentially causes tomato yield loses of up to 80% (Adhikari *et al,* 2017; Awan and Shoaib, 2019). Necrotrophic fungus in the genus Alternaria that include *Alternaria alternate, Alternaria solani* and *Alternaria tomatophila,* have been vindicated as the causative agent of early blight diseases (Awan and Shoaib, 2019; Wang *et al.,* 2022). Plants diseases limit chlorophyll levels and enhances build up injurious reactive oxygen species such as superoxide anion ($O2^{-}$), hydrogen peroxide (H_2O_2) which damage biomolecules such as lipids and nucleic acids (Sallam *et al*., 2021; Gulzar *et al.,* 2021). According to Sallam *et al*. (2021) The early blight pathogens further enhances ascorbic acid synthesis while limiting synthesis of antioxidant (Glutathione and soluble phenolic compounds), salicylic acid a stimulant, and catalysts such as polyphenol oxidase and phenylalanine ammonialyase which are necessary in plant protection in times of stress.

Tomato production is practised in different agro-ecological zones (AEZs) of Kirinyaga County ranging from zone LM4 to UM2 (Ogolla *et al.,* 2022a). These AEZs vary in climatic conditions (Jaetzold *et al.,* 2007) and tomato cultivation practices such as variety grown among other factors (Ogolla *et al.,* 2022b). Variations in microclimate i.e. humidity, temperature, light duration may influence pathogen selection, pathogen development and disease progress (Dillon and Meentemeyer, 2019; Gulzar *et al.,* 2021). Due to environment fluctuations which drives change in plant pathogen behaviour, adaptations and morphologies, the burden of disease control relies on positive and accurate identification of causing pathogen (Francisco *et al.,* 2019). According to Bhunjun *et al.* (2021), accurate pathogen identification is fundamental to farmers as it link the disease-causing pathogen with the already available information necessary for its management. Traditionally, identification of fungal pathogen entails an elaborate process that include pathogen isolation and characterization (Simmons 2007; Tan *et al.*, 2008; Sharma *et al.,* 2013; Lawrence *et al*., 2016). Phenotypic characterization has been predominantly used in identification of fungal pathogen include conidial shape, colour and septation (Meena *et al*., 2005; Kaur *et al*., 2007; Ramjegathesh and Ebenezar, 2012). However, phenotypic characterization provides variable results for different fungal phytopathogens. Kumar *et al*. (2008) evaluated characteristics of *Alternaria solani* from different agro-climatic conditions and observed yellow, brown, black, brown to greenish black pigments on potato dextrose agar medium. The conidiogenous hyphae varied in thickness from 1.17 to 9.56 µm. In the majority of the isolates, mycelial growth was smooth having circular or irregular margins that did not form concentric rings. Regarding cultural traits, variation in *Alternaria solani* strains from Jordan valley was reported with mycelial width range of 0.8 to 1.5 µm, conidia breadth of 35-75 µm, length of 10-20 µm, 2 to 7 transverse septa and 1 to 4 longitudinal septa (Alhussaen, 2012). In Limpopo, Mphahlele (2017) reported variability of *Alternaria solani* isolated from tomato leaves collected from four tomato production regions. Isolates were highly varied phenotypically with reference to the pigmentation of mycelial, conidia length, septa number and diameter of the colonies. In Kenya, despite reported cases of tomato early blight in Mwea (Mugao *et al.,* 2021), little work has been done on characterization of the causative pathogens from different AEZs. Use of morphologies for identification of plant pathogens are tedious, require expertise and are hindered by fungal inconsistency and phenotypic plasticity (McCartney *et al.*, 2003; Toumatia *et al.,* 2015; Naroei and Salari, 2015; Luchi *et al.*, 2020). To resolve this inadequacy, there is need for development and use of advanced identification criteria, such as molecular techniques that allows comparative genome analyses to distinguish closely-related tomato pathogens is necessary (Adhikari *et al*., 2017). A combination of phenotypic and molecular based identification approaches can provide more comprehensive knowledge of tomato foliar fungal pathogen in Kirinyaga County to facilitate management.

Improved tomato varieties such as Terminator F_1, Kilele F_1, Hansal F_1 and Riotinto F_1 have been introduced to farmers in Kirinyaga County. However, cases of early blight and other fungal diseases have persisted (Nuwamanya *et al*., 2021; Mugao *et al,* 2021; Ogolla *et al.,* 2022a). Success in breeding for the EB resistance have been slowed down by limited available effective genes for resistance particularly among the cultivated tomato (Chaerani, 2006). Therefore, ssusceptibility tests of tomato varieties to early blight disease may serve as guidelines for breeding of disease resistant cultivars, hence improving tomato production and income to farmers. According to Odilbekov (2015) and Adhikari *et al.* (2017), knowledge gap exists on the susceptibility of varieties of tomato grown by farmers to disease that include early blight among others. Thus, screening of local tomato varieties against early blight diseases in different pathosystems such as AEZ of Kirinyaga County is necessary to bridge the existing knowledge gap on their susceptibility to the pathogen in the area.

MATERIALS AND METHODS

Study Area

The fungal pathogen used in this study were isolated from diseased tomato leaves collected from farms in Kirinyaga County (Figure 2). Kirinyaga County is located in the Southern Mt. about 100 km North East of Nairobi (Serede *et al.,* 2015) at latitudes 0° 37'S and 0° 45'S, longitudes of between 37° 14'E and 37° 26'E with altitude ranges of 1,100 m to 1,200 m above sea level. The average annual rainfall is 940 mm with long and short rains occurring between April and May, and October to November, respectively (Jaetzold *et al*., 2007). Temperatures range from 12°C to 26°C with an average of 20°C. The AEZ in Kirinyaga County are grouped from Tea Dairy Zone LH 1 at the base of Mount Kenya National park, three coffee zones (UM 1, UM 2, UM 3), Marginal Cotton Zone in zones LM 3 and LM 4 [(Table 1) Jaetzold *et al*., 2007]. As shown in Table 1, soils types in Kirinyaga County differ within and across AEZs. For instance, whereas AEZ UM2 and UM3 comprises majorly of humic nitosols soil type, LM4 comprises of three soil types i.e. humic nitosols, eutric nitosols and pellic vertisols (Jaetzold *et al*., 2007). Preliminary studies carried out prior to actual study showed that tomato is grown in coffee zones, tea zones and largely in cotton zones through irrigation.

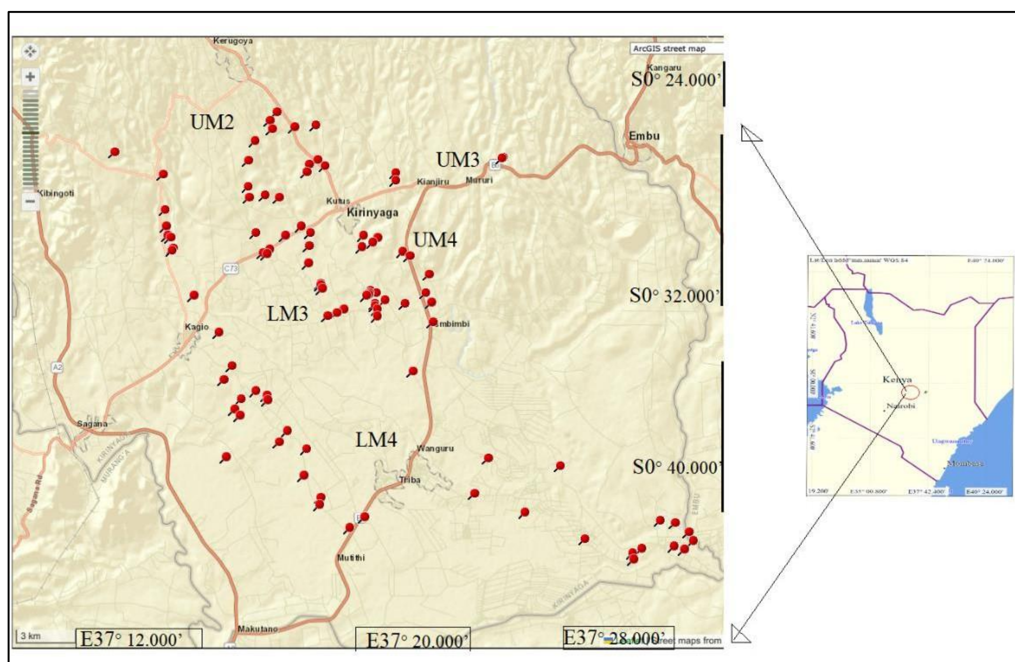

Figure 1: Map of Kirinyaga County showing agro-ecological zones (UM2, UM3, UM4, LM3 and LM4) surveyed for foliar diseases of tomato; where UM = Upper midland (1, 2 and 3), LM = Lower midland (3 and 4).

Specifically, the diseased tomato leaves used in this study were collected in five tomato growing AEZs of Kirinyaga namely LM 3, LM 4, UM 4, UM3 and UM 2 (Figure 2).

Table 1: Features of agro-ecological zones where diseased tomato leaves were collected in Kirinyaga County

*AEZs	Soil type	Altitude (m)	Temp (°C)	Subzone	Rainfall (mm)
UM2	Humic Nitosols			m/l + m/s	1220-1500
		1400-1580	19.0-20.1	m + s/m	1200-1250
UM3	Humic Nitosols	1340 - 1400	20.1-20.6	m/s + s	1100 - 1250
UM4	Humic Nitosols	1280 - 1340	20.4-20.9	s/m + s	950 - 1200
	Eutric Nitosols			s + s	350 - 960
LM3	Humic Nitosols	1220 - 1280	20.9-21.2	s /m+ s	950 -1200
				s + s	350 -960
LM4	Humic Nitosols, Eutric Nitosols Pellic Vertisols	1090 - 1220	21.2-22.0	s + s/vs	850 - 950

*AEZ = agro-ecological zones, in the subzones, *m*= medium rainfall, *s*= short rainfall, l= long rainfall, *vs*= very short rainfall, UM = Upper midland (1, 2 and 3), LM = Lower midland (3 and 4) Jaetzold *et al.* (2007).

Experimental Designs

To determine the susceptibility of tomato varieties to foliar fungal pathogens 5 × 4 factorial experiment laid out in complete randomized design (CRD) and replicated 6 times was used under greenhouse conditions. There were two factors, i.e., tomato varieties 5 levels (Hansol F_1, Kilele F_1, Rambo F_1, Riotinto F_1 and Terminator F_1) and fungal isolates 2 levels (Early blight and control). Control treatment comprised of un-inoculated tomato plant for each treatment.

Isolation of Fungus Early Blight Leaf Spot Pathogen

A small piece of diseased leaf tissue (3 x 3 mm) at the margin of early blight lesion and healthy leaf part was cut using sterile blade. The cut lesion was surface disinfected with 0.3% sodium hypochlorite for 1 min, rinsed in 3 changes of distilled water to remove the detergent. The excisions were surface sterilized in 70% ethanol for 30 s, rinsed with sterile distilled water 3 times then blot dried using sterilized Whatman filter paper. Water free excised leaf parts were placed on PDA in the petri dishes and incubated for 1week at 25°C to realize optimal fungal growth. Upon growth of the colonies were viewed under the microscope and pure cultures prepared.

Purification and Identification of Fungus

Pure fungal pathogens were obtained by sub culturing in fresh PDA media. A flame sterilized scapel was used to cut the culture media with grown mycelia at the periphery and transferred in fresh media and were re-incubated for 2 weeks. After the pure mycelia had completely covered the plates, phenotypic characterization was done based on external cultural morphologies and by use of microscopy following scotch tape method (Kali *et al.,* 2014). In culture plates fungal phenotypic traits such as spore colour (SP), culture pigmentation, colour of colony margins (M-col), growth rate (GR)

and Mycelial fluffiness [(MF) for late blight pathogen] were recorded during the growth phase of the fungal isolates to facilitate identification (Table 2). Scotch tape method involved picking of the spore using transparent adhesive tape where the spores were picked from the plates using the adhesive side of the tape then placing the tape on the lactophenol cotton blue stain on the slide (Kali *et al.,* 2014). The slide was then observed under LCD phase contrast microscope light microscope (Model: OMAX 40X-400X) at × 40 objective lenses and microscopic details of the fungus observed, measured and the images photographed. Fungal characteristics assessed under the microscope were; conidial length in µm, number of septa, and existence of beak and arrangement of conidia [(C-Arr) Table 2].

Table 2: Aspects of early blight pathogen isolates used for Phenotypic characteristic

Cultural characteristic early b light pathogen isolates		
Growth rate (GR)	Colony margin (M-col)	Spore colour (SP)
1= Slow 2= Medium 3= Rapid	1= Brownish 2= Greyish 3= Whitish	1= Greenish 2= Greyish 3= Dark
Presence of Beak (PB)	No. transverse septor (T-Spt)	No. longitudinal septa (L-Spt)
1= Beak present 2= Beak absent		
Conidia arrangement (C-Arr)	1=Single 2 = Chains	

The reference materials used for identification of early blight pathogen isolates included published material from related work (Kumar *et al.*, 2008; Loganathan *et al.,* 2016; Kaur *et al.,* 2020; Mugao *et al.*, 2021). Pathogenicity test was carried out for selected pathogen isolates for further identification and characterization. All the conidial measurements were taken in micro-meter (µm).

Pathogenicity Test and Aggressiveness of Early Blight Fungal Pathogen Isolates

Pathogenicity test was carried out as required by Koch's postulate (Agrios, 2005) on disease causing organism in a pot experiment in the greenhouse at Chuka University. Spore suspension (10^3 spores/ml) prepared from two weeks old pure cultures of early blight disease pathogen. The spores on the plates were scraped gently with an aid of scalpel and suspended in 100 ml distilled water then filtered through threefold cheese cloth. Haemocytometer (30 x 70 x 4mm) was used to standardize the inoculum to 10^3 spores/ml. Two-month old Terminator F_1 tomato variety was used in the pathogenicity test (Koch's postulate experiment). Terminator tomato variety was selected for pathogenicity test because it is grown in most areas where the pathogens were obtained. The leaves of tomato were sprayed with respective fungal pathogen isolates and were monitored for two weeks for the occurrence of early blight like disease symptoms.

To determine the aggressiveness of the early blight pathogens, detached leaf method described by Pryor and Michailides (2002) and Tymon *et al.* (2015) was used. Ten well developed mature and diseases free tomato leaves from Terminator F_1 were aseptically collected from green house. The leaves were transported to the laboratory in moist sterile zip lock bags. At the laboratory, the leaves were rinsed under running tap water and surface sterilised with 70% alcohol for 5 seconds and rinsed again in three changes of sterile water to remove the alcohol. The water on leaf surface wiped out using sterile blotting paper. The five sets leaves were pricked using sterile needle and inoculated with pathogen suspension (20 µl) of respective disease pathogens. The suspension was dropped at the point of the lesion and one leaf that served as the control was inoculated with 20 µl distilled water. Tomato leaves were then placed on sterile clean grid in a transparent glass desiccator (Diameter 300 mm height 240 mm). The desiccator had 250 ml of distilled water underneath to maintain humidity. The inoculated leaf area was monitored for seven days for development of lesion. The length (L) and width (l) of the lesion were measured for each leaflet inoculated. The lesion size (S in cm^2) was calculated using the formula below (Goufo *et al*., 2010).

$$S = \frac{\pi(L + 1)^2}{4}$$

Molecular Identification of Selected Isolates

Molecular analysis of fungal pathogens isolated in this study was done at the University of Nairobi's Centre for Biotechnology and Bio-informatics. The DNA was extracted from 20 mg mycelia harvested by scraping the colony surface. The mycelia were ground into fine pieces using motor and pestle and placed into a 1.5 mL micro centrifuge tube containing 800 µL cetyltrimethyl-ammonium bromide (CTAB) extraction buffer (Final concentration: 2% (w/v) CTAB, 200 mMTris–HCl pH 8.0, 20 mMEDTA pH 8.0, 1.4 MNaCl, 1% (w/v) poly-vinylpyrrolidone, 1% (v/v) b-mercaptoethanol) (Lee and Taylor, 1990). The DNA extracts were dissolved in 50 µL of sterile distilled water and stored at 20°C for further use.

Polymerase chain reaction (PCR) was done using Thermocycler (Model C1000, Touch Biorad). Polymerase chain reaction was done in a 0.5 ml Eppendorf tube containing 10.5 µL ddH_2O, 12.5 µL Premix Ex Taq (Containing 0.625 U Taq DNA polymerase, 200 µM dNTP and 2 mM$MgCl_2$), 0.5 µL each of the two primers (10l M) and 1 µL DNA template (100 µgmL^{-1}). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of the isolates was amplified using primer set ITS1 [(50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50- TCCTCCGCTTATTGATATGC-30) White *et al.,* 1990; Lee and Taylor, 1990]. Amplification reactions was done with an initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 40 s, annealing at 58°C for 40 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. Polymerase chain reaction products were checked using gel electrophoresis on 1% agar rose gel stained using ethidium bromide. The DNA amplicon (1µl) mixed with loading dye was assayed alongside 1kb 0 gene ruler plus ruler with initial reading at 75kb. The DNA loading bands were viewed on gel documenter imager (Bio- Rad). Positive DNA PCR products were cleaned using Quick cleaning kit supplied by Gene Script Company in readiness for senger sequencing. Sequencing of the DNA amplicons was done at the International Livestock Research Institute in Nairobi, Kenya using Sanger sequencer (Model: ABI 3730 genetic analyser) and The Big Dye Terminator v3.1.

The sequences (PCR fragment ITS rDNA) were compared with the record at the NCBI/Gene Bank database (www.ncbi.nlm.nih.gov/blast/). Multiple sequence alignment of fungal species obtained from the database were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) program method in the Mega X version 11.0 (Tamura *et al.,* 2021). A phylogenetic tree of the pathogen sequences and those of related fungal species downloaded from the gene bank was generated using Neighbor-joining program and bootstrapping performed with 1000 replication to estimate the stability the branches (Saitou and Nei, 1987). The evolutionary distances of the species in the phylogenetic tree was computed using the p-distance method and output presented in units of the number of base substitutions per site (Tamura *et al*., 2018).

Susceptibility of Tomato Varieties to Different Fungal Pathogen Isolates

Five tomato varieties used for susceptibility screening were obtained from Kenya Seed Company in Nairobi. The nurseries of the 5 tomato varieties (Kilele F_1, Hansol F_1, Rambo F_1, Riotinto F_1 and Terminator F_1) were germinated separately in sterile germination trays in the greenhouse at Chuka University. The trays were watered every for three weeks before transplanting. Calcium Ammonium Nitrate (*CAN*) and Diammonium phosphate (*DAP*) was used to supplement the tomato growth. The growth bags used for growing tomatoes were 45 x 30 x 30 cm in size. The containers were filled with oven sterilized soil made up of sand, silt and compost (Ratio 3:1:1), respectively (Aslam *et al*., 2017). The three weeks old tomato seedlings were individually transferred to the plant growth bags (One plant per container) with each treatment being replicated six times. The distance between each tomato plant and the next was 0.5 meter. Watering was done by sprinkler every morning and evening to maintain humidity. Three weeks after transplantation, each tomato variety was inoculated (By spraying) with 50 ml of respective fungi pathogen culture containing 10^3 CFU/ml determine by haemocytometer.

After inoculation, the tomato plants were watered at alternative days and plants inspected for the development of disease symptoms. At the end of 28 days after inoculation, the diseases symptoms were scored using the criteria and scale described in section 3.5.2 for individual diseases. The scores for different pathogens were used to calculate disease severity that represented the susceptibility of individual tomato variety used in the screening experiment. Susceptibility to *Septoria* leaf spot was categorised as resistant where the severity score ranged from 1 to 10%; Moderate resistant where severity ranged between 11 to 20%; Moderately susceptible where severity ranged between 21 to 40% and lastly, highly susceptible where severity score was above 40% (Raina and Razdan, 2010).

Data Analysis

Phenotypic Characterization of Fungal Pathogen Causing Tomato Foliar Diseases in Different Agro Ecological Regions of Kenya

Cluster analysis of data collected on the phenotypic traits was done using factoextra R package in R studio version 4.0.3. The normality test evaluated using Shapiro-Wilk test. Given that the normality test for conidial length and breadth gave non-significance ($p > 0.05$) Shapiro-Wilk test results, their means were subjected to the ANOVA using general linear model and significance means were separated using LSD in SAS version 9.4 at $\alpha = 0.05$.

Susceptibility Test of Tomato Varieties to Different Foliar Fungal Disease Pathogens

Data on the susceptibility based on percentages of severity of early blight had skewness value of 0.0119, Septoria leaf spot had skewness value of 0.003 and lastly late blight had skewness value of 0.210. Additionally, the normality test for all the tests had a $p > 0.05$ Shapiro-Wilk test result indicating that the data was normally distributed. Therefore, the data

on susceptibility were subjected to the analysis of variance using the general linear model procedure in SAS version 9.4. Significant means were separated using least significant difference (LSD) at α= 0.05 in SAS.

RESULTS

Phenotypic Characterization of Early Blight Pathogen

A total of 30 early blight pathogen isolates were obtained from diseases tomato leaves from different AEZ. Most on the isolates on potato dextrose agar were not different in terms of spore's colour on PDA. The isolates had green- dark grey spores and had dark pigmentation. The cultures had either regular or irregular margins (Table 3).

Table 3: Phenotypic characteristics of early blight pathogen isolated from different agro-ecological zones of Kirinyaga County

AEZ [1]	Isolate	GR[2]	SP[3]	PB[4]	Mcol[5]	L-Spt[6]	T-Spt[7]	C-Arr[8]
LM4	EB _Gtg6	3	1	2	1	3	4	1
	EB _KBU3	2	3	1	2	0	1	1
	EB _ND1	2	2	1	1	0	5	2
	EB _ND4	3	3	1	1	2	4	2
	EB _ND7	2	3	1	2	0	3	1
	EB _WNG4	3	1	2	1	2	4	1
LM3	EB _KDG1	2	2	1	2	2	5	2
	EB _KDG9	3	1	2	1	1	4	1
	EB _KDO9	3	2	1	1	2	3	1
	EB _NYT4	2	3	1	2	0	6	1
	EB _NYT6	1	2	2	2	2	4	1
	EB _SRG2	1	2	2	2	1	5	1
UM4	EB _KGG1	2	2	1	1	2	3	2
	EB _KGG9	2	1	2	1	0	3	1
	EB _NDM3	3	3	1	3	3	5	1
	EB _NDM7	1	3	1	3	1	4	1
	EB _NJI2	2	1	2	1	1	4	2
	EB _NJI6	1	2	1	1	3	5	1
UM3	EB _GTH3	1	1	2	2	2	4	1
	EB _GTH5	1	2	2	2	2	3	1
	EB _KAM2	2	1	1	3	0	4	2
	EB _KAM3	2	1	1	3	0	4	2
	I EB _KAM8	3	2	1	1	1	4	2
	EB _THR9	1	2	2	2	2	3	1
UM2	EB _GEO3	2	2	1	3	0	3	2
	EB _GEO6	2	3	2	2	0	4	1
	EB _KER2	2	1	1	3	2	3	1
	EB _KER7	3	1	1	1	1	3	1
	EB _KRG1	1	2	2	3	0	4	1
	EB _KRG9	3	3	2	3	2	3	1

[1]Agro-ecological zones: UM = Upper midland, LM = Lower midland, [2]Growth rate (1= Slow, 2= Medium, 3 =Rapid), [3]Spore colour (1= Greenish, 2= Greyish, 3= Dark), [4]Presence of beak (1= Beak present, 2= Beak absent), [5] Margin colour (1= Brownish, 2= Greyish, 3= whitish), [6]Number of longitudinal septa, [7] Number of Transverse septa, [8] Conidia arrangement (1=Single, 2 = Chains),

Conidiophores of the *Alternaria* spp isolates were solitary or in groups and were either straight or flexuous. Morphologically, conidia differed in breath, length and presence of beak. The shape of conidia was straight or slightly curved muriform or ellipsoid. Conidia were tapering towards one end and were olivaceous brown in colour (Plate 1). The

conidia's transverse and longitudinal septa varied ranging from 1 to 6 and from 0 to 2, respectively. The conidia appeared in chains or solely and some had beaks which were short, long or had no beak (Plate 1).

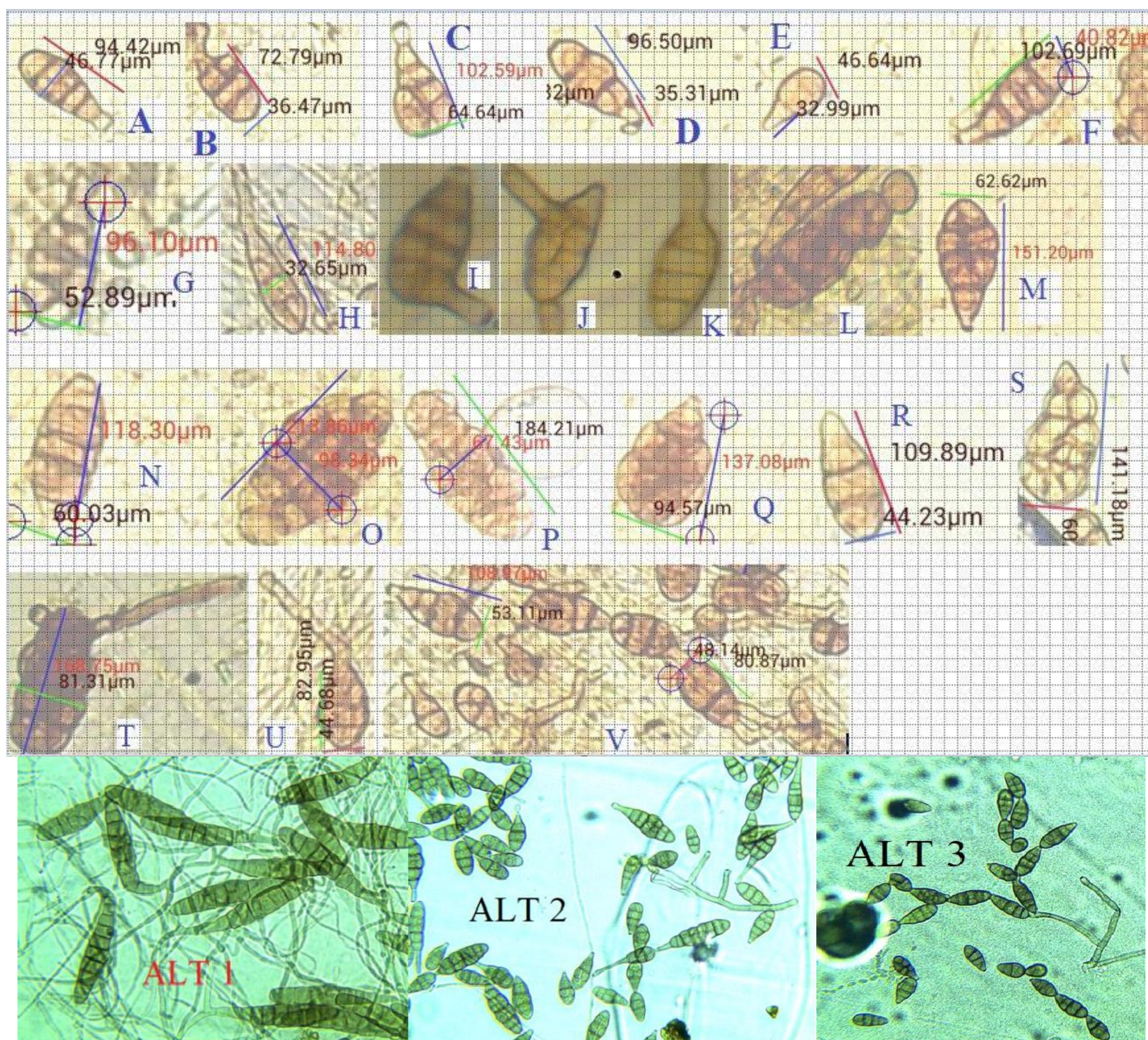

Plate 1: Selected conidia morphologies for the early blight pathogen (*Alternaria* spp) isolates from tomato leaves from Kirinyaga County, Kenya,

NOTE: Muriform shape (A, C, F, R, S), Pyriform (I, E, O, P), Cuneate shape (Q), Conidia with short beak (A, C, D, E), Medium curved beak (B and D), long beak (H and U) no beak (M, N, O, P, Q, R, S), ALT 1= Isolate EB-KAM8, ALT2= Isolate EB EB-NJ12-, ALT3= Isolate EB-ND4

Thirty early blight pathogen isolates from five AEZ (Table 3) were grouped in three clusters by euclidean distance constructed using phenotypic characteristics. The first and third clade comprised of 13 isolates each of which were further divided into two sub clades. The smallest clade comprised of isolates EB_KBU3 isolated from AEZ, isolates EB_LM4, EB_KD09 isolated from AEZ UM2 and isolate EB_KER2 and EB_KER7 both isolated from AEZ LM3 and AEZ LM4 and lastly, isolate NDM7 isolated from AEZ UM4 [(Table 3) Figure 2]. Isolate EB_KD09 and EB_KER7 had similar distance while EB_KBU3 formed a separate sub sub clade. Isolates EB_KD09 and EB_KER7 had similar growth rate, had beak, had similar colony margin, same conidia arrangement and number of transverse septa as outline in preceding Table 3.

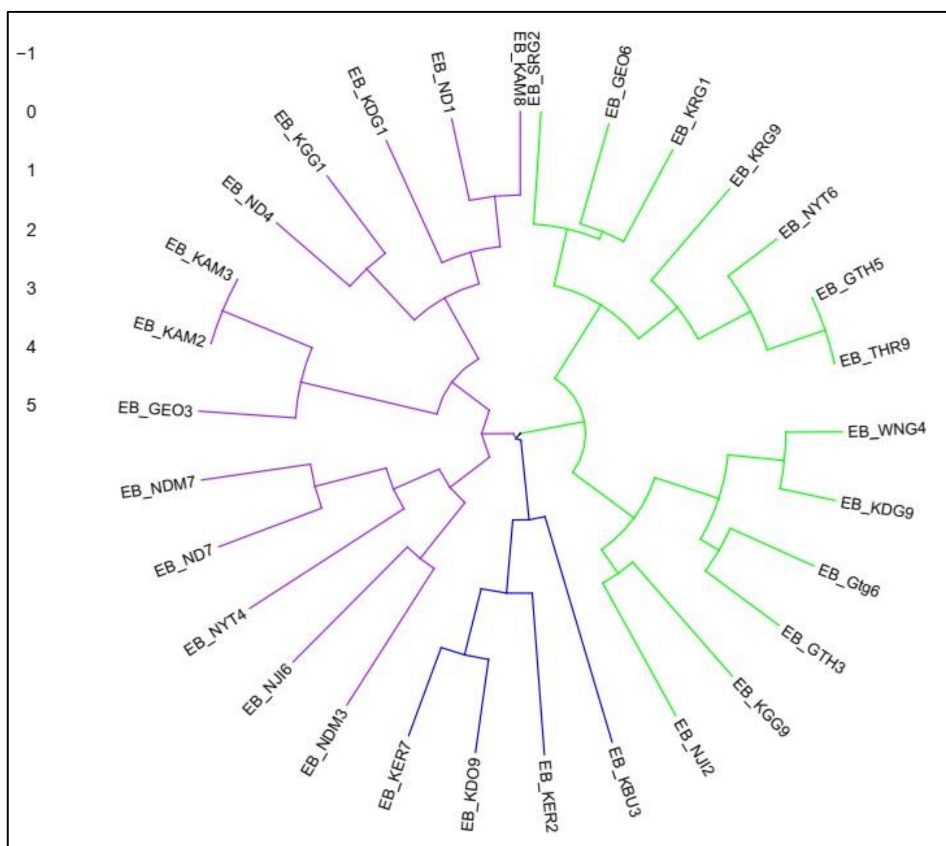

Figure 2: Cluster membership based on phenotypic traits of early blight pathogens isolated from tomato leaves from different agro-ecological zones of Kirinyaga County

There was a significant (F (29, 210) =8.25, p <0.0001) difference among the conidial lengths of early blight pathogen isolates from infected tomato leaves from different AEZ. In LM4, the lengths of early blight pathogen isolates were significantly (F (5, 42) =22.31, p <0.0001) different with the minimum and maximum conidial lengths measured being 52.8 and 194.2 µm, respectively (Table 4). Isolate EB_ND4 had longer conidia (148.37 µm) while isolate EB_KBU3 had smallest length of the conidia at 78.52 µm (Table 4). In LM3, the lengths of the conidia of the early blight pathogen did not differ significantly (F (5, 42) =2.05, p <0.0909). However, conidia lengths ranged from 95.60µm to 204.6 µm, respectively. In AEZ UM4, the lengths of the conidia of the early blight pathogen were significantly (F (5, 42) =3.93, p <0.0051). different where the minimum and maximum length of conidia lengths measured were 85.2 and 184.2 µm, respectively. Isolate EB_NJI2 had longer conidial length (131.17 µm) while isolate EB_ NDM7 had short conidial length measuring 101.17 µm. The length of conidia for early blight pathogen were significantly (F (5, 42) =5.30, p <0.0007). different in UM3 where the minimal and maximum conidia lengths measured were 64.2 and 159.4 µm, respectively. Isolate EB_KAM3 had longer conidial length (123.62 µm) while isolate EB_ GTH5 had short conidial length measuring 92.75 µm (Table 4). Conidia lengths in AEZ UM2 differed significantly (F (5, 42) =2.59, p <0.0398). with the minimum and maximum lengths measured being 64.6 *µm* and 138.4 *µm,* respectively. Isolate EB_KER7 was significantly longer measuring 112.37 *µm* (Table 4).

Table 4: Conidia length and breadth of early blight pathogens isolated from diseased tomato leaves collected from different agro-ecological zones of Kirinyaga County

AEZ [a]	Isolate	N	Length (µm)	Breadth (µm)	Length (µm)		Length
					Min[b]	Max[c]	Anova Statistics
	EB_Gtg6	8	123.72 ^{edf}	39 ^{h}	99.00	149.00	
	EB _KBU3	8	78.52 ^{m}	38.7 ^{h}	66.00	94.40	Cv = 17.86
LM4	EB _ND1	8	83.95 ^{km}	48.83 ^{de}	52.80	109.00	Mean= 115.71
	EB _ND4	8	148.37 ^{abc}	42.65 ^{efgh}	104.20	194.20	$p < 0.0001$
	EB _ND7	8	97.97 ^{hijkl}	42.68 ^{efgh}	84.40	114.80	LSD = 20.85
	EB _WNG4	8	161.72 ^{a}	45.6 ^{efgh}	133.20	207.20	
	EB _KDG1	8	139.85 ^{abcd}	41.1 ^{fgh}	104.80	180.40	
	EB _KDG9	8	138.50 ^{abcd}	41.77 ^{fgh}	108.40	174.60	Cv = 20.89
LM3	EB _KDO9	8	150.3 ^{ab}	41.3 ^{fgh}	96.60	204.60	Mean= 130.49
	EB _NYT4	8	124.5 ^{edf}	40.5 ^{fgh}	95.60	156.20	$p = 0.0246$
	EB _NYT6	8	126.77 ^{cdef}	40.58 ^{fgh}	89.40	171.80	LSD = 27.52
	EB _SRG2	8	103.05 ^{ghijh}	44.15 ^{efgh}	81.60	137.80	
	EB _KGG1	8	102.65 ^{ghij}	47.05 ^{def}	88.40	141.60	
	EB _KGG9	8	102.72 ^{ghij}	46.23 ^{efg}	85.20	130.20	Cv = 17.33
UM4	EB _NDM3	8	121.8 ^{edfg}	62.43 ^{b}	89.80	161.00	Mean= 114.58
	EB _NDM7	8	101.17 ^{hijk}	46.78 ^{edf}	89.80	112.60	$p = 0.0051$
	EB _NJI2	8	131.17 ^{bcde}	71.1 ^{a}	91.80	184.20	LSD = 20.04
	EB _NJI6	8	128.0 ^{bcde}	58.75 ^{bc}	114.60	167.80	
	EB _GTH3	8	99.2 ^{ijkl}	45.07 ^{efgh}	77.80	159.40	
	EB _GTH5	8	92.75 ^{jklm}	40.65 ^{fgh}	64.20	111.00	CV= 15.28
UM3	EB _KAM2	8	107.0 ^{fghij}	45.43 ^{efgh}	95.80	125.20	Mean= 107.93
	EB _KAM3	8	124.9 ^{bcdef}	60.58 ^{b}	109.40	158.20	$p = 0.0007$
	EB _KAM8	8	123.62 ^{cdef}	69.97 ^{a}	113.20	152.80	LSD = 16.65
	EB _THR9	8	100.15 ^{hijk}	40.83 ^{fgh}	85.20	128.60	
	EB _GEO3	8	100.07 ^{hijk}	44.58 ^{efgh}	80.80	138.40	
	EB _GEO6	8	91.5 ^{klm}	39.43 ^{gh}	64.60	132.80	CV= 14.93
UM2	EB _KER2	8	94.0 ^{ijkl}	44.43 ^{efgh}	84.60	100.60	Mean= 101.16
	EB _KER7	8	112.37 ^{efgh}	52.98 ^{cd}	93.20	126.40	$p = 0.0398$
	EB _KRG1	8	98.4 ^{hijk}	44.8 ^{efgh}	84.80	121.60	LSD 15.24
	EB _KRG9	8	110.62 ^{efghi}	40.23 ^{fgh}	78.20	124.60	
Means (µm)			113.97	46.91			
LSD (p <0.05)			20.034	7.02			
Cv (%)			17.832	15.18			

[a]Agro-ecological zones: UM= Upper midland, LM = Lower midland, [b] Minimum conidia lengths, [c] Maximum conidia lengths,

[d] Means followed by the same letter in columns is not significantly different at $\alpha = 0.05$

There was a significant (F (4, 235) =8.25, p <0.0001) different in the length of conidia of the early blight pathogen isolated in the five AEZs. Conidia of the isolates in AEZ LM3 were significantly longer (130.5 µm) while those from AEZ UM2 were significantly shorter measuring 101 µm (Table 5).

Table 5: Overall lengths of conidia of early blight pathogens isolated in different agro-ecological zones of Kirinyaga County

AEZ [a]	N	Length (µm)	Minimum (µm)	Maximum (µm)
LM3	48	130.5 [a]	81.60	204.60
LM4	48	115.71 [b]	52.80	207.20
UM2	48	101.16 [c]	64.60	138.40
UM3	48	107.94 [bc]	64.20	159.40
UM4	48	114.59 [b]	85.20	184.20
Means (µm)		113.97		
LSD ($p < 0.05$)		10.59		
Cv (%)		23.11		

[a]Agro-ecological zones, UM = Upper midland, LM = Lower midland, N = Sample size,

[b] Mean followed by the same letter in columns is not significantly different at α = 0.05

Pathogenicity test using isolate EB_WNG4 (Fast growing isolate) produced symptoms identical to those observed in tomato leaves leaf samples from famers fields (Lesion with visible concentric rings) on the tomato inoculated with the pathogen isolates. The pathogens re-isolated from the lesion were phenotypically similar to the initial pathogen isolates in terms of spores' colour and conidia shape.

Inoculation of the tomato leaves under detached leaf experiment with selected 10 isolates showed that there was significantly (F (13, 36) =12.48, p <0.0001) differed in the lesion sizes formed by the isolates (Table 6). Isolate EB_KAM8 was highly aggressive with lesion size of 122.17cm^2 and was followed by isolate EB_NDM3 110.17cm^2. Isolate EB_KDG1 had smaller lesion size of 17.10 cm^2 and was followed by isolate EB_ND4 with lesion size 20.09 cm^2 (Table 6)**.**

Table 6: Aggressiveness of early blight pathogen isolates isolated from tomato leaves in Kirinyaga County

Isolate	N	Aggressiveness (cm^2)
EB-KAM8	5	12.27 [a]
EB_NDM3	5	110.17 [a]
EB_KER7	5	49.85 [b]
EB_WNG4	5	46.01 [b]
EB_KAM3	5	45.15 [b]
EB_KRG9	5	43.20 [b]
EB_KDO9	5	37.31 [b]
EB_NJI2	5	20.73 [c]
EB_ND4	5	20.09 [c]
EB_KDG1	5	17.10 [c]
Mean (cm^2)		41.27
LSD ($p < 0.05$)		1.67
Cv (%)		24.33

[a] Means followed by the same letters are not significantly different at α = 0.05

Molecular Characterization of Selected Early Blight Pathogen Isolates

The agar rose gel analysis of DNA amplicon PCR products (ITS rDNA) of tomato foliar pathogen isolates produced bands at different locations between 500 to 700 bp based on 1kb gene ruler plus ladder (Plate 2).

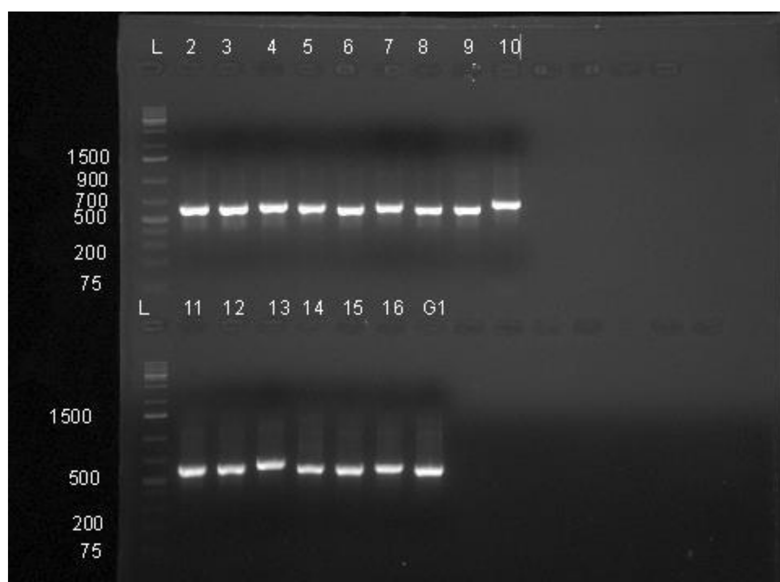

Plate 2: The agar rose gel analysis of DNA amplicon PCR products (ITS rDNA) of tomato foliar pathogen isolates isolated from diseases leaf samples from Kirinyaga County
where L= ladder (1kb gene ruler plus), 2= KRG-F02, 3= EB-KAM8., 4= EB-WNG4, 5= KRG-F05, 6= EB_NDM3., 7= KRG-F07, 8= KRG-F08, 9= EB-KD09, 10= KRG-F13, 11= EB-NJ12, G1= KRG-G01. 12= EB_KER7, 13= KRG-F06, 14= KRG-F08, 15= EB_KER7, 16= EB-KAM8; Isolates EB_KER7, EB-KAM8 were assayed twice

The blastin analysis of DNA amplicon Sanger sequenced nucleotides results (ITS rDNA sequences) revealed presence of different pathogens causing early blight like symptoms, which included *A. solani*, *A. altanata* and *Fusarium* spp (Table 7). The similarity identities of the nucleotide from pathogen isolates and fungal species in the gene bank ranged from 98 – 100% for *Alternaria* spp and for *Fusarium* spp. The phylogenetic tree of the sequenced nucleotides and their similar fungal species sequences available in the GenBank gave two main distinctive clades. Twelve isolates (HeB331-8, EB-KAM8, TgAa1, AC82, StrainL-2, EB-NDM3, EB-WNG4, EB-KER7, Strain AS-L-1, EB-ND4, NY003, EB-NJ12) comprising mainly of *Alternaria* spp were clustered together. On the other hand, fourteen isolates majorly *Fusarium* spp (10 isolates), two *Nigrospora* spp and *Pestalotiopsis* spp, respectively were clustered in a separate clade. All the species on the phylogenetic tree was rooted on *Rattus norvegicus* that served as an out group (Figure 3). The evolutionary distances matrix is shown in Table 8.

Table 7: Molecular identification of some tomato foliar pathogens using sequenced nucleotides and similar sequences in the gene bank

Isolate	Place of isolation	Close match	GenBank Accession No.	% Sim [a]	County	E values
KRG-F01	Gechenjo	1	MT035918	99.81	Egypt	0
KRG-F02	Kianganga	2	MT560323.1	100	China	0
EB-KAM8	Kiumbu	3	MG012289.1	99.82	China	0
EB-WNG4	Wanguru	4	OM980193.1	100.00	India	0
KRG-F05	Geotheri	5	MN898027.1	100.00	Israel	0
EB_NDM3	Thumaita	6	LC440583.1	99.64	Japan	0
KRG-F07	Chemise	7	MG664736.1	100	Uganda	0
KRG-F08	Kiangunga	8	NR_147556.1	91.33	Portugal	0
EB_KER7	Kidaruni	9	MK690429.1	99.33	China	0
KRG-F13	Kerigo	10	KY318497.1	91.22	South Africa	0
EB-NJ12	Thumaita	11	MN636300.1	99.82	Kenya	0
EB-ND4	Ndindiruki	12	MK656442.1	100	China	0
KRG-G01	Nguvaine	13	MH028084.1	100	Oman	0

[a] Percentage Similarity, [1]*Fusarium verticillioides*, [2]*Fusarium equiseti*, [3] *Alternaria solani*, [4]*Alternaria alternate*, [5]*Fusarium equiseti*, [6] *Alternaria alternata*, [7] *Fusarium equiseti*, [8] *Pestalotiopsis portugallica*, [9] *Alternaria alternata*, [10]*Fusarium equiseti*, [11] *Alternaria* sp, [12] *Alternaria alternata*, [13]*Nigrospora sphaerica*,

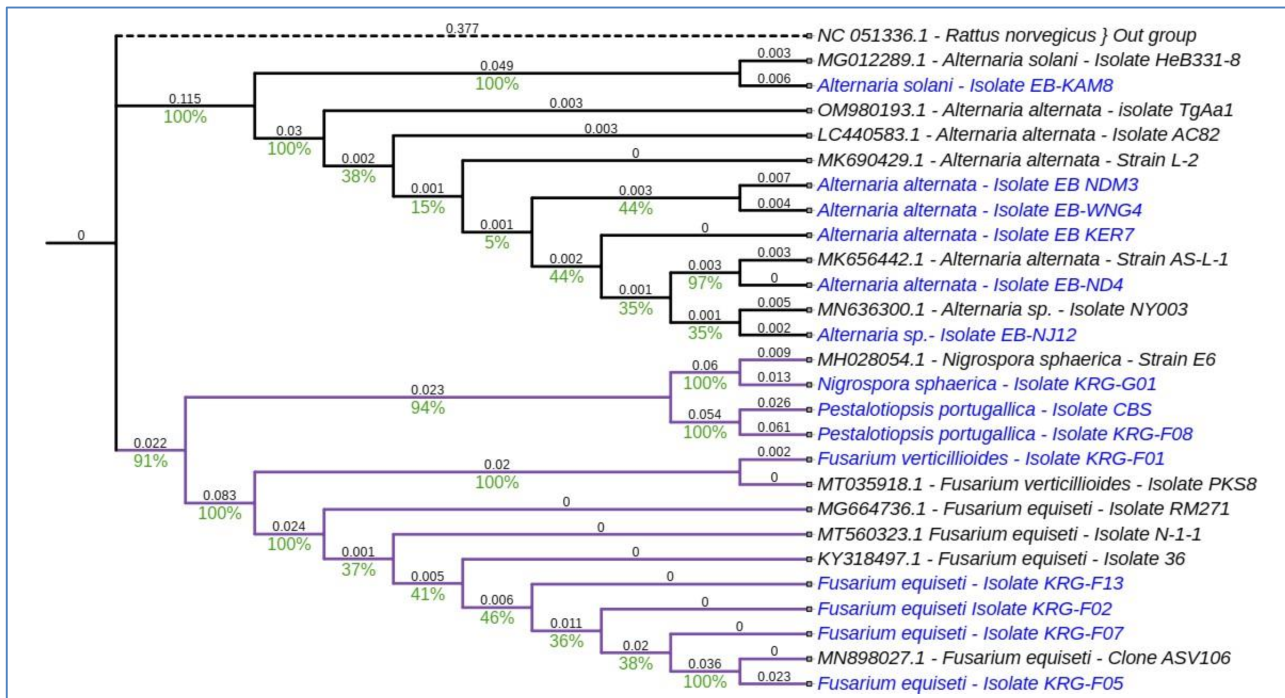

Figure 3: Phylogenetic analysis of sequences of some tomato foliar pathogens and similar sequences in the gene bank using MEGA X 11 software, neighbor-joining method and 1000 bootstrapped replications.
where: The species in blue font are the fungal pathogens species isolated in the current study

Susceptibility of Tomato Varieties grown in Kirinyaga County to Early Blight Foliar Fungal Disease Pathogen Isolates

There was a significant [(F (19, 100) = 4.73, p <.0001] difference on the susceptibility of different tomato varieties to early blight pathogen isolates. Kilele F$_1$ variety expressed most severe symptoms of early blight (78.24%) to isolate EB-KAM8 followed by Riotinto F$_1$ (72.22 %) while Terminator F$_1$ had least (58.33%) severe symptoms (Table 9). Inoculation with isolate EB_KER7 triggered severe symptoms in Kilele F$_1$ variety (69.91%) followed by Rambo F$_1$ (67.59%) and the symptoms was less severe (54.17%) in Terminator F$_1$. Isolate EB_NDM3 caused severe symptoms in Kilele F$_1$ variety (70.83%) followed by Riotinto F$_1$ (68.06%) and least in Terminator F$_1$ (58.33%). Inoculation with isolate EB_WNG4 induced severe symptoms in Kilele F$_1$ variety (76.39 %) followed by Riotinto F$_1$ (62.2%) and the symptoms was less severe (58.33 %) in Hansol F$_1$ (Table 9).

Rambo F$_1$ tomato variety did not exhibit significantly [(F (3, 20) = 0.56, p = 0.649] difference susceptibility towards early blight pathogen isolates (Table 10). Riotinto F$_1$ tomato variety screened exhibited significantly [(F (3, 20) = 4.36, p = 0.0162] different susceptibility towards early blight pathogen isolates where isolate S_NDM7 had higher severity mean of 55.86 % and lower 50.31% in isolate S_KGG9 (Table 10). Hansol F$_1$ tomato variety screened did not exhibit significantly [(F (3, 20) = 1.57), p = 0.229] difference susceptibility towards early blight pathogen isolates. Kilele F$_1$ tomato variety screened did not exhibit significantly [(F (3, 20) = 2.16, p = 0.124] difference susceptibility towards early blight pathogen isolates (Table 10). Terminator F$_1$ tomato variety screened did not exhibit significantly [(F (3, 20) = 1.36, p = 0.283] difference susceptibility towards early blight pathogen isolates (Table 10).

Table 8: The evolutionary distances matrix of the fungal pathogens and fungal species from gene bank in the phylogenetic tree computed using the p-distance method

	Fungal species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	MT035918.1 – *F. verticillioides*	0																						
2	* *F. verticillioides*	0.00																						
3	* *F. equiseti*	0.05	0.05																					
4	MT560323.1 – *F. equiseti*	0.05	0.05	0.00																				
5	* *A. solani*	0.30	0.30	0.30	0.30																			
6	MG012289.1 – *A. solani*	0.29	0.30	0.30	0.28	0.01																		
7	* *A. alternata*	0.28	0.28	0.28	0.28	0.08	0.09																	
8	OM980193.1 – *A. alternata*	0.28	0.28	0.28	0.27	0.09	0.08	0.01																
9	* *F. equiseti*	0.06	0.06	0.00	0.00	0.46	0.46	0.46	0.46															
10	MN898027.1 – *F. equiseti*	0.06	0.06	0.00	0.00	0.39	0.38	0.37	0.36	0.00														
11	* *A. alternata*	0.29	0.29	0.29	0.30	0.09	0.10	0.01	0.02	0.48	0.37													
12	LC440583.1- *A. alternata*	0.27	0.27	0.28	0.26	0.09	0.08	0.01	0.01	0.46	0.36	0.02												
13	* *F. equiset*	0.05	0.05	0.00	0.00	0.30	0.30	0.28	0.28	0.00	0.00	0.29	0.28											
14	MG664736.1 – *F. equiseti*	0.04	0.04	0.00	0.00	0.30	0.29	0.28	0.27	0.00	0.00	0.29	0.26	0.00										
15	* *P. portugallica*	0.26	0.26	0.26	0.27	0.31	0.31	0.31	0.31	0.30	0.28	0.31	0.32	0.26	0.26									
16	NR_147556.1 – *P. portugallica*	0.21	0.21	0.22	0.21	0.29	0.28	0.28	0.28	0.25	0.25	0.29	0.27	0.22	0.21	0.09								
17	* *A. alternata*	0.28	0.28	0.29	0.29	0.09	0.08	0.01	0.00	0.46	0.37	0.01	0.00	0.29	0.29	0.31	0.29							
18	MK690429.1 – *A. alternata*	0.28	0.28	0.28	0.28	0.09	0.08	0.01	0.00	0.46	0.36	0.01	0.00	0.28	0.28	0.31	0.28	0.00						
19	* *F. equiseti*	0.05	0.05	0.00	0.01	0.31	0.30	0.29	0.29	0.01	0.01	0.29	0.29	0.00	0.00	0.26	0.22	0.29	0.29					
20	KY318497.1-*Fusarium equiseti*	0.05	0.05	0.01	0.01	0.30	0.29	0.28	0.27	0.01	0.01	0.30	0.28	0.01	0.01	0.26	0.22	0.29	0.28	0.01				
21	* *Alternaria* sp.	0.28	0.28	0.28	0.29	0.10	0.09	0.02	0.01	0.47	0.37	0.01	0.01	0.28	0.28	0.30	0.28	0.00	0.00	0.28	0.29			
22	MN636300.1 - *Alternaria* sp	0.27	0.27	0.28	0.29	0.10	0.10	0.01	0.02	0.47	0.37	0.02	0.01	0.28	0.29	0.31	0.29	0.00	0.01	0.29	0.29	0.01		
23	* *A. alternata*	0.28	0.28	0.28	0.29	0.10	0.09	0.02	0.01	0.46	0.37	0.01	0.00	0.28	0.28	0.31	0.28	0.00	0.00	0.29	0.29	0.01	0.01	

24	MK656442.1- *A. alternata*	0.27	0.28	0.28	0.30	0.10	0.10	0.02	0.02	0.46	0.36	0.01	0.02	0.28	0.29	0.31	0.29	0.00	0.01	0.29	0.29	0.01	0.01	0.00
25	* *N. sphaerica*	0.19	0.19	0.18	0.20	0.29	0.29	0.27	0.27	0.22	0.21	0.27	0.27	0.18	0.20	0.20	0.15	0.27	0.27	0.18	0.19	0.27	0.27	0.26
26	MH028054.1 – *N. sphaerica*	0.19	0.19	0.18	0.17	0.29	0.28	0.27	0.26	0.22	0.21	0.27	0.26	0.18	0.17	0.20	0.13	0.27	0.27	0.18	0.18	0.27	0.28	0.27
27	NC_051336.1 – *R. norvegicus*	0.49	0.49	0.50	0.50	0.55	0.55	0.53	0.53	0.54	0.53	0.53	0.53	0.50	0.50	0.52	0.52	0.53	0.53	0.50	0.50	0.53	0.53	0.53

Isolates marked with asterix are the pathogen isolated in the current study, and, A= Alternaria, F= Fusarium, N= Nigrospora, P = Pestalotiopsis

Table 9: Susceptibility of selected tomato varieties to early blight pathogen isolated from tomato leaves in Kirinyaga County

Isolates	Varieties	N	Severity (%)	Lsd ($p < 0.05$)	Cv (%)	Mean (%)
	Hansol F_1	6	64.81 [bc]			
	Kilele F_1	6	78.24 [a]			
EB-KAM8	Rambo F_1	6	66.20 [bc]	8.43	10.44	67.96
	Riotinto F_1	6	72.22 [ab]			
	Terminator F_1	6	58.33 [c]			
	Hansol F_1	6	64.81 a			
	Kilele F_1	6	69.91 [a]			
EB_KER7	Rambo F_1	6	67.59 [a]	8.57	11.18	64.44
	Riotinto F_1	6	65.74 [a]			
	Terminator F_1	6	54.17 [b]			
	Hansol F_1	6	61.11 [bc]			

	Kilele F_1	6	70.83 [a]			
EB_NDM3	Rambo F_1	6	66.67 [abc]	8.49	10.98	65
	Riotinto F_1	6	68.06 [ab]			
	Terminator F_1	6	58.33 [c]			
	Hansol F_1	6	58.33 [c]			
	Kilele F_1	6	76.39 [a]			
EB_WNG4	Rambo F_1	6	61.57 [bc]	7.57	9.83	64.72
	Riotinto F_1	6	66.20 [b]			
	Terminator F_1	6	61.11 [bc]			
Mean (%)			65.53			
LSD ($p < 0.05$)			3.99			
Cv (%)			10.62			

[a] Means followed by the same letters are not significantly different at α = 0.05

Table 10: Susceptibility of selected tomato varieties to different isolates of early blight pathogen isolated from tomato leaves in Kirinyaga County

Susceptibility level (%)					
Isolate	Riotinto F_1	Hansol F_1	Rambo F_1	Kilele F_1	Terminator F_1
EB-KAM8	51.23 [bc]	64.82	66.20	78.24	34.87
EB_WNG4	54.32 [ab]	58.33	61.57	76.39	37.96
EB_NDM3	55.86 [a]	61.11	66.67	70.83	40.12
EB_KER7	50.31 [c]	64.81	67.59	69.91	37.96
Mean (%)	68.06	62.26	65.51	73.84	57.98
LSD ($p < 0.05$)	3.67	7.43	10.60	8.22	7.23
Cv (%)	9.73	9.91	13.44	9.24	10.35

[a] Means followed by the same letters in column are not significantly different at α = 0.05

DISCUSSION

Phenotypic Characterization of Early Blight Pathogen

Morphological, biochemistry and metabolites feature of fungal pathogens are useful in identification and diagnosis of disease pathogen (Simmons 2007; Sharma *et al.,* 2013; Lawrence *et al.,* 2016). Fungal pathogens' phenotypic traits predominantly used in pathogen identification are conidial shape, colour and septation (Meena *et al*., 2005; Kaur *et al*., 2007; Ramjegathesh and Ebenezar, 2012). The spores of early blight isolates were consistent with those reported by Mugao *et al.* (2021) in a study carried out in Mwea in Kirinyaga County in Kenya. As this study, Mugao *et al.* (2021) observed that the early blight pathogen isolates appeared greyish to greenish in colour and darkened as the culture aged, margins of the cultures were either regular or irregular pattern. Pigmentation and colony texture of early blight isolates in this study corroborated with other studies where greenish, dark and brownish pigmentation have been observed (Kumar *et al.*, 2008; Loganathan *et al.,* 2016; Mugao *et al.*, 2021). Surface texture of the colonies exhibited slight variations with zonation at margins observation which were similar to those observed in other studies involving early blight pathogens (Kumar *et al.*, 2008; Loganathan *et al.*, 2016; Kaur *et al.,* 2020).

Microscopic observation revealed variation of early blight isolates in their conidia morphology and concides with the findings of Mugao *et al.* (2021) where variability of tomato early blight pathogen in Mwea was reported. Further, morphological traits are in agreement with other studies carried out elsewhere where conidia have differed in breath, length and beak and have reported the conidia to be straight, slightly curved, muriform or

ellipsoid (Rotem, 1994; Singh *et al.,* 2007; Blanchard, 2012; Ismael and Omer, 2018; Ragupathi *et al.,* 2020). The conidial transverse and longitudinal septa varied ranging from 1 to 6 and from 0 to 2, respectively which was in tandem with other studies (Rotem, 1994; Singh *et al.,* 2007; Blanchard, 2012; Ismael and Omer, 2018). The length of conidia of the early blight pathogen ranged from 78.52 µm to 161.72 µm. Conidia lengths observed in this study were within range of 105-220 µm reported earlier by Nolla (1927). However, conidia lengths were longer than those reported earlier by Neergaard (1945) and Abubakar and Ado (2009) ranging from 10.26 - 77.52 µm.

Molecular characterization of some of the early blight pathogen which showed variation in phenotypic traits revealed involvement of *Alternaria solani* and *A. alternate* spp. Some fungal species particularly *Fusarium equiseti* and *Fusarium verticillioides* were associated with leaf blight in this study. *Fusarium* spp isolated from this study have earlier been isolated from tomato leaves in various studies (Amini *et al.,* 2013; Micah *et al.,* 2018).

Evaluation of Aggressiveness of Early Blight Foliar Fungal Pathogens of Tomato

Knowledge on the aggressiveness level of different pathogen isolates in an area is useful for the evaluation on the magnitude of crop damage that can be caused by individual isolate (Bessadat *et al.,* 2014). Pathogens isolates differed significantly in their aggressiveness level. Differences observed in the aggressive level of early blight pathogen isolates may have been attributed to the effect of factors such as environmental conditions influenced by AEZs, variation in farming practices and a possible occurrence of different strains of pathogens. According to reports aggressiveness of early blight pathogens such as *Alternaria solani* is influenced by environmental conditions that include temperature stress and moisture and farm practices like the cropping system (Tsedaley 2014; Tymon *et al*., 2016; Hussain *et al*., 2019). For instance, mono-cropping or mixed cropping may influence pathogen aggressiveness due to impact on pathogen structure. Differences in the aggressiveness of the isolates may be an indication of existence of difference races of the pathogen. Occurrence of different races of pathogen may circumstantially cause synergistic interactions leading to elevated level of crop damage (Bonde 1929; Bessadat *et al.,* 2017). Variation in the level of aggressiveness of early blight pathogen isolates in this study is in tandem with the finding of van der Waals *et al*. (2004). Variation in aggressiveness of pathogen in an area influences the level of susceptibility of the crop varieties (Bessadat *et al.,* 2014).

Susceptibility of Tomato Varieties to Foliar Fungal Disease Pathogen Isolates from Kirinyaga County

Cultivation of disease tolerance/resistant crop varieties lowers cost of managing crop diseases in addition to protecting the environmentally against continuous use of harmful fungicides. Screening of tomatoes against disease pathogen in different niches may aid in identification and selection of varieties that may be explored in breeding for resistance and as well as adoption by farmers to lower mitigate high production cost associated with fungicide use.

The tomato varieties screened against various early blight pathogens in this study showed significant variation on their susceptibility to early blight. Kilele F_1 variety was found to be the most susceptible to early blight pathogens isolates (73.84%) while Terminator F_1 was least susceptible (57.99%). Difference in the susceptibility of tested tomato variety depicts variation in their genetical makeup. Varieties with high susceptibility rating may lack genes that confer resistant to early blight infection Chaerani *et al.,* 2006; Ramathani *et al.,* 2021). These results may be compared to the findings of Mphahlele *et al.* (2020) who observed variation in the susceptibility of Money-maker and Rodade tomato variety grown in South Africa to early blight isolates. The findings indicate that tomato varieties grown in different regions are susceptible to early blight infection.

There was no significant difference in the effect of isolates on the susceptibility in individual tomato varieties screened in this study. This may be attributed to the narrow genetic diversity of isolates used as they were selected on the basis of being the most aggressive during the detached leaf experiment. The findings of this study imply that tomato varieties screened do not have complete resistance towards early blight pathogens in Kirinyaga County. However, Terminator F_1 variety displayed reduced susceptibility when compared to the rest of varieties screened. The results deviate from the findings of Mphahlele *et al.* (2020) in Limpopo Province where a significance effect of early blight isolates on the susceptibility of individual tomato varieties was observed.

Further the results of this study differed to those of Bessadat *et al*. (2014) and Ragupathi *et al.* (2020) which reported variation in the susceptibility of tomato variety to early blights in Algeria and India respectively.

CONCLUSION AND RECOMMENDATIONS

Early blight pathogen isolated from tomato leaves from different AEZ differs in their growth rate, aggressiveness and on the conidia aspects such as number of transverse and longitudinal septa. For instance, conidia lengths ranged from 52.8 µm for isolate EB_ND1 to 207.2 µm for isolate EB _WNG4 both isolated in AEZ in LM4. Further, morphological and molecular characterization confirmed the involvement of *Alternaria solanai* and *Alternaria alternata* as the causative agent of early blight in different AEZ of Kirinyaga County. The *Septoria* leaf spot pathogen isolated from tomato leaves different AEZ differed in their phenotypic characteristics such as rate of growth, number of transverse septa longitudinal septa and on conidia traits. Conidia lengths of the isolates ranged from 32.3 µm for isolate S_GEO6 in UM2 to 102.4 µm for isolate S_KDO9 in AEZ LM3. Late blight pathogen isolated from tomato leaves from different AEZ differs in their phenotypic characteristics that include conidia lengths and breadth. For instance, conidia lengths of these pathogens ranged from 25.5 µm for isolate L_KDG1 isolated from AEZ LM3 to 49.3 *µm* for isolate L_NDM7 in UM3. Molecular characterization revealed that other fungal pathogens such as *Fusarium verticillioides*, *Fusarium equiseti*, *Pestalotiopsis portugallica* and *Nigrospora sphaerica* also also asspociated with symptom that ressembles late blight in tomato leaves. Susceptibility screening showed that none of the tomato varieties screened was sufficiently resistant towards fungal pathogens under artificial inoculation in the greenhouse setup. However, Kilele F_1 and Terminator F_1 were found to be the most and least susceptible varieties, respectively to the early blight pathogens.
Thus, Terminator F_1 may be adopted by farmers in Kirinyaga County due to its low susceptibility to early blight infection.

Early blight fungal pathogen isolates differed on their phenotypic characteristics and on their aggressiveness, which may point at race variation thus, these isolates should further be assessed determine their races and the impact of environmental variation on their pathogenicity. Given that all the tomato varieties screened were susceptible to foliar fungal disease isolates at different levels, under artificial inoculation, further studies using varied concentration of pathogens is recommended. Further studies are also necessary to determine physiological and biochemical response of tomato varieties towards different isolates of early blight fungal pathogens in Kirinyaga County.

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