Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Plant Pathology Journal

ISSN 1812-5387
DOI: 10.3923/ppj.2021.29.40

Research Article
Morphological and Molecular Characterization of *Alternaria solani* and *Phytophthora infestans* Isolates from Tomato Farms in Kenya

L.G. Mugao, P.W. Muturi, B.M. Gichimu and A.K. Kamiri

Department of Agricultural Resource Management, University of Embu, P.O. Box 6-60100, Embu, Kenya

Abstract

Background and Objective: Early and late blight caused by *Alternaria solani* and *Phytophthora infestans* respectively, are the world's most important diseases of tomato. The objective of the study was to assess the morphological and molecular diversity of *A. solani* and *P. infestans* in tomato growing farms in Kirinyaga, Kenya. **Materials and Methods:** Infected tomato leaf samples were obtained from tomato farms and cultured using V8 Agar for *P. Infestans* and PDA for *A. solani* to facilitate isolation of the pathogens. The isolates were then subjected to morphological characterization using microscopic and macroscopic features and molecular characterization through PCR amplification of their ITS regions. The PCR products were then sequenced and blasted using NCBI database. **Results:** The results showed high morphological and molecular diversity within *A. solani* but low genetic variability within *P. infestans*. At least four clones of *A. solani* were found to exist in the study area but only one strain of *P. infestans* was identified. Other disease-causing pathogens were also isolated from the samples including *A. alternata*, a fungus that causes leaf spot and other diseases in plants and *Fusarium equiseti*, a soil-borne fungus that causes wilt disease in different vegetable plants. **Conclusion:** These findings are useful in the development of sustainable strategies to manage the early and late blight and other related diseases in tomato growing areas in Kenya.

Key words: *Alternaria solani*, *Phytophthora infestans*, genetic diversity, late blight, sustainable strategies

Citation: Mugao, L.G., P.W. Muturi, B.M. Gichimu and A.K. Kamiri, 2021. Morphological and molecular characterization of *Alternaria solani* and *Phytophthora infestans* isolates from tomato farms in Kenya. Plant Pathol. J., 20: 29-40.

Corresponding Author: L.G. Mugao, Department of Agricultural Resource Management, University of Embu, P.O. Box 6-60100, Embu, Kenya

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable in Kenya after Brassicas[1]. In Kenya tomato contributes 14% of the entire vegetable produce and 7% of the entire horticultural produce[2]. The crop is cultivated for its fruits which are consumed by almost all the families to provide vitamins A, C and lycopene. Kenya is one of the major tomato producers in Sub-Saharan Africa (SSA) producing 410,033 tonnes of tomatoes annually[3]. Tomato also takes the lead in processed vegetable crops in Kenya[4]. However, tomato cultivation in Kenya is strained by a myriad of biotic and abiotic stresses, key among them being pests and diseases. Nowick *et al*.[5] reported that over 200 pests and diseases attack the tomato plant thus hampering its production. Biotic agents of economic influence in tomato growing in Kenya include pests and diseases caused by bacteria, fungi, viruses and nematodes[6].

Early and late blight caused by *Alternaria solani* and *Phytophthora infestans* respectively, are the most important diseases on tomato in the world[6]. Early blight is the most catastrophic disease-causing production and post-harvest losses resulting in 50-86% fruit yield cutback[7]. According to Desta and Yesuf[8], high yield losses are recorded depending on disease severity. Torrential rain, high humidity and temperature range of 24-29°C creates a conducive environment for early blight development in tomatoes and can result to complete damage to the plant leaves[9]. Late blight is also a very disastrous tomato disease globally, causing remarkable economic losses annually[5] especially under cool weather conditions[10]. In Kenya, the late blight together with the early blight was estimated to cause up to 95.8% of all the pre-harvest tomato losses[11].

Alternaria solani is a soil-inhabiting fungus and spreads to host plants through air and rain splash[9]. The pathogen can survive for more than a decade in the soil, seed or crop remains at optimum temperature[9]. The pathogen produces toxins such as alternaric acid, altersolanol, macrosporin and zinniol that act on the protoplasm of the host and distract plant defence mechanisms[12]. Genetically, *A. solani* exhibits high variation between isolates from tomato and potato crops and different countries[13]. Isolates from unrelated host plant species vary in terms of aggressiveness, physiology and genetic diversity when inoculated in different plants[13].

On the other hand, *P. infestans* is a diploid, obligate, biotrophic and heterothallic pathogen with two mating types: A1 and A2[14]. The pathogen is an oomycete and its asexual lifecycle is distinguished by alternating phases of hyphal growth, sporangial germination and sporulation[5]. Sexual reproduction results in oospores that are thick-walled to enable them to overcome severe climatic conditions such as low temperatures, chemical fumigations and biodegradation, thus conserving the inocula for the subsequent years[15]. The pathogen has low levels of diversity and its population structure and distribution is influenced by host preference[16].

Studying genetic variation within and among populations of related disease-causing agents is important in understanding pathogen-host co-evolution, disease epidemiology, development of sustainable control methods and preventing the development of host resistance[17]. For example, gene recombination through sexual reproduction can result in virulent genes thus complicating the management of diseases[16]. Genetic diversity of *A. solani* and *P. infestans* has been studied using various methods such as vegetative compatibility, virulence analyses, biochemical analyses and molecular analyses[17]. The most commonly used methods are molecular techniques such as isozyme analysis and PCR methods including Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) markers[13,18,19].

Different previous studies reported significant genetic variation within isolates of *P. Infestans* and *A. solani*. Van der Waals *et al*.[17] observed an exorbitant level of genetic differences in *A. solani* using Random amplified microsatellite markers which were not typical of a species that is assumed to reproduce asexually only. Based on RAPD-profiles, Leiminger *et al*.[20] reported the occurrence of significant genetic heterozygosity in *A. solani* isolates from potatoes in Southern Germany. Similar reports of the high degree of genetic diversity between isolates of *A. solani* were made by Nikam *et al*.[21]. Contrastingly, Cardenas *et al*.[22] reported low genetic diversity among *P. infestans* isolates from crops within diverse Colombian regions and Venezuela. Similarly, Wu *et al*.[23] observed low genetic diversity among 134 strains of *P. infestans* from four provinces in China. Generally, minimal studies have been conducted on the genetic diversity of *P. Infestans* and *A. solani* in Kenya despite the significance of these pathogens in tomato and potato production. The current study targeted to examine the morphological and molecular diversity of *A. solani* and *P. infestans* isolated from tomato plants in farmers' fields in Kirinyaga County, Kenya.

MATERIALS AND METHODS

Study area: The study was conducted between May to December, 2020. Sampling was done in Mwea, Kirinyaga County because it is an area renowned for tomato cultivation and blight diseases are rampant. The area lies within 0.6897°S,

37.3400°E. It is characterized by annual rainfall ranging between 800-1250 mm and is usually received in two seasons. The annual range of temperature is between 19.6-26.3°C. It has gentle rolling slopes with black cotton soils.

Collection of infected samples: Stratified random sampling was employed during the collection of samples whereby four tomato growing villages within the Sub-County were selected. Random sampling was done on fifteen tomato producing farms from each village for the collection of infected samples. Through visual examination, unhealthy tomato leaves showing symptoms of early and late blight were identified and randomly collected from the targeted farms. The collected diseased samples were put in cool boxes and transported to the University of Embu where they were preserved in a refrigerator at 4°C in the Microbiology Laboratory awaiting the pathogen isolation process.

Isolation of target pathogens: The isolation of *P. Infestans* and *A. solani* from the leaves that were infected was conducted following the modified approach of Naik *et al.*[18] as adopted by Mugao *et al.*[24]. The tomato leaves bearing blight symptoms were washed under clean running tap water first before being surface sterilized in 1% sodium hypochlorite for three minutes. Rinsing was then done in three changes of sterilized distilled water and sterilized blotting paper was used to blot them dry. Infected leaf tissues of 3×3 mm size were cut using a sterilized scalpel towards the healthy tissues where the blight pathogens were suspected to be more active. Direct plating of the surface-sterilized tissues was done on the sterilized PDA and V8 agar for early and late blight independently and then incubated in the laboratory for three days at room temperature (25°C). Pure cultures were obtained through single spore isolation using a hyphal segment from the three-day-old colonies of each of the pathogens. The hyphal sections were introduced into a sterilized growth medium (PDA and V8 agar) and the incubation was done at room temperature.

Morphological characterization of the pathogen isolates: Identification of the pathogens was done 8 days after single spore isolation using morphological features based on established keys[25] to verify the identity of the target pathogens. Morphological identification was based on visual observation of pathogen growth patterns, mycelia colours, margin colours and microscopic assessment of reproductive and vegetative structures[25]. Colonies with similar morphological characteristics were considered to be of the same species.

DNA extraction from pathogen isolates: The DNA extraction followed the modified procedure of Aamir *et al.*[26]. Sterilized wooden tooth sticks were used to aseptically scrap the mycelia of the 5-day old culture of *A. solani* and *P. infestans* isolates into separate Eppendorf tubes. In every tube, 1.5 mL Lysis buffer (50 mM Tris [pH 8.5], 20 mM EDTA [pH 8.0], 3% SDS) and 200 mg L^{-1} of 10 µL proteinase K were added and the content mixed by inversion. Vortexing of the mixture was done and then incubated at 65°C for 60 min. To the mixture, an equal volume of phenol-chloroform was added and the content was centrifuged at 13200 rpm for 5 min at 4°C. The supernatants were transferred to new tubes where 150 µL of sodium acetate (pH 5.2) was added and their volumes noted. An equal amount of isopropyl alcohol was added to the mixture and the content was mixed by inverting gently. The tubes were then spun at 13,200 rpm for 10 min and the supernatant discarded. To the resulting DNA pellets, 500 µL of 70% ethanol was added to wash them and then they were centrifuged for 1 min at 10,000 rpm and the supernatant was discarded. The DNA pellets were washed again with 70% ethanol and then re-suspended in 50 µL PCR water and stored at -20°C for further processing.

PCR amplification and DNA sequencing: PCR analysis was done in a 25 µL reaction mixture comprising 1 µL of genomic DNA, 0.5 µL each of the forward and reverse primers (ITS1 and ITS4), 0.125 µL of Taq polymerase, 2.5 mM dNTPs (2.5 µL), 0.125 µL of premix Taq buffer and 2.5 µL of 10x dream Taq buffer ($MgCl_2$). The final volume was topped up with 17.75 µL of molecular grade water to make the 25 µL. Amplification was performed in a PCR with the following cycling conditions: an initial hybridization at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 2 min. The amplicons were resolved by gel electrophoresis in 1.2% agarose gel stained with ethidium bromide (0.5 µg mL^{-1}). The DNA bands were visualized and photographed under UV light. The sizes of the amplicon were estimated by comparing them with a commercial 1 kb ladder RTU 1151021805 on the agarose gel. The primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCCTCC GCT TAT TGA TAT GC-3′) were used to amplify the Internal Transcribed Sequences (ITS) region. The PCR products were then sequenced and blasted using the NCBI database. Related species were identified based on more than 99% similarity between the query and reference sequence[27,28].

RESULTS

Morphological identification of *Alternaria* isolates: Twenty-four *Alternaria* isolates suspected to be *A. solani* varied in their morphological characteristics. Conidia characteristics varied significantly between the isolates although they were all solitary. The observed morphological features characterized the isolates into five groups in Table 1. Group one was the largest comprising of twelve isolates that were characterized by an irregularly shaped colony of greenish-brown colour with dark brown substrate colour and greyish white margin in Fig. 1a-b. These isolates had septate conidia with four transverse septa and elongated but unbranched beak in Fig. 1c. Group two had four isolates characterized by a circular dark brown colony with dark grey substrate colour and brownish white margin in Fig. 2a-b. Members of this group had muriform septate conidia with three transverse septa, one longitudinal septum and an unbranched slender short beak in Fig. 2c. Group three comprised of two isolates with circular dark grey colony having a black substrate and a white margin in Fig. 3a and b. Their conidia were septate with three transverse septa, one longitudinal septum and elongated branched beak in Fig. 3c. Group four contained only one isolate (A23) with a circular colony of grey colour, brown zoned substrate and grey margin in Fig. 4a-b. The isolate had septate conidia with four transverse septa and an unbranched slender short beak in Fig. 4c. Group five consisted of five isolates with a circular greyish brown colony with a brownish white margin Fig. 5a-b. Their conidia were muriform with two transverse septa and one longitudinal septum and elongated branched beak in Fig. 5c. The mycelia for all the groups were septate, straight, hyaline and branched while the colour of the conidia was brown in the entire group.

Morphological identification of *Phytophthora infestans*: All the sixteen isolates suspected to be *P. infestans* did not vary in their morphological characteristics. The isolates had a white colony (front) colour and creamish substrate (reverse) colour. The growth pattern was circular with white margin colour in Fig. 6a-b. There were oospores with oogonia and amphigynous antheridia in Fig. 6c. The mycelia were aseptate, multinucleate and heterothallic in Fig. 6d.

Molecular identification of *Alternaria solani*: The PCR amplification of the ITS region of the twenty-four *A. solani* isolates resulted in a product of about 580 bp. The band size did not vary between the fungal isolates in Fig. 7. However, isolate A24 did not amplify and thus could not be positively identified through sequencing.

Table 1: Morphological variability of *Alternaria* isolates

Groups	Isolates	Colony color	Substrate color	Margin color	Margin growth	Transverse conidial septa	Longitudinal conidial septa	Beak elongation	Beak branching
1	A1, A2, A8, A10, A12, A13, A14, A15, A16, A18, A20, A22	Greenish brown	Dark brown	Greyish white	Irregular	Four	None	Elongated	Unbranched
2	A4, A6, A9, A17	Dark brown	Dark grey	Brownish white	Circular	Three	One	Slender short	Unbranched
3	A5, A11	Dark grey	Black	White	Circular	Three	None	Elongated	Branched
4	A23	Grey	Brown with zonation	Grey	Circular	Four	None	Slender short	Unbranched
5	A3, A7, A19, A21, A24	Greyish brown	Greyish brown	Brownish white	Circular	Two	One	Stout short	Unbranched

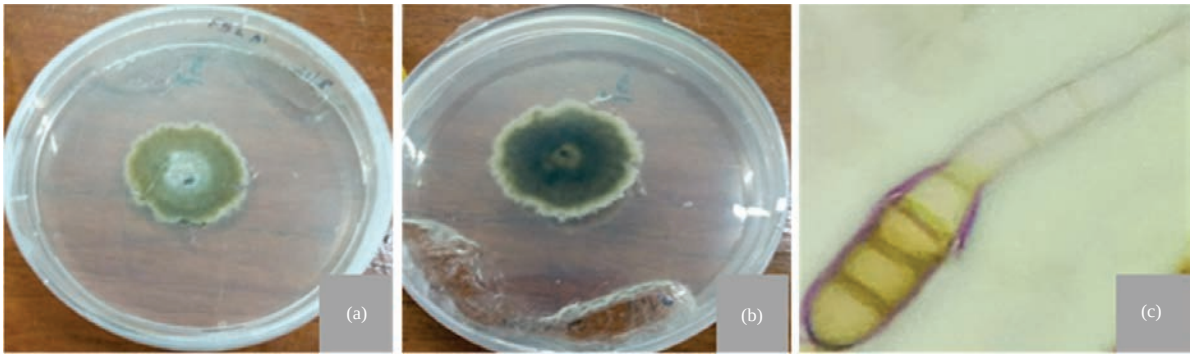

Fig. 1(a-c): Group one of the *Alternaria* isolates (a) Upper and (b) Lower and (c) Conidium

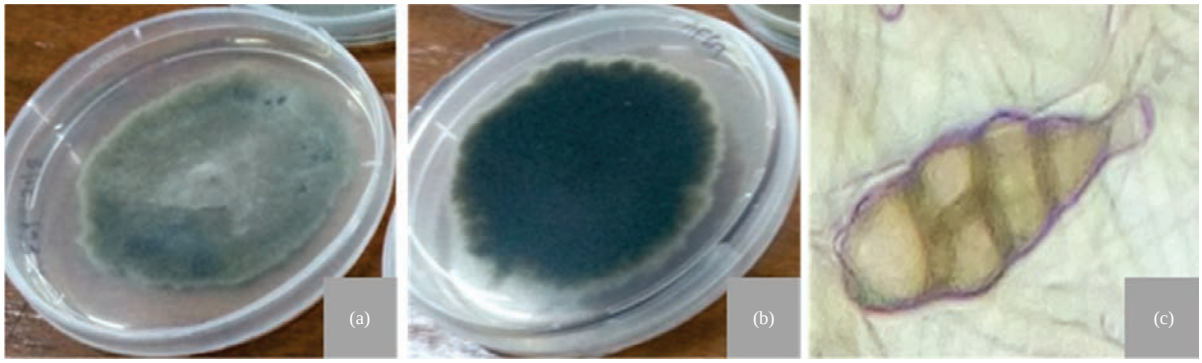

Fig. 2(a-c): Group two of the *Alternaria* isolates (a) Upper, (b) Lower and (c) Conidium

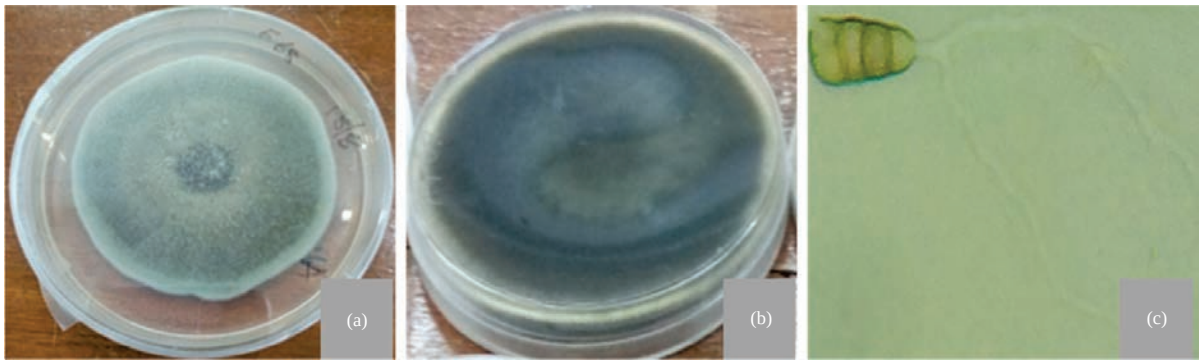

Fig. 3(a-c): Group three of the *Alternaria* isolates (a) Upper, (b) Lower and (c) Conidium

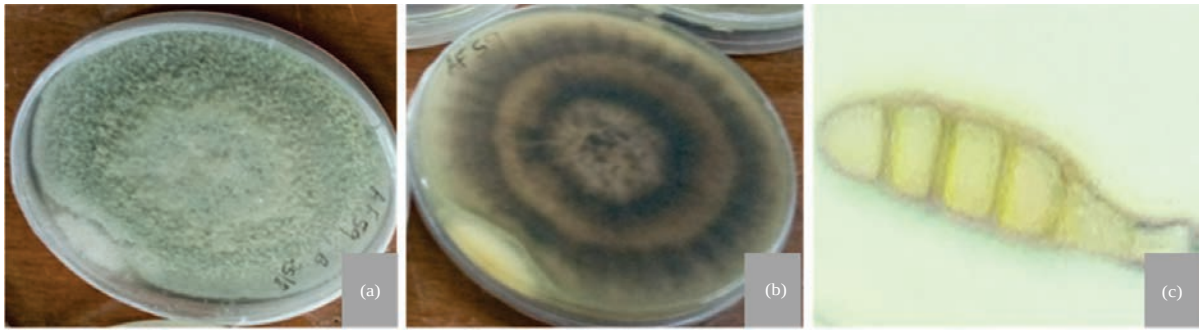

Fig. 4(a-c): Group four of the *Alternaria* isolates (a) Upper, (b) Lower and (c) Conidium

Fig. 5(a-c): Group five of the *Alternaria* isolates (a) Upper, (b) Lower and (c) Conidium

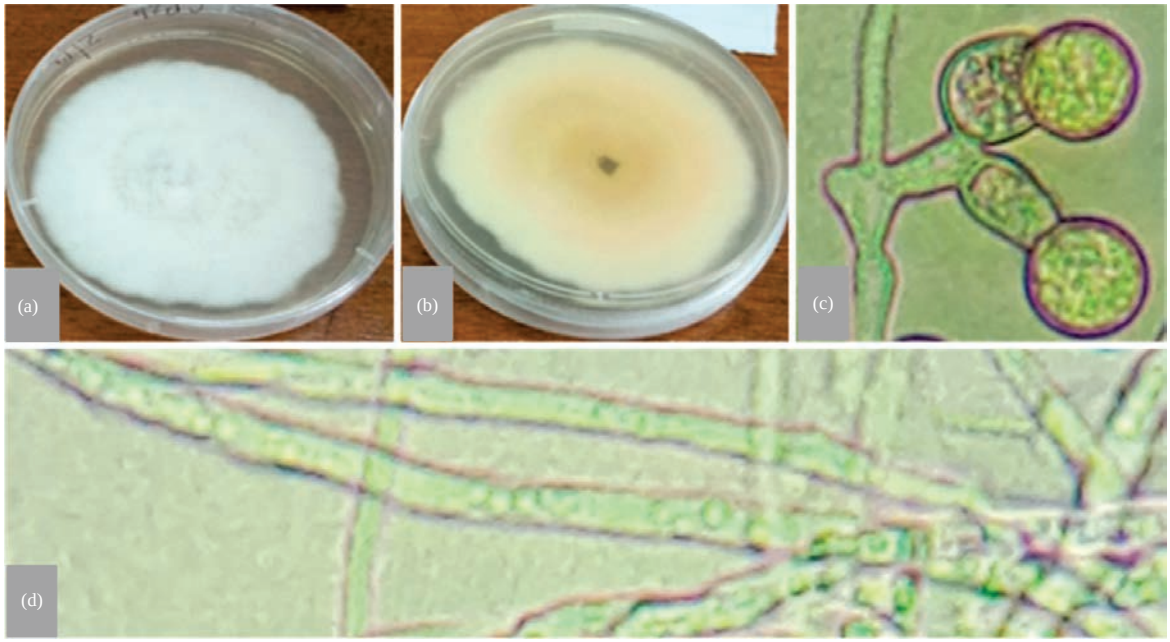

Fig. 6(a-d): (a) Upper, (b) Lower colour of *P. infestans* colony, (c) Sporangiophore with oospores and (d) Mycelia

Sequence similarity searches of the 23 isolates whose ITS fragment amplified successfully was carried out using the nucleotide BLAST program in the NCBI database. Nineteen of them were positively identified as *A. solani* while the other four were identified as *A. alternata* in Table 2. Eleven *A. solani* isolates showed 100% nucleotide similarity to Genebank clone 105 with accession number MN871613 together with isolate A1 which showed 99.42% nucleotide similarity to clone 105 in the Genebank. These had earlier been grouped under morphological group 1. Four other *A. solani* isolates (A4, A6, A9, A17) which were members of the morphological group 2 were found to be 100% similar to the Genebank clone 185 with accession number MN871616.

Isolates A5 and A11 which belonged to the morphological group 3 had 100% similarity with Genebank clone 43 with accession number MN871610 (Table 2). Isolate A23 which was the only one in morphological group 4 was found to have 99.81% nucleotide similarity with *A. solani* accession number LN879928 in the Genebank but its closest clone could not be identified. The accession MN871613 was the most prevalent with a total percent frequency of 52.18% followed by MN871616 (17.39%) and MN871610 (8.68%). The four isolates (A3, A7, A19, A21) that were identified to belong to *Alternaria alternata* resembled clones with accession numbers MN822496, MN822565, KY570321 and MW009021 respectively and they showed a 100% perfect match with different Genebank strains as shown in Table 2. The four belonged to morphological group 5 together with isolate 24 whose ITS fragment was not amplified.

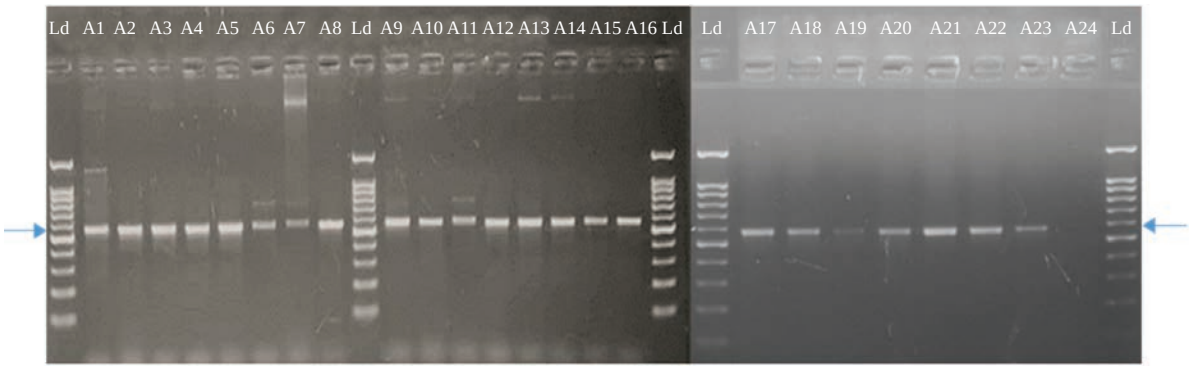

Fig. 7: Gel image showing 580 bp (arrowed) DNA fragment amplified by ITS (1 and 4) primer in *A. solani* isolates on 1.2% agarose gel electrophoresis
Ld is the I kb RTU 1151021805 ladder

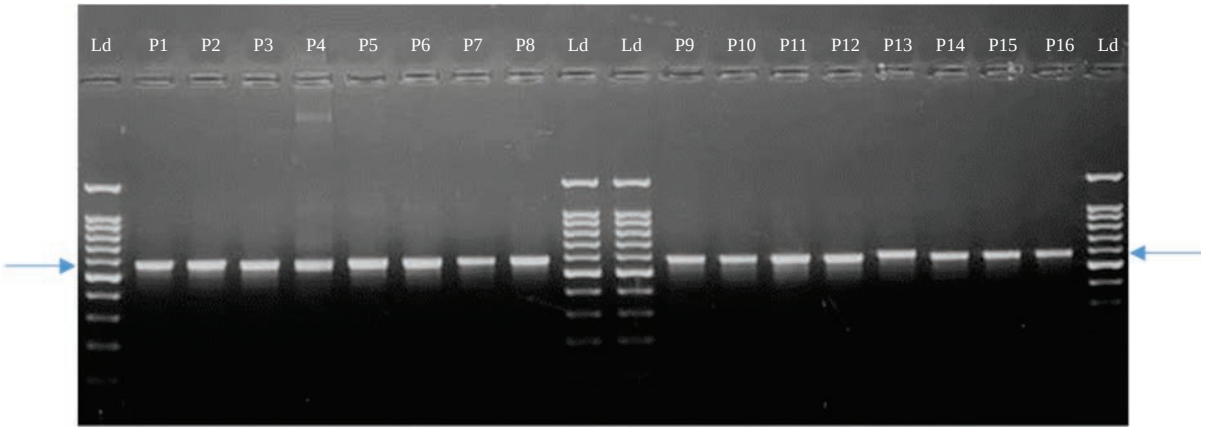

Fig. 8: Gel image showing 580 bp (arrowed) DNA fragment amplified by ITS (1 and 4) primer in *P. infestans* isolates on 1.2% agarose gel electrophoresis
Ld is the I kb RTU 1151021805 ladder

Table 2: Molecular variability of *Alternaria* isolates

Groups	Isolate code	Species Identity	Accession number	Closest match	Similarity (%) to Genebank accessions	Frequency (%)
1	A1	*A. solani*	MN871613	Clone 105	99.42	4.35
	A2, A8, A10, A12, A13, A14, A15, A16, A18, A20, A22	*A. solani*	MN871613	Clone 105	100	47.83
2	A4, A6, A9, A17	*A. solani*	MN871616	Clone 185	100	17.39
3	A5, A11	*A. solani*	MN871610	Clone 43	100	8.68
4	A23	*A. solani*	LN879928	Unidentified	99.81	4.35
5	A3	*A. alternata*	MN822496	BJ-YZ-14	100	4.35
	A7	*A. alternata*	MN822565	BJ-SB-41	100	4.35
	A19	*A. alternata*	KY570321	Strain Te 19	100	4.35
	A21	*A. alternata*	MW009021	DT1884-B	100	4.35

Molecular identification of *Phytophthora infestans*: The ITS sequence amplification of the sixteen (16) *P. infestans* isolates by PCR resulted in a product of about 580 bp and there was no variation among the isolates in Fig. 8.

Sequence similarity searches of the 16 isolates suspected to be *P. infestans* was carried out using the nucleotide BLAST program which matched the isolates with Genebank accessions. The results showed that eleven of these isolates (68.75%) were positively identified as *P. infestans* with 100% genotypic similarity Strain A2 of accession number JX666330 in Table 3. Three other isolates (P8, P15, P16) belonged to unspecified species of the genus *Phytophthora* and matched

Table 3: Molecular variability of *Phytophthora*

Groups	Isolate code	Species identity	Accession number	Closest match	Similarity (%) to Genebank accessions	Frequency (%)
1	P1, P2, P3, P4, P5, P7, P9, P10, P12, P13, P14	*P. infestans*	JX666330	Strain A2	100	68.75
2	P8, P15, P16	*Phytophthora* sp.	MT075724	Strain Phy-1i	100	18.75
3	P6, P11	*Fusarium equiseti*	MK571264	Strain P3B	100	12.5

100% with Strain Phy-1i with Genebank accession number MT075724. The other two isolates (P6, P11) were identified as *Fusarium equiseti* with 100% resemblance to Strain P3B that had Genebank accession number MK571264.

DISCUSSION

The pathogenic, cultural, morphological and molecular characterization of pathogens has been attempted by many researchers in various countries[18,21,29]. In the current study, the pathogen isolates that were suspected to be twenty-four *A. solani* and sixteen *P. infestans* isolates until molecular identification revealed that they were mixed with other closely related fungal species. Therefore, morphological characterization provided a good lead towards species identification but could not specifically identify the isolates to species level. Okayo *et al.*[30] noted that morphological classification of fungal species lacks precision but it is important in assisting the organization of the fungal isolates into groups allowing easier scrutiny by advanced methods.

Out of the 24 isolates that were initially thought to be *A. solani*, four of them were confirmed to belong to *A. alternata*, a fungus that causes leaf spot and other diseases in plants[31]. The two pathogens, *A. alternata* and *A. solani*, were reportedly isolated from blight infected plants in several previous studies. Zheng *et al.*[32] reported the association of *A. alternate* and *A. solani* in causing potato blight in China. The two pathogens were also found to cause foliar diseases in Germany[33]. Loganathan *et al.*[34] also reported *A. alternata* to cause early blight diseases in India with 80-90% disease incidence on susceptible tomato plants. However, *A. alternata* was absent in foliage with blight symptoms in Sweden[35]. On the other hand, out of the sixteen isolates that were suspected to be *P. infestans*, three of them belonged to unspecified species of the genus *Phytophthora* while two of them belonged to *Fusarium equiseti* which does a soil inhabit fungus known to cause wilt disease in different vegetable plants[36].

Morphological characteristics such as colony colour, colony texture, size and shape of the conidia have been used to differentiate *Alternaria* species[37] while asexual and sexual features are mostly used to differentiate *Phytophthora* species[38]. This study revealed high morphological variability within *A. solani* isolates but low morphological variation among *P. infestans* isolates. The macroscopic features such as growth pattern, colony (upper) colour, substrate (lower) colour and colour of the growth margin showed variability among *A. solani* isolates. Similar results were reported by Kumar *et al.*[39], Tanvil *et al.*[40], Brook and Dennis[13] and Hubballi *et al.*[41]. In addition, microscopic features of mycelia and conidia of *A. solani* were variable but similar to those reported by Najibullah *et al.*[42], Brooke and Dennis[13] and Naik *et al.*[18]. Gannibal *et al.*[43] also documented heterogeneity in various morphological attributes of *A. solani*.

High genetic diversity of *A. solani* have been reported by several authors[20,44,45]. Chaerani and Voorrips[46] reported that genetic variation may occur among isolates obtained from different lesions of the same leaflet. According to Craven *et al.*[47], genotypic variation in *A. solani* is caused by the ability of its mycelia to interconnect by bridges made through hyphal fusion that enable the distribution of nutrients, water and signalling molecules all over the colony. Genetic diversity is also contributed by mutations, selection, gene flow[48], heterokaryosis which occurs as a result of hyphal anastomosis[46], recombination and movement of the pathogen over long distances[17].

The low genetic diversity observed in this study among *P. infestans* isolates corroborates the reports of Cardenas *et al.*[22] and Wu *et al.*[23] who also reported low diversity among isolates of *P. infestans* collected in the Northern Andean region and China respectively. Njoroge *et al.*[49] also reported low variability within *P. infestans* in East Africa. The low variability among *P. infestans* isolates has been attributed to the existence of clonal populations of the pathogen in the target regions[22-23]. However, Han *et al.*[50] reported high genetic diversity among *P. infestans* field isolates in China despite their high frequency of self-fertility. The possible sources of genetic variation in *P. infestans* include mitotic crossing over, gene conversion, extrachromosomal elements[51], migration and sexual recombination of A1 and A2 mating types[52-53]. Other researchers attributed the diversity to self-sterility[54], segregation of heterokaryons[55], presence of many wild species of the host plant[56] and ideal climate for the pathogen development[57].

This study successfully identified *A. solani* and *P. infestans* isolates to species level through amplification and subsequent sequencing of their ITS region. The band size generated from the PCR product with ITS 1 and ITS 4 primers was about 580 bp for the two pathogens. These findings aligned with those of Loganathan *et al.*[34] who used ITS1 and ITS4 to amplify the DNA of *A. solani* and obtained a band of 580 bp. Zheng *et al.*[32] also obtained a 580 bp band in *A. solani* DNA amplified with primer set H3-1a/H3-1b. Reports of Manter and Vivanco[58] and Embong *et al.*[59] showed that different *A. solani* species generate bands ranging from 400-600 bp depending on the primers used. For *P. infestans*, different band sizes have been obtained by different researchers using different primers. The Genomic DNA of *P. infestans* amplified using TUBUF2 and TUBUR1 primers resulted in bands of about 990 bp[20]. Khalid *et al.*[60] amplified the genomic DNA of *P. infestans* using ITS3 and ITS4 primers and yielded a band of 612 bp.

Sequencing and similarity matching of the isolates with available accessions through blasting on the NCBI database enabled identification of *A. solani* clones and *P. infestans* strains that are available in the study area. These findings indicated that different clones of *A. solani* existed in the study area which is not typical of a species that is known to reproduce only asexually. Clone 105 was the most prevalent with a total percent frequency of 52.18% followed by clone 185 (17.39%) and clone 43 (8.68%). The results also revealed the presence of one unidentified clone of *A. solani* in the study area. Van der Waals *et al.*[17] and Leiminger *et al.*[20] also reported the presence of different clones of *A. solani* in the same region in China and Southern Germany respectively. In addition, the results portrayed a close association between *A. solani* and *A. alternata*. The close association between the two pathogens in causing blight and other foliar diseases in plants have been reported by other researchers[33-34]. All the eleven isolates that were positively identified as *P. infestans* were found to be Strain A2 indicating high dominance of this strain in the study region. The high occurrence of the A2 strain of *P. infestans* has been reported in several parts of China[50,61,62].

CONCLUSION

This study concluded that there is higher genetic variability within *A. solani* than *P. infestans* in Kirinyaga County, Kenya. At least four clones of *A. solani* were identified in the area including clone 105 which was the most dominant, clone 185 and clone 43. However, only one strain of *P. infestans* (Strain A2) was identified in the study region. The study also revealed a close association between *A. solani* and *A. alternata* as well as between *P. infestans* and other identified *Phytophthora* species. The variability of the pathogens can be studied across seasons and different host plants to understand the changes in epidemiology and host-pathogen interactions. It may also be important to investigate the nature of the association between the target pathogens and the other pathogens identified in the area. In addition, the study revealed the presence of *Fusarium equiseti* a soil-borne fungus that causes wilt disease in different vegetable plants.

SIGNIFICANCE STATEMENT

This study discovered that the early blight pathogen *A. solani* exist in more variable forms than the late blight pathogen *P. infestans* in Kirinyaga County, Kenya. These findings will be useful in the development of sustainable strategies to manage the early and late blight diseases in tomato growing areas in Kenya. The close association revealed by this study between *A. solani* and *A. alternata* as well as between *P. infestans* and another unidentified phytophthora species will form the basis of further research to determine the nature of that association.

ACKNOWLEDGMENT

The authors acknowledge the Higher Education Loans Board for providing financial support and the University of Embu for providing the laboratory space where the work was carried out.

REFERENCES

1. Onyambus, G.K., R.O. Maranga, L.M. Gitonga and M. Knapp, 2011. Host plant resistance among tomato accessions to the spider mite *Tetranychus evansi* in Kenya. Exp. Appl. Acarol., 54: 385-393.
2. Geoffrey, S.K., N.K. Hillary, K.M. Antony, M. Mariam and M.C. Mary, 2014. Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. Int. J. Bus. Manage., 9: 205-212.
3. Ochilo, W.N., G.N. Nyamasyo, D. Kilalo, W. Otieno, M. Otipa, F. Chege, T. Karanja and E.K. Lingeera, 2019. Characteristics and production constraints of smallholder tomato production in Kenya. Sci. Afr., Vol. 2. 10.1016/j.sciaf.2018.e00014.
4. Masinde, A.O.A., K.T. Kwambai and N.H. Wambani, 2011. Evaluation of tomato (*Lycopersicon esculentum* L.) variety tolerance to foliar diseases at Kenya Agricultural Research Institute Centre, Kitale in North West Kenya. Afr. J. Plant Sci., 5: 676-681.
5. Nowicki, M., E.U. Kozik and M.R. Foolad, 2013. Late blight of Tomato. In: InTranslational Genomics for Crop Breeding, Varshney, R.K. and R. Tuberosa (Eds.)., Wiley Online Library, pp: 241-265.

6. Singh, V.K., A.K. Singh and A. Kumar, 2017. Disease management of tomato through PGPB: Current trends and future perspective. 3 Biotech, Vol. 7. 10.1007/s13205-017-0896-1.
7. Kumar, P. and S. Singh, 2017. *In vitro* evaluation of fungicides and plant extract against *Alternaria solani* (Ellis) causing early blight in tomato (*Lycopersicon esculentum* Mill.). Int. J. Curr. Microbiol. Appl. Sci., 6: 820-827.
8. Desta, M. and M. Yesuf, 2015. Efficacy and economics of fungicides and their application schedule for early blight (*Alternaria solani*) management and yield of tomato at South Tigray, Ethiopia. J. Plant Pathol. Microbiol., Vol. 6. 10.4172/2157-7471.1000268.
9. Jambhulkar, P.P., N. Jambhulkar, M. Meghwal and G.S. Ameta, 2016. Altering conidial dispersal of *Alternaria solani* by modifying microclimate in tomato crop canopy. Plant Pathol. J., 32: 508-518.
10. Stroud, J.A., D.S. Shaw, M.D. Hale and K.A. Steele, 2016. SSR assessment of *Phytophthora infestans* populations on tomato and potato in British gardens demonstrates high diversity but no evidence for host specialization. Plant Pathol., 65: 334-341.
11. Ojiewo, C.O., I.S. Swai, M.O. Oluoch, D. Silué and R. Nono-Womdim *et al*., 2010. Development and release of late blight-resistant tomato varieties 'Meru' and 'Kiboko'. Int. J. Vegetable Sci., 16: 134-147.
12. Agrios, G.N., 2005. Plant Pathology. 5th Edn., Elsevier Academic Press, Amsterdam, New York, USA, ISBN-13: 9780120445653, Pages: 922.
13. Weber, B. and D.A. Halterman, 2012. Analysis of genetic and pathogenic variation of *Alternaria solani* from a potato production region. Eur. J. Plant Pathol., 134: 847-858.
14. Pacilly, F.C.A., J.C.J. Groot, G.J. Hofstede, B.F. Schaap and E.T.L. van Bueren, 2016. Analysing potato late blight control as a social-ecological system using fuzzy cognitive mapping. Agron. Sustainable Dev., Vol. 36. 10.1007/s13593-016-0370-1.
15. Judelson, H.S., A.M.V. Ah-Fong, G. Aux, A.O. Avrova and C. Bruce *et al*., 2008. Gene expression profiling during asexual development of the late blight pathogen *Phytophthora infestans* reveals a highly dynamic transcriptome. Mol. Plant Microbe Interact., 21: 433-447.
16. Zhao, W., S. Dong, W. Ye, C. Hua and H.J.G. Meijer *et al*., 2011. Genome-wide identification of *Phytophthora sojae* SNARE genes and functional characterization of the conserved SNARE PsYKT6. Fungal Genet. Biol., 48: 241-251.
17. Van der Waals, J.E., L. Korsten and B. Slippers, 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. Plant Dis., 88: 959-964.
18. Naik, M.K., Y. Prasad, K.V. Bhat and G.S.D. Rani, 2010. Morphological, Physiological, Pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. Indian Phytopthol., 63: 168-173.
19. Nandani, K. and S.K. Thakur, 2014. Randomly amplified polymorphic DNA-a brief review. Am. J. Anim. Vet. Sci., 9: 6-13.
20. Leiminger, J.H., H.J. Auinger, M. Wenig, G. Bahnweg and H. Hausladen, 2013. Genetic variability among *Alternaria solani* isolates from potatoes in Southern Germany based on rapd-profiles. J. Plant Dis. Prot., 120: 164-172.
21. Nikam, P.S, A.P. Suryawanshi and A.A. Chavan, 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria solani*, causing early blight of tomato. Afr. J. Biotechnol., 14: 872-877.
22. Cardenas, M., A. Grajales, R. Sierra, A. Rojas and A. González-Almario, 2011. Genetic diversity of *Phytophthora infestans* in the Northern Andean region. BMC Genet., Vol. 12. 10.1186/1471-2156-12-23.
23. Wu, Y., J. Jiang and C. Gui, 2012. Low genetic diversity of *Phytophthora infestans* population in potato in north China. Afr. J. Biotechnol., 11: 15636-15642.
24. Mugao, L.G., P.W. Muturi, B.M. Gichimu and E.K. Njoroge, 2020. *In vitro* control of *Phytophthora infestans* and *Alternaria solani* using crude extracts and essential oils from selected plants. Int. J. Agron., Vol. 2020. 10.1155/2020/8845692.
25. Samson, R.A., J. Varga and J.C. Frisvad, 2011. Taxonomic Studies on the Genus *Aspergillus*. CBS-KNAW Fungal Biodiversity Centre, Netherlands, ISBN-13: 9789070351861, Pages: 96.
26. Aamir, S., S. Sutar, S.K. Singh and A. Baghela, 2015. A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods. Plant Pathol. Quarantine, 5: 74-81.
27. Pandey, A.K., M.S. Reddy and T.S. Suryanarayan, 2003. ITS-RFLP and ITS sequence analysis of a foliar endophytic *Phyllosticta* from different tropical trees. Mycol. Res., 107: 439-444.
28. Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.
29. Singh, A., V. Singh and S.M. Yadav, 2014. Cultural, morphological and pathogenic variability of *Alternaria solani* causing early blight in tomato. Plant Pathol. J., 13: 167-172.
30. Okayo, R.O., D.O. Andika, M.M. Dida, G.O. K'Otuto and B.M. Gichimu, 2020. Morphological and molecular characterization of toxigenic *Aspergillus flavus* from groundnut kernels in Kenya. Int. J. Microbiol., Vol. 2020. 10.1155/2020/8854718.
31. Gat, T., O. Liarzi, Y. Skovorodnikova and D. Ezra, 2012. Characterization of *Alternaria alternata* causing black spot disease of pomegranate in Israel using a molecular marker. Plant Dis., 96: 1513-1518.

32. Zheng, H.H., J. Zhao, T.Y. Wang and X.H. Wu, 2015. Characterization of *Alternaria* species associated with potato foliar diseases in China. Plant Pathol., 64: 425-433.
33. Leiminger, J.H., B. Adolf and H. Hausladen, 2014. Occurrence of the F129L mutation in *Alternaria solani* populations in Germany in response to QoI application and its effect on sensitivity. Plant Pathol., 63: 640-650.
34. Loganathan, M., V. Venkataravanappa, S. Saha, A.B. Rai and S. Tripathi *et al*., 2016. Morphological, pathogenic and molecular characterizations of *Alternaria* species causing early blight of tomato in Northern India. Proc. Nat. Acad. Sci., India Section B: Biol. Sci., 86: 325-330.
35. Odilbekov, F., E. Edin, L. Garkava-Gustavsson, H.P. Hovmalm and E. Liljeroth, 2016. Genetic diversity and occurrence of the F129L substitutions among isolates of *Alternaria solani* in South-Eastern Sweden. Hereditas, Vol. 153. 10.1186/s41065-016-0014-0.
36. Akbar, A., S. Hussain and G.S. Ali, 2018. Germplasm evaluation of tomato for resistance to the emerging wilt pathogen *Fusarium equiseti*. J. Agric. Stud., 6: 174-189.
37. Simmons, E.G., 2007. Alternaria: An Identification Manual. CBS Fungal Biodiversity Centre, Netherlands, ISBN-13: 9789070351687, Pages: 775.
38. Ho, H.H., 2018. The taxonomy and biology of phytophthora and pythium. J. Bacteriol. Mycol.: Open Access, 6: 40-45.
39. Kumar, S.P., M.K. Mishra and P.R. Mishra, 2018. *In vitro* efficacy of botanicals and biocontrol agents against early leaf blight in tomato. Int. J. Curr. Microbiol. Appl. Sci., 7: 1340-1345.
40. Kaur, T., A.N. Yadav, S. Sharma and N. Singh, 2020. Diversity of fungal isolates associated with early blight disease of tomato from mid Himalayan region of India. Arch. Phytopathol. Plant Prot., 53: 612-624.
41. Hubballi, M., S. Nakkeeran, T. Raguchander, T. Anand and R. Samiyappan, 2010. Effect of environmental conditions on growth of *Alternaria alternate* causing leaf blight of noni. World J. Agric. Sci., 6: 171-177.
42. Rahmatzai, N., A.A. Zaitoun, M.H. Madkour, A. Ahmady, Z. Hazim and M.A.A. Mousa, 2016. Morphological, pathogenic, cultural and physiological variability of the isolates of *Alternaria solani* causing early blight of tomato. Int. J. Adv. Res., 4: 808-817.
43. Gannibal, P.B., A.S. Orina, N.V. Mironenko and M.M. Levitin, 2014. Differentiation of the closely related species, *Alternaria solani* and *A. tomatophila*, by molecular and morphological features and aggressiveness. Eur. J. Plant Pathol., 139: 609-623.
44. Lourenco, Jr., V., T.T. Rodrigues, A.M. Campos, C.A. Braganca and K.K. Scheuermann *et al*., 2011. Genetic structure of the population of *Alternaria solani* in Brazil. J. Phytopathol., 159: 233-240.
45. Meng, J.W., W. Zhu, M.H. He, E.J. Wu, L.N. Yang, L.P. Shang and J. Zhan, 2015. High genotype diversity and lack of isolation by distance in the *Alternaria solani* populations from China. Plant Pathol., 64: 434-441.
46. Chaerani, R. and R.E. Voorrips, 2006. Tomato early blight (*Alternaria solani*): The pathogen, genetics and breeding for resistance. J. Gen. Plant Pathol., 72: 335-347.
47. Craven, K.D., H. Velez, Y. Cho, C.B. Lawrence and T.K. Mitchell, 2008. Anastomosis is required for virulence of the fungal necrotroph *Alternaria brassicicola*. Eukaryotic Cell, 7: 675-683.
48. McDonald, B.A. and C. Linde, 2002. Pathogen population genetics evolutionary potential and durable resistance. Annu. Rev. Phytopathol., 40: 349-379.
49. Njoroge, A.W., B. Andersson, A.K. Lees, C. Mutai, G.A. Forbes, J.E. Yuen and R. Pelle, 2019. Genotyping of *Phytophthora infestans* in Eastern Africa reveals a dominating invasive European lineage. Phytopathology, 109: 670-680.
50. Han, M., G. Liu and J.P. Li, 2013. *Phytophthora infestans* field isolates from Gansu province, China are genetically highly diverse and show a high frequency of self-fertility. J. Eukaryotic Microbiol., 60: 79-88.
51. Abu-El Samen, F.M., G.A. Secor and N.C. Gudmestad, 2003. Variability in virulence among asexual progenies of *Phytophthora infestans*. Phytopathology, 93: 293-304.
52. Grunwald, N.J., W.G. Flier, A.K. Sturbaum, S.E. Garay, T.B.M. Van Den Bosch *et al*., 2001. Population structure of *Phytophthora infestans* in the Toluca valley region of central Mexico. Phytopathology, 91: 882-890.
53. Cooke, D.E.L., V. Young, P.R.J. Birch, R. Toth and F. Gourlay *et al*., 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). Plant Pathol., 52: 181-192.
54. Zhu, W., L.L. Shen, Z.G. Fang, L.N. Yang, J.F. Zhang, D.L. Sun and J. Zhan, 2016. Increased frequency of self-fertile isolates in *Phytophthora infestans* may attribute to their higher fitness relative to the A1 isolates. Sci. Rep., Vol. 6. 10.1038/srep29428.
55. Elansky, S.N. and D.I. Milyutina, 2007. Heteroplasmosis in *Phytophthora infestans*. Russian J. Genet., 43: 255-258.
56. Spooner, D.M., R.G. van den Berg, A. Rodríguez, J. Bamberg, R.J. Hijmans, S.I. Lara and Cabrera, 2004. Wild Potatoes (Solanum Section Petota; Solanaceae) of North and Central America. The American Society of Plant Taxonomists. Syst. Bot. Monogr. 68:1-209. http://www.jstor.org/stable/25027915.
57. Janiszewska, M., S. Sobkowiak, E. Stefanczyk, E. Stefanczyk and J. Sliwka, 2020. Population structure of *Phytophthora infestans* from a single location in Poland over a long period of time in context of weather conditions. Microbial. Ecol., 81: 746-757.

58. Manter, D.K. and J.M. Vivanco, 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. J. Microbiol. Methods, 71: 7-14.
59. Embong, Z., W.H.W. Hitam, C.Y. Yean, N.H.A. Rashid and B. Kamarudin *et al*., 2008. Specific detection of fungal pathogens by 18S rRNA gene PCR in microbial keratitis. BMC Ophthalmol., Vol. 8. 10.1186/1471-2415-8-7.
60. Khalid, H., A. Grover and S.K. Dwivedi, 2018. PCR-based methods for identification and detection of *Phytophthora infestans* in infected leaves of tomato. Defence Life Sci. J., 3: 41-44.
61. Li, Y., T. van der Lee, J.H. Zhu, G.H. Jin and C.Z. Lan *et al*., 2013. Population structure of *Phytophthora infestans* in China - geographic clusters and presence of the EU genotype blue_13. Plant Pathol., 62: 932-942.
62. Tian, Y.E., J.L. Yin, J.P. Sun, Y.F. Ma, Q.H. Wang, J.L. Quan and W.X. Shan, 2015. Population genetic analysis of *Phytophthora infestans* in Northwestern China. Plant Pathol., 65: 17-25.