Full Length Research Paper

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

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

**Key words:** Diversity, morphology, pathogenecity, *Pyricularia grisea*.

**INTRODUCTION**

Finger millet blast disease, caused by *Pyricularia grisea* (Teleomorph; *Magnaporthe grisea* (T.T Herbert, (M. E Barr)), is the most economically important disease of finger millet (Mgonja et al., 2007). It is known to cause significant losses in yield and utilization of finger millet. Worldwide losses of above 50% yield have been reported.
in finger millet and above 30% in rice production (Esele, 2002; Prajapati et al., 2013). In India, an increase in % infection in the neck and finger results to a corresponding increase of 0.32 and 0.084% in yield losses and grain losses of 6.75 to 87.5%, respectively (Rao, 1990). In East Africa, Pagliaccia et al. (2018) reported yield losses that exceeded 80%, while in rice, P. grisea has been known to cause yield loss of 60-100% (Kihoro et al., 2013). Blast infects finger millet at all stages from seedling stages all through panicle formation (Sreenivasaaprasad et al., 2004). Effect on the panicle on susceptible genotypes is drastic and may lead to total seed loss of entire finger millet crop (Gashaw et al., 2014). Muumba-Kankolongo (2018) reported that favorable weather conditions (temperature of 25°C and 80% humidity) precedes infection of blast diseases, which starts when a three-celled conidia lands on a leaf surface. This leads to formation of an appressorium which later forms a penetration peg, punctures the cuticle allowing entry to the epidermis. Formation of lesions then follow which later spreads to the whole plant through the epidermis forming diamond shaped grey lesions with brown or black margins. Infection from the leaves begins from the tip backwards. The disease has a wide range of hosts especially grasses and sedge species including rice (Oryza sativa), wheat (Triticum aestivum), pearl millet (Setaria italica), and foxtail millet (Setaria italica). Blast affects production and utilization of these crops leading to a substantial decrease in production in Southern Asia, Eastern and Southern Africa (Takan et al., 2012).

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

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

MATERIALS AND METHODS

Isolation of P. grisea

Four samples each of diseased tissues (leaves and panicle) were picked from five different regions (Bomet, Baringo, Busia, Makueni and Nakuru) and ported to Egerton biotechnology laboratory for isolation of the fungus using Tuite (1969) and Aneja (2005)’s standard tissue isolation procedure with minor modifications. The margins of the infected tissues were cut in triangular shapes of 5-10 mm and surface sterilized with 0.5% sodium hypochlorite solution and dipped in sterile distilled water for 2 s to saturate the specimen and dealkoholize. The tissues were then placed in glass plates with filter paper to dry the excess water. They were then plated on growth media containing Oat meal Agar amended with 60mg/l Neuronycine sulphate to avoid bacterial contamination (Tredway et al., 2003) and incubated at 25 ± 1°C in artificial light on a 12 h light/dark photoperiod for 15–25 days for sporulation and growth of the fungi (Aneja, 2005). Pure colonies were obtained from each region through five subsequent sub-culturing.

Microscopic characterization of P. grisea isolates

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

**Pathogen spore preparation**

Spores and conidia from a 14 day cultured fungi were harvested by flooding five plates representing the five regions with sterilized distilled water and scraping the growth by a spatula and placed on glass Petridish (Getachew et al., 2014). A spore solution of 10 µl was pipetted and placed on a haemocytometer and the number of spores counted on the chambers A, B, C, D and E. The numbers found were then used to determine the number of spores per ml by multiplying the value by 2000. The spore suspension was then adjusted to desired concentration of 1x10^8 spores/litre with the help of hemocytometer using the C1V1 formula and 0.01% Tween 20, a surfactant which amends the properties of the carrier to ensure it dissolves. The suspension was then sieved through a double layer of muslin sleeve poured in calibrated hand sprayers.

**DNA extraction of *P. grisea***

Pure cultures of *P. grisea* strains grown on oat Meal Agar (OMA) for 5 days were used. DNA extraction was done using a modification of the Dellaporta (1994) protocol. The modifications included use of a single SDS buffer (Modified SDS Buffer Components; 20g SDS, 100ml of 1M Tris-HCl (pH 8.0), 50ml of 0.5M EDTA (pH 8.0), 20g PVP, 10g Sodium Sulphite and 82g Sodium Chloride), omission of the sodium acetate, potassium acetate and mecarptoethanol. 70% ethanol was used instead of 80% and subsequent extraction procedure modified as follows: 50-70 mg of fungal mycelia was scrapped from the Petridish and placed in a 2ml microcentrifuge tube. 1000 µl of 2% SDS buffer was added and placed in a water bath at 65°C for 1 h. Centrifugation was then done at 13000 rpm for 10 min. 750 µl of the supernatant was then pipetted out and placed on a sterile empty 2 ml microcentrifuge tube and an equal amount of Chloroform: Isopropanol was used instead of Chloroform: isoamyl alcohol. This was then incubated in a freezer (−20°C) for 12 h and then centrifuged at 13,000 rpm for 10 min.

After centrifugation, the supernatant was carefully decanted leaving a pellet in the tubes. 700 µl of 70% alcohol was added and centrifuged at 13,000 rpm for 10 min. Ethanol was carefully decanted leaving the pellets. The pellets were then air-dried for 30 min, 100 µl of sterile ddH2O added and samples incubated at 65°C for 1 h to dissolve the pellets. The samples were then stored at 4°C.

**Table 1. Primers used with annealing temperature, forward and backward primer-sequences.**

| Name    | AT | Forward primer (5'-3')                | Backward primer (5'-3')                |
|---------|----|---------------------------------------|----------------------------------------|
| MGM200  | 55 | AAGGTTAATGATGGCTACATTGC               | GCTGATTTGTTTGGCTGTT                    |
| MGM436  | 55 | GACCTTATCGATGCGTGT                    | CACACAGTGCCACTAAG                     |
| MGM437  | 55 | GCCCTCAATAGATCGTCAA                   | ACTGCCGATTTAACC                      |
| MGM451  | 55 | TCTCAAGTGGCTTTGAAATTGA                | CTTGATTGGTGTTGTTT                     |
| MGM454  | 55 | GCAAATAACATAGAAAAACG                  | AGAAAGCAAAACACTGG                    |
| Pyrms 15-16 | 61 | TTCTTCCATTTCCTCTCGTCTC                | CGATTGTGGGTTATGTGATAG                  |
| Pyrms 61-62 | 61 | GAGGCACTTGGGCTACATTACC                | TGGATTACAGATGGCTTGC                   |

*AT=Annealing Temperature.

Neck blast severity was based on the relative lesion size on the neck; 1 = no lesions to pin head size of lesions on the neck region, 2 = 0.1 to 2.0 cm size of typical blast lesion on the neck region, 3 = 2.1 to 4.0 cm, 4 = 4.1 to 6.0 cm, and 5 = >6.0 cm size of typical blast lesion on the neck region.

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

Test plants were planted in a CRD design with three replicates representing the five regions and three pots for control. Eighteen small pots measuring 20 cm diameter and 40 cm height were filled with sterile soil autoclaved at 121 Pa pressure and 21°C for 15 min DAP of 15.5 g added per pot. Three seeds from the most susceptible variety (KNE 741) were then sown in the pots and allowed to germinate and grow for two weeks. A spore suspension of the *P. grisea* strains from each of the five regions (Bomet, Nakuru, Kolbake, Alupe and Makueni) were used to do the pathogenicity test. Five calibrated hand sprayers were used to spray the pathogen strains to the susceptible variety. The pots were then covered with a parchment bag for 48 h to create humidity required for the growth of the pathogen. After a period of 7 days the symptoms were recorded from each variety and the most virulent pathogen determined on disease severity. Each observed symptom was assigned group I-III where; I- Highly pathogenic, II- Moderately pathogenic III- Mild pathogenic.

Disease severity rating (DSR) (% damage) was done on the first four leaves (flag) where five plants were randomly tagged per plot. Disease severity on tagged plants was recorded at tillering, flowering, and physiological maturity stages on KNE 741 using modified Cobb scale (Babu et al., 2013). Leaf blast scored based on percent surface area of the infected leaves was evaluated as *P. grisea* severity was used as an indication of severity (Table 1).

**DNA confirmation by gel electrophoresis**

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

**DNA quantification**

DNA quantification was done using a 1% agarose prepared using 1X sodium borate and pre-stained with Gelred dye. The gel was placed in an agarose gel tank (Model CBS Scientific) containing sodium borate. 5 µl of DNA was mixed with 3 µl of lading dye and...
Table 2. Colony diameter (mm) of *P. grisea* incubated at 25°C±1 on oat meal agar for 10 days period and spore diameter (µm, Magn x40) at 20 day growth observed under a microscope.

| Site      | Colony diameter (mm) | Spore diameter (µm) Magn x40 |
|-----------|----------------------|-----------------------------|
|           | 3-day                | 5-day                       | 7-day                       |
| Alupe     | 17.0±0.9             | 31.3±1.6                    | 75.0±2.6                   |
| Bomet     | 27.8±2.2             | 36.3±2.1                    | 81.0±1.1                   |
| Kolbatek  | 20.7±0.5             | 27.6±1.4                    | 76.6±2.0                   |
| Makueni   | 17.2±2.1             | 36.0±2.0                    | 75.0±2.6                   |
| Nakuru    | 20.8±1.4             | 29.6±0.8                    | 72.1±1.7                   |
| LSD (5%)  | 0.5                  | 0.54                        | 0.66                       |
| CV%       | 14.6                 | 10.05                       | 5.14                       |
| R²        | 75.7                 | 68.5                        | 53.3                       |

*Means of three replicates per site ± standard error of the mean. Means followed by the same letters are not significantly different (P<0.01)

RESULTS

Cultural and morphological characteristics of *Pyricularia grisea*

Colony color

There was variation in colony color of all the pathogens collected from Alupe, Bomet, Koibatek, Nakuru and Makueni regions. Variation in color occurred mostly 3 to 5 days and 5-14 days at initial and later stages, respectively. At day 10, most pathogens had varied grey and black color on both the front and the back side of the petri-dish (Figure 1). Blast isolate 1 (Alupe) was white at initial growth and finally changed to grey color at mid-stage from day 10, it was greyish-white in color. The colony had smooth margins with 5 rings observed only at the front of the Petri-dish. It had a unique appearance of red pigmentation during its growth which disappeared at later stages. Blast
isolate 2- (Bomet) was white at initial stages of growth which turned to grey color at the final stages with a buff color observed at the back of the Petri-dish. It has smooth margins with 6 rings observed on the front and the back of the Petri-dish. Blast isolate 3- (Koibatek) had numerous variations in color. White and red pigmentation was observed during the initial stages of growth. The color later changed to greyish white at the final stages. It grows with smooth margins with 4 rings observed only at the front of the Petri-dish. Blast isolate 4- (Makueni) had grey color at the initial stage of growth on both sides of the Petri-dish which later changes to a total black color. It had no rings both at the back and the front of the Petri-dish with irregular margin as it grows. Blast isolate 5- (Nakuru) had a clear white color at the initial stages of growth with smooth margins and 5 centric rings. It finally changed to greyish white color at later stages (Figure 1).

Conidia characteristics

Microscopic observation showed that spore diameter of P. grisea was measured and the results indicated that there was significant difference (P<0.01) in spore sizes on the 20 days cultures. P. grisea from Makueni had the highest mean of 14.37 µm. The spores were long, pyriform with four septa at 20 days culture, more mature in terms of growth. Nakuru had two septate conidia with a rounded shape. The septae had separate margin. Alupe had a two medium sized pyriform spore with a rounded apex. Bomet had the lowest 4.57 µm with 2-4 septate conidia which had a smooth margin at the apex (Figure 2). The spores had a highest diameter of 5.34 µm when fully mature at 25 days. There was no significant difference in spore sizes from Alupe, Koibatek, Nakuru and Bomet at full maturity (Table 2). All P. grisea isolates from all the sites had pyriform shape with varied apex; round/sharp and round/flat, number of cells; from 2-4 celled conidia of various sizes and shapes. The middle cells were larger compared to the apex and the base cells. There was also varied growth from being large and
fully mature at 20th day (Isolate D) to slow development of spore characterized by small and immature spore (Isolate E) (Figure 2).

**Pathogenicity test**

There was variation in growth patterns, shape and color of *P. grisea* collected from the five regions. The effect of the *P. grisea* from the various sites on test plants indicated the pathogenicity levels of the pathogen. The symptoms were noted 7-20 days after inoculation as small white lesions which grew into diamond shape at the 10th day, greyish white symptoms with brown spots which later developed to be black at the 15th day and leaf necrosis by yellowing which was typical from the apex as it progressed downwards at the 20th day (Figure 5) (Wekesa et al., 2019). These symptoms were a clear indication of *P. grisea* especially when compared to control which was not sprayed. *P. grisea* from Bomet and Makueni had the highest virulence (Highly pathogenic- Group I) while those from Alupe and Nakuru and finally Koibatek *P. grisea* had the least virulence (Mild pathogenic- Group III) (Table 3).

**Virulence of pathogens strains from selected regions**

Pathogen strains from different regions had different indication of the symptoms and infection to the susceptible variety KNE 741. From the ANOVA table, site was significant (p<0.005) for both leaf and neck blast severity (Table 4).

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

![Figure 2. Micro-images of a twenty day culture of *P. grisea* isolates; Magn x40 collected from different growing regions of Kenya. Key: A= Blast 1- Alupe; B= Blast 2- Bomet; C= Blast 3- Koibatek; D= Blast 4- Nakuru; E= Blast 5- Makueni.](image-url)
Table 4. Morphological characterization of Pyricularia grisea from five selected counties of Kenya.

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

Figure 3. Comparison of leaf and neck severity of P. grisea on KNE 741. Means with the same letters are not significantly different (P<0.01).

(Figure 3).

Relationship between neck and leaf blast

Pearson’s correlation coefficient indicated that there was a positive correlation of leaf and neck blast since a one-unit increase in leaf blast with all the factors held constant led to an increase of 0.9003 with 88.2 % of neck blast (Figures 4 and 5).

Molecular characterization of P. grisea from five selected major growing areas of Kenya

Confirmation of the DNA by gel electrophoresis

The bands of 30 isolates of P.grisea collected from the five sites were confirmed to be present after visualization under a UV transilluminator (Vilber Lourmat) (Figure 6).

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

A simple numeric scoring was used to denote the presence (1) and absence (0) of allele for each loci (Table 5). The observed allele number was two for all the selected primers indicating that only one locus was amplified by all the markers. Effective number of alleles \( \left\{ A_E=1/ (1-H_{exp}) \right\} \) ranged from 1.3006 (MGM 437) to the highest (1.9912) (Pyrm 61-62). Major allele frequency ranged from 0.5333 to 0.8667. Polymorphic Information Content (PIC) varied between 0.2044 and 0.3739 for primers MGM 437 and Pyrm 61-62 respectively. When P. grisea diversity was assessed, gene diversity ranged between 0.2311 and 0.4978 with a mean of 0.4.
Factorial analysis revealed that the samples from all the five sites clustered randomly with no distinct pattern observed (Figure 7). Most isolates clustered as individuals showing that they were genetically distinct while a few clumped together.

The phylogenetic analysis grouped the samples into two main clusters and six sub-clusters. Cluster I comprised 2 sub clusters. Sub-cluster 1 comprised isolates 2, 3, 4 and 5 from Nakuru, 3 and 4 from Alupe and 3 and 6 from Koibatek which appeared as duplicates and isolate 6 from Bomet which clustered as a distinct individual. Sub cluster two had only two isolates Makueni 1 and Alupe 6 which appeared distinct. Cluster II comprised 4 sub clusters. Sub cluster I comprised isolate 4 and 5 from Bomet which appeared as duplicates and isolate 1 from Koibatek. Sub cluster II comprised isolate 1 and 2 from Bomet and 2 and 5 from Koibatek which appeared as duplicates and isolate 5 from Alupe appeared as distinct. Sub cluster III comprised distinct isolates 6 and 1 from Makueni and Nakuru respectively. Sub cluster IV had 5 duplicate isolates Makueni (2, 3, 4 and 5) and distinct isolate 1 from Alupe, 3 from Bomet and 6 from Nakuru. There was no particular pattern deduced from the grouping; however, some strains from different regions were observed to cluster in same sub clusters as duplicates with few appearing distinct (Figure 8).

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

Analysis of Molecular variance (AMOVA)

Results of AMOVA (Table 6) revealed there was huge diversity within the *P. grisea* isolate populations (87%) compared to among the population (13%) with a P value of 0.053.

DISCUSSION

Morphological diversity of *P. grisea* in major finger millet growing areas in Kenya

*P. grisea* isolates collected from Alupe, Bomet, Makueni, Koibatek and Nakuru showed high variation both morphologically and genetically. There was variation in colony color, colony diameter and structure in growth patterns both at the petridish and microscopically. Description of the *Ascomycete* fungi has been described by conidia and conidiophore morphology as the main characteristics of the fungi (Choi et al., 2013). The difference in color maybe due to different growth stages of the spores which tend to vary with blast isolate and patterns of growth. Meena (2005); Getachew et al. (2014); and Shahriar et al. (2020) revealed that *Magnaporthe grisea* tends to vary due to sexual hybridization which shows variability in form and color at different asexual stage of the fungi. Colony structure on the microscope revealed significant morphological differences on the structure of conidia and conidial appendages of *P. grisea* from different regions. *P. grisea* had several flared pigmented conidiophores which are aseptate in nature. The findings are similar to those reported earlier by a study done by Klaubauf et al. (2014) who noted that the fungi have septate conidia of varying shape. This could be due to effects of different environments and ecological conditions under which the various *P. grisea* strains were collected which could have influenced the size, septation and form of the conidia. Getachew et al. (2013) reported similar findings and noted that environment affects the growth of the fungi size and shape. Conidia appendages aid in attachment to substrates, dispersal of spores and acclamation to new environments, which affect their variability in shape, mode of development, color and infectivity. Klaubauf et al. (2014) also noted different isolates differ with respect to the factors which also are a determinant of their variability in shapes, mode of development, color and infectivity.
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Figure 5. Effect of *P. grisea* isolates on susceptible KNE 741 variety. Where letters represent the pathogens sprayed on susceptible KNE-741: A= Bomet isolate, B= Makueni isolate, C= Alupe isolate, D=Nakuru isolate, E=Koibatek isolate and F=Control (Non-sprayed)

Infectivity to the host plant. The pathogen from Bomet and Makueni had higher growth on the petri-dish compared to those of Nakuru, Koibatek and Alupe. This could be due to the pathogens' aggressiveness to mature faster and produce leading to more colonization on the host plant; these types of pathogen tend to gain resistance faster and can breakdown easily hence gaining resistance. These findings are in accordance with Saleh et al. (2014) who studied the origin, diversity and, dispersion of rice blast fungi and found that faster evolution and resistance of *Magnaporthe grisea* is linked to the faster growth of the pathogen.

Pathogenic diversity of *P. grisea* in major finger millet growing areas in Kenya

Pathogenicity test which was a measure of the virulence of the pathogen collected from five different major growing areas showed that there is variation in symptoms of various *P. grisea*. This may be due to the existence of diversity that existed within the pathogens and their expression on the host. This was because an attack on the vegetative cycle of the plant can easily be translocated on the neck. Similar to the findings, Ghatak et al. (2013) observed the aggressiveness of *P. grisea*
The presence of *P. grisea* isolates showing presence of DNA bands as viewed under a UV trans-illuminator.

Table 5. Summary statistics of genetic diversity indices of 30 samples of *P. grisea* in the five selected sites.

| Marker     | Sample size | Observed allele number | Effective number of alleles | Major allele freq. | PIC | Gene diversity | Shannon's information index |
|------------|-------------|------------------------|-----------------------------|--------------------|-----|----------------|-----------------------------|
| MGM 200    | 30.0000     | 2.0000                 | 1.3846                      | 0.8333             | 0.2392 | 0.2778        | 0.4506                      |
| MGM 436    | 30.0000     | 2.0000                 | 1.9651                      | 0.5667             | 0.3705 | 0.4911        | 0.6842                      |
| MGM 437    | 30.0000     | 2.0000                 | 1.3006                      | 0.8667             | 0.2044 | 0.2311        | 0.3927                      |
| MGM 451    | 30.0000     | 2.0000                 | 1.6423                      | 0.7333             | 0.3146 | 0.3911        | 0.5799                      |
| MGM 454    | 30.0000     | 2.0000                 | 1.9651                      | 0.5667             | 0.3705 | 0.4911        | 0.6842                      |
| PYRM 15-16 | 30.0000     | 2.0000                 | 1.7241                      | 0.7000             | 0.3318 | 0.4200        | 0.6109                      |
| PYRM 61-62 | 30.0000     | 2.0000                 | 1.9912                      | 0.5333             | 0.3739 | 0.4978        | 0.6909                      |
| Mean       | 30.0000     | 2.0000                 | 1.7104                      | 0.6857             | 0.3150 | 0.4000        | 0.5848                      |

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

Genetic diversity of *P. grisea* in major finger millet growing areas in Kenya

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

In this study, genetic diversity indicated the existence of different strains collected from different environments.

The average number of observed and effective number of alleles reported in the study (2 and 1.71) and indication of the number of frequent alleles that would achieve expected heterozygosity in the population. This finding was slightly lower than that reported by Babu et al. (2013) (6.18). This variation in the number of alleles has been reported in other studies (Kaye et al., 2003; Zheng et al., 2008; Fujita et al., 2009). This could be due to few number of markers and samples, nature of MGM markers which were more specific to rice than finger millet and possible difference in the level of genetic variation in different areas (Salem et al., 2010).

MGM markers had lower scores of PIC and genetic diversity compared to *Pyrm*. Among the MGM markers, MGM 454 scored high PIC (0.3705) and genetic diversity (0.4911) while MGM 437 had the lowest 0.2044 and 0.2311 for PIC and genetic diversity respectively. MGM 454 has a higher motif number (ct-29) compared to MGM 437 (ct-11) (http://ibi.zju.edu.cn/pgl/MGM/index.html). Higher repeat motif is linked to the coverage area of the primer which directly impacts primer amplification and detection (Wang et al., 2009). The PIC and genetic diversity values in this study were similar to those reported by Anjum et al. (2016) for MGM 454 (0.40, 0.32) and higher for MGM 437 (0.08, 0.08).

Markers *Pyrm* 15-16 had PIC values of 0.3318 and 0.3739 respectively while *Pyrm* 61-62 had 0.785 and 0.760 respectively. These values were lower than those reported by Babu et al. (2013). The higher values reported by Babu et al. (2013) could be due to the fact that the isolates of *M. grisea* used were from a combination of finger millet, foxtail millet and rice. Anjum et al. (2016) who incorporated the use of the same markers as those in the study recorded a 0.59 and 0.54 on *Pyrm* 15-16 and *Pyrm* 61-62 respectively from isolates of *P. grisea* from finger millet. The higher values reported by Babu et al. (2013) could be due to the fact that their study used rice, foxtail millet and finger millet while in this study only finger millet is used.

Genetic diversity for *Pyrm* 15-16 and *Pyrm* 61-62 was 0.42 and 0.49 respectively which was almost similar to the findings of Anjum et al. (2016) (0.59 and 0.54); while Babu et al. (2013) reported higher values of 0.803 and 0.780 for the same markers. The higher value reported could be due to genetic diversity of the pathotypes collected from different crops; while this study’s own is in contrast to Anjum et al. (2016) who used *P. grisea* isolates collected from finger millet only. This variation in values with the same pathogen assessed could be due to host-pathogen specificity and adaptation of the pathogen to a particular host; this in turn could have influence on the variability of the pathogen hence higher values recorded (Tribble et al., 2013).

The results on factorial analysis showed that the samples from all the five sites clustered randomly without any distinct pattern observed. For example, Cluster 1 comprised 2 sub clusters. Sub-cluster 1 comprised isolates 2, 3, 4 and 5 from Nakuru, 3 and 4 from Alupe and 3 and 6 from Koibatek which appeared as duplicates and isolate 6 from Bomet which clustered as a distinct individual. This finding is in line with other numerous studies on finger millet pathogen which found out that there was geographical differentiation between strains (Rebib et al., 2014). This suggests the non existence of sexual reproduction among strains of *P. grisea* and therefore pathogen changes with time through evolution (Fry et al., 2015; Bengtsson, 2003).

Phylogenetic analysis revealed that *P. grisea* in the study regions was diverse with no geographical grouping with some strains from different regions observed to cluster in same sub clusters as duplicates with few appearing distinct. This observation was similar to findings of Longya et al. (2020) who also failed to deduce regional differentiation of rice *P. grisea* in Thailand. Similar findings have been documented by Anjum et al. (2016) and Singh and Kumar (2010).

AMOVA indicated there was huge diversity within *P. grisea* isolates (87%) and low diversity among the selected regions (13%). This implies that majority of the observed variation in the *P. grisea* was due to genetics rather than geography. The pathogen varied genetic diversity has challenges in development of management and control of the pathogen (Mia, 2013). Similar to the findings, Kumar et al. (1999) and Rebib et al. (2014) reported a huge diversity occurring within populations as opposed to among populations of *Magnaporthe grisea* of rice, which was mainly linked to the varied genetic make up of the pathogen.

**CONCLUSION AND RECOMMENDATIONS**

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

**CONFLICT OF INTEREST**

The authors have not declared any conflict of interests.

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