International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Pathogenicity of *Colletotrichumkahawae* in Kenya

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Abstract: ***This study was aimed at determining the virulence diversity of Colletotrichumkahawae, and their correlation with coffee growing regions and interaction with coffee varieties in Kenya. A total of 34 single conidia isolates were obtained and subjected to variation analysis using DNA banding patterns. The test varieties included Rume Sudan, Catimor, K7 and SL 28 (susceptible). Seedlings were individually scored for disease symptoms and mean grade of infection was computed. The mean grade data was then used to perform Analysis of Variance (ANOVA) using the random effects model. The results were used to determine the correlation between molecular polymorphism and diversity in virulence. The variety x isolate interaction effects although significant (p<0.05) did not conclusively reveal the existence of races because the isolate effect was not significant. The virulence tests revealed that variation was due to main effects of varieties. Rume Sudan was highly resistant with a mean grade of 4.75. Catimor with a mean grade of 7.66 showed medium resistance. K-7 showed medium resistance with a mean grade of 9.97. SL 28 was highly susceptible with a mean grade of 11.75. The growing regions had no influence on the genetic and virulence diversity since C. kahawae isolates from all regions were pathogenic on the tested coffee cultivars. All the isolates were significantly (p<0.05) more aggressive on coffee cultivar SL28, followed by K7 and Catimor in that order. Rume Sudan showed high resistance to all the tested isolates irrespective of the region. It is concluded that variation in Colletotrichumkahawae population is largely due to differences in aggressiveness of the isolates.***

Keywords: *Colletotrichumkahawae, Coffeaarabica,* pathogenicity, resistance, races, aggressiveness, polymorphism, isolates

1. Introduction

Coffea is a large genus containing about 100 species of flowering plants in the family *Rubiaceae* with over 6000 species (Wrigley, 1988)(1). Coffee 'beans' are widely cultivated in the tropical countries in plantations for both local consumption and export to temperate countries. Coffee ranks as one of the world's major commodity crops and is the major export product of many countries. Economic production of arabica coffee in Kenya is greatly hindered by coffee berry disease (CBD). It is believed that breeding for resistance to CDB may provide a sustainable long-term management of the disease (Omondi, 1998)(2). Since the release of resistant Ruiru 11 in 1985, efforts have been devoted to the improvement of the genetic base of resistance, but this has faced the problem of possible pathogen variation (Omondi, 1998)(2). Some strains of *Colletotrichumkahawae*have been isolated from Ruiru 11, thus investigation into the possible physiological specialization of the fungus and pyramiding of resistance genes is necessary (Omondi, 1998)(2). A good understanding of CBD pathogen's genetic diversity could lead to development of cultivars with sufficient disease resistance. Host – plant resistance is therefore relatively cheap, biologically safe and self sustaining (Hogenboom, 1993)(3). This study was aimed at determining the diversity of the CBD pathogen population, demonstrating pathogen diversity in correlation with location and coffee varieties in Kenya.

2. Materials and Methods

Infected berries were obtained from areas with CBD epidemics. The areas included coffee growing regions to the East and West of Rift Valley. The locations were representative of the agroecological zones where arabica coffee is grown in Kenya. Arabica varieties in these regions include Rume Sudan, Catimor, K7 and SL28 (susceptible). Diseased berries were sampled from susceptible and resistant plants from which 34 single conidia isolates were derived. In locations where both resistant and susceptible varieties were grown, a larger proportion of the berries were obtained from the resistant varieties to increase the chances of obtaining different pathotypes.

DNA was extracted from lyophilized mycelia of 34 isolates using the protocol of Moller *et al.,* (1992)(4), from which 5 isolates which showed polymorphism with 9 primers were used to inoculate different coffee varieties. The PCR products were separated on a 1.5% agarose gel in TAE (Tris-Ammonium EDTA buffer). The gel was stained in ethidium bromide, visualized and photographed under UV light 260 nm. The DNA marker used was 100 bpladder. RAPDs generated by single primer PCR were used to compare relationship among 34 isolates. For each isolate, a data record was constructed in which each band of a particular molecular weight, as generated by each primer. Binary matrix was constructed combining all the data records for each isolate - primer combination which yielded reproducible bands.

The isolates were C3, C11, C14, C23 and C27. Seeds from the four *Coffeaarabica* varieties (Rume Sudan, Catimor, K7 and SL28), were obtained and germinated to obtain the hypocotyls for inoculations. For each of the 5 isolates, 100 seeds of each variety were sown in 3 replications in moist sterilized sand in plastic boxes with closely fitting transparent lids.

Conidia suspension was prepared from 10 days old monoconidial cultures and standardized to 2 x 10^6 conidia/ml by haemocytometer counting followed by serial dilution.

After 6 weeks the seedlings were inoculated, incubated and individual scored after 3 weeks for disease symptoms developed on the hypocotyls stem using the scale of Van der vossen*et al*., (1977)(5).The susceptible SL28 genotype was used as the control.The mean grade of infection was computed for each box and then used to perform an Analysis of Variance. The mean grade of infection was computed for each box. The mean grade data was then used to perform an Analysis of Variance (ANOVA) according to random effects model (Steel and Torrie, 1981)(6).
Mean grade of infection (G) was computed for each box as follows;

$$G = \frac{1}{N}\sum_{i=1}^{12} in_i$$

Where *i* is the disease grade, $n_i$is the number of seedling in the grade *i* and *N* is the total number of seedlings scored. The laboratory data on the *C. kahawae*virulence/ aggressiveness on various coffee varieties was analyzed using MINITAB version 13.0 Computer package.

3. Findings and Analysis

3.1 Genetic diversity of *Colletotrichumkahawae*

A total of 9 primers assayed showed amplification with the 34 isolates. Isolates C3, C11, C14, C23 and C27 were selected based on their polymorphic reaction when subjected to DNA analysis by RAPD. Plate1a and 1b show RAPD profiles generated by primer J-19 on the 34 isolates.

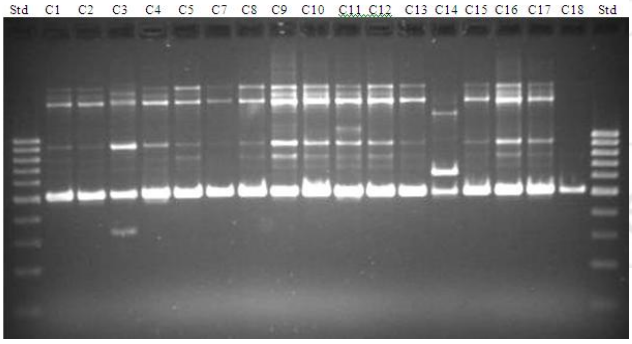

Plate1a:RAPD profiles generated by Primer J – 19 on a first set of 17 isolates.

C1-C35 represents *Colletotrichumkahawae* isolates; Std. represents 100 bp ladder marker (Lambda 3)

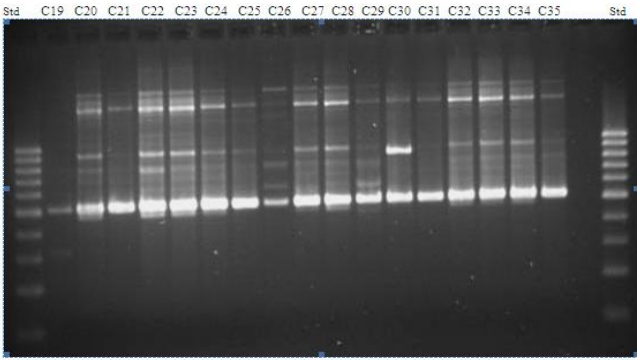

Plate 1b: RAPD profiles generated by Primer J – 19 on a second set of 17 isolates.

RAPD analysis therefore indicated that variation within *Colletotrichumkahawae*was relatively of low magnitude as indicated in Plate 1a and b. The uniformity of the DNA bands observed from PCR agrees with the DNA patterns of *Colletotrichumkahawae* documented so far.

Omondi (1998)(2), conducted protein electrophoresis and noted that protein profiles of *Colletotrichumkahawae* were highly identical indicating lack of genetic diversity (lack of races) among *Colletotrichumkahawae* while the non-pathogenic, *Colletotrichumacutatum* had protein profiles distinctly different from *Colletotrichumkahawae.* Phylogenetic analysis using multi-gene data showed that C*olletotrichumkahawae*is genetically distinct from other closely related species in the complex (Prishastuti*et al*., 2009)(7). Grouping of Kenyan *Colletotrichumkahawae* isolates using vegetative compatibility showed that all the C*olletotrichumkahawae* isolates belonged to one vegetative compatibility group (Gichuru*et al*., 1999)(8). Molecular studies by Loureiro*et al*., (2007)(9), using RAPD techniques did not show polymorphism within the isolates tested. However other research (Loureiro*et al*., 2007)(9) using isoelectric focusing electrophoresis (IFE) and polyacrylamide gel electrophoresis (PAGE) detected some polymorphism.

3.2 Virulence of different isolates of *Colletotrichumkahawae* on a set of coffee varieties.

The results of mean separation in Table 1, indicate that Rume Sudan was highly resistant; Catimor and K7 were partially resistant while SL28 was susceptible. Rume Sudan carries resistance on the dominant R - and the recessive k - loci. Catimor and K7 carry resistance on the dominant T- and the recessive k - locus respectively. Combination of two or more genes as in the case of Rume Sudan appears to enhance resistance. The variety x isolate interaction effect was significant at ($P \leq 0.05$) (see table below). This could be attributed to some isolates being more aggressive on some varieties than others. Isolate C23 was more aggressive on Rume Sudan than C3, C11, C14 and C27. On the other hand, it was the least aggressive on Catimor compared to C3, C11, C14 and C27. C3 was less aggressive on K7 than C23, C11, C14 and C27. The interaction effect cannot be attributed to existence of races because the isolate effect was not significant.

Reaction of some selected coffee differentials with different isolates.

Isolates	Coffee differentials				
	Catimor	Rume Sudan	K7	SL 28	Mean
C3	8.33b	4.37c	8.40b	11.33a	8.11
C23	6.23b	5.30b	10.13a	11.73a	8.35
C11	7.27c	4.70d	10.53b	11.83a	8.58
C14	8.60b	4.57c	10.57ab	11.80a	8.88
C27	7.87c	4.80d	10.23b	11.93a	8.71
Mean grade	7.66c	4.75d	9.97b	11.73a	

Mean score in the same row denoted by similar letters are not significantly different at P≤ 0.05

Omondi*et al.* (1998)(2), who performed pathogenicity tests on 11 genotypes of *Coffeaarabica* using single-isolate suspension of *Coletotrichumkahawae* obtained from 90 monconidial isolates and found that a large part of the variation in the pathogen population was due to aggressiveness. They observed that the differential effects were too small to suggest conclusively that races exist. Waller *et al.*, 2009(10), further observed that isolates of coffee berry disease pathogen taken from across its range of distribution in Africa have common morphological, biochemical and pathogenic characteristics distinguishing them from other species of *Colletotrichum.* This could explain the lack of variation observed among the studied isolates.

3.3 Correlation between pathogen diversity and growing regions

Sampling sites for *Colletotrichumkahawae* were divided into Western, Central and Eastern regions of Kenya. *Colletotrichumkahawae* isolates C11 and C27 were sampled from Western region of Kenya, C23 and C14 were sampled from Central region while isolate C3 was sampled from Eastern part of Kenya. These findings indicate that the isolates from all the regions had similar reaction on the tested coffee cultivators. However, an analysis of their aggressiveness showed that isolates from Central Kenya (C14 and C23) had the highest mean grade (8.82) followed by isolates from Western (C11 and C27) with a mean of 8.63. Isolate C3 from Eastern had the lowest mean grade of 8.12, (Table 2). The results indicate that Rume Sudan recorded highest resistance (mean grade 4.68) followed by Catimor (mean grade 7.77), K7 (mean 9.71) and SL28 recorded the highest mean grade of 11.66 when inoculated with *Colletotrichumkahawae* from different regions. Omondi, (1998)(2), observed that the apparent lack of variety specific variation associated with existence of races was probably due to the fact that the pathogen co-evolved with a genetically narrow based host species. Bridge *et al.*, (2008)(11) observed that geographic variability within *Colletotrichumkahawae* isolates is very small leading to genetic uniformity among the isolates. Rodrigues *et al.*, (1991)(12), also reported that variation was due to both aggressiveness and some cultural characters such as rates of sporulation and growth.

4. Conclusions

The DNA banding patterns using RAPD indicate predominant genetic uniformity among *Colletotrichumkahawae* with minor differences. There was minor pathogen diversity detected by RAPD analysis which could not be attributed to differences in virulence or aggressiveness. Virulence tests revealed that the reaction of isolates on the varieties tested was to a large extent uniform indicating that there were no races. However, minor differences were observed in disease score which can be attributed to difference in aggressiveness.

It was also observed that there was uniformity in pathogen population across the regions from where the isolates were collected. Location or origin had little significance on virulence of *Colletotrichumkahawae*tested. There was no evidence indicating variation in virulence across the regions. Resistant varieties are likely to be deployed for wide adaptability without risk of breakdown of resistance.

5. Acknowledgements

I sincerely acknowledge the Management and Board of Directors of the Coffee Research Foundation (CRF), Ruiru, for availing all the materials and facilities with which this research was carried out. I thank my supervisors, Dr. Fredrick M. Njoka Dean School of Agriculture (Embu University) Dr. Chrispine O. Omondi of KenyaSugar Research Foundation (KESREF) and Dr. Elijah K. Gichuru of Coffee Research Foundation (C.R.F), Ruiru,for their tireless guidance during my research. My sincere gratitude also go to Professor Ndiritu of Plant and Microbial Sciences Department, Kenyatta University and Dr. Paul Omanga for their professional advice during my research. Many thanks also go to Mr. Peter Gocho and his colleagues at the CRF Molecular Biology Laboratory for their assistance during extraction of DNA and running of Polymerase Chain Reaction (PCR) analysis.

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