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Status of Coffee Leaf Rust Resistance on Kenyan Commercial Resistant Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. The research project was conducted by author SGL, who also wrote the first draft of the manuscript. The contribution of the co-authors was performed as follows: EKG and OK assisted in the development of the concept note for this study and provided technical advice on design and layout of the experiment. Author BMG analyzed and interpreted the data and contributed greatly in the preparation of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Coffee Leaf Rust (CLR) is a fungal disease caused by *Hemileia vastatrix* Berk. and Br. The pathogen is constantly evolving leading to rapid break down in resistance of once resistant coffee varieties. To date, more than 49 races of the pathogen have been characterized all over the world and new races are continuously being characterized some of which are able to infect Robusta derivatives.
Aim: The objective of this study was therefore to re-examine the status of CLR resistance on Kenyan commercial resistant cultivars and investigate the pathogenic interaction between *H. vastatrix* isolates and their host genotypes.
Methodology: *Hemileia vastatrix* isolates were collected from naturally infected leaves of the host

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coffee genotypes and were inoculated on one Robusta coffee genotype and eight Arabica genotypes comprising of three Kenyan commercial cultivars (SL28, Ruiru 11 and Batian) and five museum genotypes (HDT, Mundo Novo, Pretoria, 110/2 and Bourbon) using leaf disks inoculation method. An infection scale of 1-6 was used to score the virulence of the pathogen isolates.

Results: There was significant variation among isolates on their virulence against the different genotypes. SL28, Pretoria and Mundo Novo were the most susceptible to most isolates while none of the isolates infected Ruiru 11, HDT and Robusta. All the isolates were able to infect Batian but none reached the stage of sporulation. Isolates from K7, SL34 and SL28 were found to be more virulent than those from Batian and Blue Mountain. Unlike the host genotype, the region from which the isolates were obtained was not found to play any role on the virulence of the isolates.

Conclusion: Although six additional races of *H. vastatrix* were recently detected in Kenya some of which are able to infect Robusta derivatives, the study confirmed that Kenyan genotypes in this group are still resistant against most races of *H. vastatrix* in Kenya. It was therefore deduced that either these new races are not yet wide spread in all coffee growing areas in Kenya or that there are other major and minor genes conditioning the coffee-rust interactions besides the SH genes.

Keywords: Coffea arabica; Coffea canephora; Coffee leaf rust; SH genes; Kenya.

1. INTRODUCTION

The genus *Coffea* consists of approximately 130 species and belongs to the family *Rubiceae* which has over 6000 species [1]. According to Pearl et al. [2], *Coffea* species that are under commercial cultivation are Arabica coffee (*Coffea arabica* L.) which commands 80% of world coffee trade and Robusta (*Coffea canephora* Pierre) which takes the bulk of the remainder. *Coffea liberica* and *Coffea excelsa* contribute less than 1%. C*offea arabica* is self-pollinating thus limiting its genetic variability unlike Robusta coffee which is cross-pollinating [3]. Arabica coffee is known for the production of very high quality beverage but is more susceptible to major diseases of coffee [4,5,6]. Robusta is more tolerant to major coffee diseases and insect pests but with inferior cup quality [7,6]. The main coffee producing regions in Kenya are on deep, fertile and acidic volcanic soils found in the highlands between 1400 to 2000 meters above sea level. These regions produce high quality, milder Arabica coffees that are known for their intense flavour, full body and pleasant aroma [8]. Over 90% of the total coffee acreage in Kenya is under Arabica coffee while the rest is occupied by Robusta coffee [9].

Approximately 350 different diseases infect coffee globally [10]. The major coffee diseases in Kenya include Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* Waller and Bridge*,* Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* Berk. and Br.*,* and Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* pv. *garcae* van Hall [11]. The CLR fungus is found in all the coffee-growing countries worldwide, causing losses between 10% and 40% [12] unlike CBD which is a major constraint to Arabica coffee production in Africa [13] and BBC which has been described in Brazil, Kenya, Uganda and China [14]. Much of the world coffee is still produced by traditional cultivars of *C. arabica* (66%) and *C. canephora* (34%), most of which are susceptible to CLR [15]. In Kenya, CLR is the second most important disease after CBD, and breeding to obtain new resistant coffee varieties has been a priority [16]. The main damages caused by the disease are premature defoliation, resulting in a reduced leaf area and withered lateral branches, leading to a gradual weakening of the infected plant [17] and reduced yields [18].

CLR resistance in the coffee plants is conditioned by at least nine major dominant genes (SH1-SH9) that act singly or in association [19]. By the same theory, it was possible to infer 9 genes of virulence (v1-v9) in *Hemileia vastatrix* [12]. This allows coffee genotypes to be classified in resistant groups according to the physiological races of the rust pathogen [20]. The genes SH1, SH2, SH4 and SH5 are found in pure Arabicas originating from Ethiopia; the gene SH3 in *Coffea liberica;* and the genes SH6, SH7, SH8 and SH9 found exclusively in Robusta and its introgressed derivatives [19,16]. Previously, only six races of the pathogen (races I, II, VII, XV, XX and XXIV) and four virulence genes (*v2, v3, v4,* and *v5*) had been identified in Kenya [21]. Recently, six more races (races III, XVII, XXIII, XLII, XXXVI and XLI were recently detected revealing three new virulence genes (*v1*, *v7*, *v8*) and possibly *v9* [16]. This poses a great danger of breakdown of the CLR resistance in resistant

Kenyan cultivars whose resistance is conferred by genes SH6 – SH9. The purpose of this study was therefore to re-examine the status of CLR resistance on Kenyan commercial resistant cultivars and investigate the pathogenic interaction between *H. vastatrix* isolates and their host genotypes.

2. MATERIALS AND METHODS

2.1 Coffee Leaf Rust Pathogen Inoculum Collection

H. vastatrix isolates were collected from naturally infected leaves of nine host genotypes from six coffee growing counties (Table 1). The isolates were from single host coffee tree that was sampled with a high level of CLR infection. The bulk samples from each host tree were kept separately, sealed and stored under ice to maintain viability. An isolate constituted bulk collection of urediniospores from each plant [22].

2.2 CLR Isolates Inoculation and Evaluation

Excised pieces of leaves (1·8 cm diameter) cut with a cork borer, were taken from healthy full-grown leaves of nine test genotypes (Table 2) and placed in plastic boxes on sterilized foam moistened with distilled water.

The nine genotypes (Table 2) were inoculated with the nine *H. vastatrix* isolates. Double distilled water was used as the control. Each treatment was replicated three times in a completely randomized design. Each leaf disc was inoculated with one droplet of 0·025 mL *H. vastatrix* spore suspensions (1 mg spores per mL). The boxes were closed with a transparent glass cover and kept at 24°C without illumination. Glass lids were removed after 24 h to allow for evaporation of the inoculum. The discs were then slightly rewetted with distilled water and further incubated for 12 hours light period of approximately 1000 lux intensity of artificial light, at a temperature of 22±2°C and relative humidity of above 90%. Disease scoring was done 30 days after inoculation using a 6-point scale [23] described below:

1 = Absence of symptoms
2 = Small chlorotic lesions
3 = Medium chlorotic lesions, without spores formation
4 = Chlorotic lesions, with few urediniospores formation (urediniospores occupying <25% of the lesion area)
5 = Sporulation occupying between 25 and 50% of the lesion area; and
6 = Sporulation occupying >50% of the lesion area.

Table 1. *H. vastatrix* isolates that were tested for virulence

Isolates	Host genotype	County
Isolate 1	SL28	Kiambu
Isolate 2	Blue Mountain	Kiambu
Isolate 3	Blue Mountain	Kisii
Isolate 4	K7	Kiambu
Isolate 5	SL28	Trans Nzoia
Isolate 6	SL34	Kisii
Isolate 7	SL34	Meru
Isolate 8	Batian	Kericho
Isolate 9	K7	Bungoma

From the above scale, the genotypes were classified in three phenotypic groups based on their level of sporulation: Those whose leaves scored 1-2 (absence of urediniospores) were considered resistant; those with scores of 3-4 (medium chlorotic lesions without sporulation to presence of urediniospores occupying up to 25% of the lesion area) were considered moderately susceptible; those with scores of 5-6 (urediniospores occupying more than 25% of the lesion area were considered susceptible.

Table 2. Coffee genotypes inoculated with Isolates

	Variety	Status	Origin
1.	Robusta	CLR Resistant Gene bank Accession	Kenya
2.	HDT	CLR Resistant Breeders Material	Timor Island
3.	Ruiru 11	CLR Resistant Commercial Cultivar	Kenya
4.	Batian	CLR Resistant Commercial Cultivar	Kenya
5.	SL28	CLR Susceptible Commercial Cultivar	Kenya
6.	Mundo Novo	CLR Susceptible Gene bank Accession	Latin America
7.	Pretoria	CLR Susceptible Gene bank Accession	Guatemala
8.	110/2	CLR Susceptible Gene bank Accession	Portugal
9.	Bourbon	CLR Susceptible Gene bank Accession	Reunion

2.3 Data Analysis

The data were subjected to analysis of variance (ANOVA) using XLSTAT 2014 software and effects declared significant at 5% level. Students-Newman Keuls ($SNK_{5\%}$) was used to separate the means. The data were presented in tables, figures and plates.

3. RESULTS

Inoculated genotypes recorded significant variation in their average reaction to the tested *H. vastatrix* isolates (Fig. 1). Mundo Novo, Pretoria and SL28 which were the most susceptible were not significantly different in their general susceptibility to *H. vastatrix* isolates. These were then followed by Bourbon, 110/2 and Batian in that order. Ruiru 11, HDT and Robusta showed complete resistance to all the isolates recording a mean infection score of 1.0 (absence of symptoms). The genotypes thus fell into two phenotypic groups: Robusta, HDT and Ruiru 11 were resistant while the rest were moderately susceptible.

The tested *H. vastatrix* isolates were significantly different in their virulence against the nine coffee genotypes ($P<0.05$). The isolates separated into two significantly different groups based on their general virulence against the nine coffee genotypes that were inoculated. Isolate 7 which was obtained from Meru County from an SL34 host was the most virulent with an infection mean of 2.96 followed by isolates 4 (2.91) and 9 (2.83) from Kiambu and Bungoma both from a K7 host. These were not significantly different from isolates 1 (2.76) and 5 (2.80), both of which were isolated from an SL28 host from Kiambu and Transnzoia, respectively, and isolate 6 (2.70) from SL34 host from Kisii. In the other lesser virulent group were isolates 2 (2.29) and 3 (2.24) both of which were isolated from Blue Mountain variety from Kiambu and Kisii, respectively. Their average virulence was statistically similar to that of isolate 8 (2.38) from Kericho County isolated from Batian host. Double distilled water which was used as a control remained disease free thus confirming differential pathogenecity of the nine isolates (Fig. 2). Therefore, isolates from K7, SL34 and SL28 were found to be more virulent than those from Batian and Blue Mountain. Unlike the host genotype, the region from which the isolates were obtained was not found to play any role in the virulence of the isolates.

Different isolates sporulated at different rates on different coffee genotypes (Fig. 3). There was therefore a significant ($p<0.05$) interaction between *H. vastatrix* isolates and the inoculated coffee genotypes. SL28, Pretoria and Mundo Novo were the most susceptible to most isolates (Fig. 3) while none of the isolates infected Ruiru 11, HDT and Robusta (Fig. 3; Plate 1). All the isolates were able to infect Batian but none reached the stage of sporulation. Isolates 4 and 9 both from K7 were the most virulent on Batian.

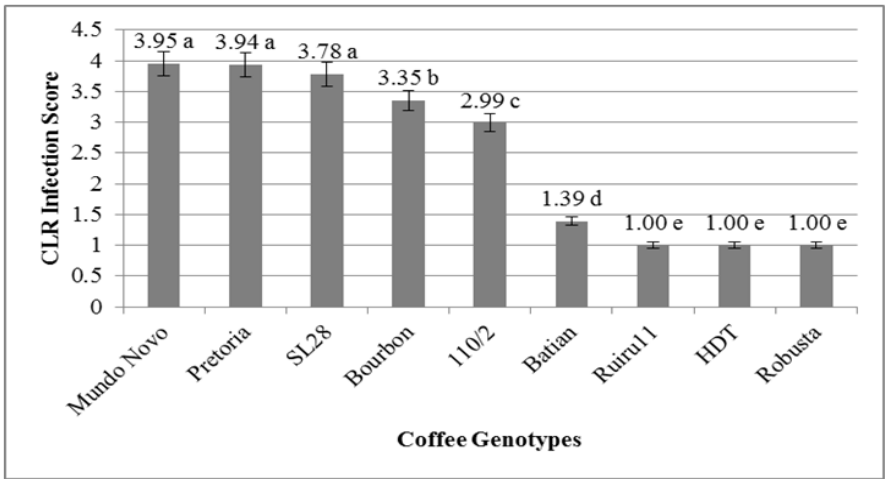

Fig. 1. Host genotype interaction with *H. vastatrix* isolates. The error bars represent the standard error of the means. The means marked with the same letters were not significantly different at p=0.05 according to Student-Newman Keuls test

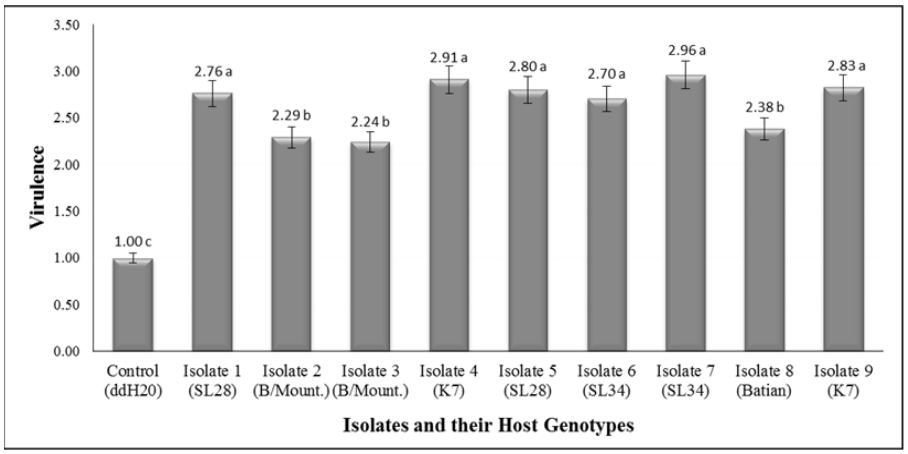

Fig. 2. Comparative general virulence of *H. vastatrix* isolates against nine coffee genotypes. The error bars represent the standard error of the means. The means marked with the same letters were not significantly different at p=0.05 according to Student-Newman Keuls test

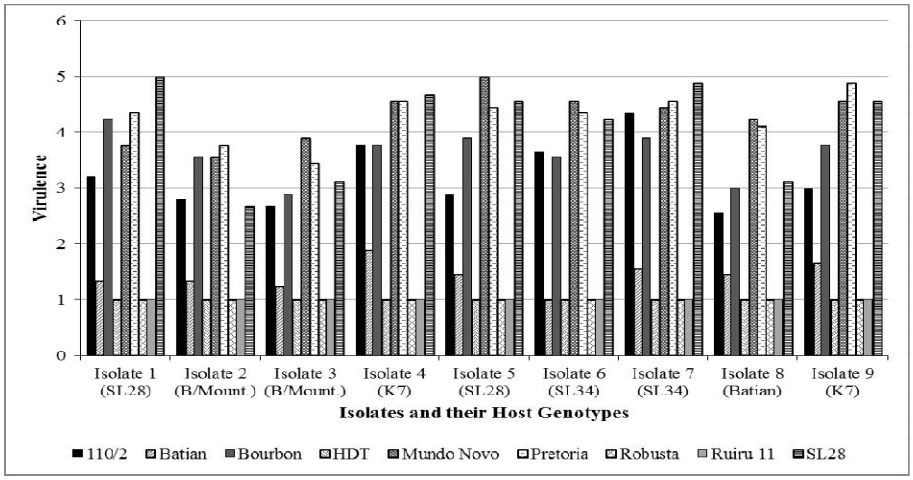

Fig. 3. Interaction between CLR pathogen isolates and the genotypes based on the rate of sporulation of the isolates on each coffee genotype. The genotype legend is arranged in the same order as the bars appear in the figure

4. DISCUSSION

The tested genotypes reacted differently to the tested isolates. This can be attributed to a differential interaction between the genotypes (SH genes) and the isolates (v genes). According to (Herrera et al. [20], resistance of coffee genotypes to CLR pathogen varies and is determined by resistance genes in the coffee genotype (SH1-SH9) and the virulence genes of the isolate. None of the isolates infected Robusta, HDT and Ruiru 11. Robusta, HDT and their derivatives belong to resistant group A and are known to contain resistance genes SH6-SH9 [12]. Therefore, Ruiru 11 and Batian are also considered to contain these genes introgressed from Robusta through HDT. The resistant spectra in HDT can only be annulled by a combination of virulence genes (V5-V9) present in different races of the fungi. Although all the isolates were able to infect Batian, the type of resistance in this cultivar managed to arrest the pathogen preventing sporulation to take place. It can therefore be inferred that resistance in Ruiru 11 and Batian is still active against most races of *H. vastatrix* in Kenya. Another possibility is that the most recent races to be characterized that are able to infect derivatives of Timor Hybrid are not yet wide spread in all coffee growing areas in Kenya. Besides these SH genes, it is likely that

other major and minor genes might be conditioning the coffee-rust interactions [12].

The relatively low resistance in Batian as compared to Ruiru 11 may have been caused by gene dilution which may have occured in the process of back crossing during Batian development. Batian may also contain resistance gene SH5 inherited from Rume Sudan which confers adequate resistance to CLR, especially under field conditions [24]. This is likely because isolates 4 and 9 both from K7 were the most virulent on Batian. K7 is known to contain resistance genes SH2 and SH5 [25] and is also considered to have partial resistance to CLR which is often pathogen nonspecific and involves both constitutive and induced defence mechanisms [26]. All the other varieties that were tested (Mundo Novo, Pretoria, SL28, Bourbon and 110/2) were found to be susceptible to all the isolates though at different magnitudes. Historically, cultivated Arabica coffee is derived from Bourbon and Typica types [27] most of which contain SH1, SH2, SH4 and SH5 genes and they are known to confer resistance only to some races of *H. vastatrix* [28].

The isolates of *H. vastatrix* portrayed a significant diversity in their pathogenicity on different coffee genotypes. The differences in pathogenicity of isolates from the same region portrayed the possibility of having different races in the same region and in the same host genotype. This agrees with the findings of Gichuru et al. [16] that virulence genes in CLR pathogen isolates are highly evolving leading to formation of new virulence genes. This change in the virulence genes is associated with a continued interaction with resistant coffee genotypes leading to breakdown of once resistant coffee genotypes. Therefore, isolates from K7, SL34 and SL28 were found to be more virulent than those from Batian and Blue Mountain. Although this could not be explained, it was evident that different host genotypes were harbouring different races of the fungi. Unlike the host genotype, the region from which the isolates were obtained was not found to play any role on the virulence of the isolates. Mutation of CLR isolates is therefore more predisposed by the host genotype and not the region. However, the region and its environmental conditions may also play a minor role in formation of new races by creating conducive environment for recombination.

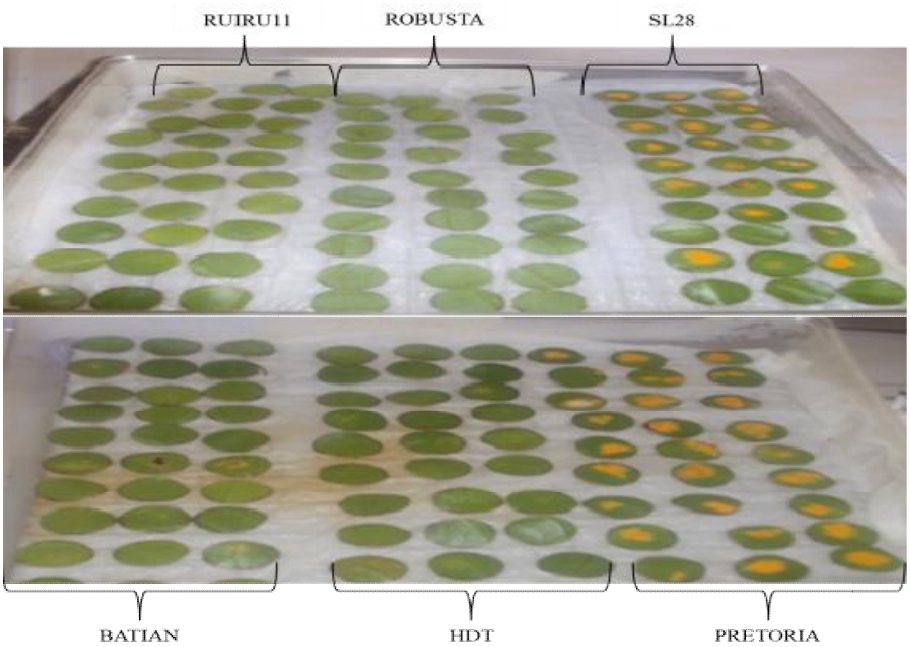

Plate 1. Experimental layout and interaction of CLR pathogen isolates with coffee genotypes

5. CONCLUSION

From this study, it was evident that resistance of coffee genotypes to CLR pathogen varies and is determined by resistance genes in the coffee genotype (SH1-SH9) and the virulence genes of the isolate. Although six additional races of *H. vastatrix* were recently detected in Kenya some of which are able to infect derivatives of Robusta and HDT, the study confirmed that genotypes in this group are still resistant against most races of *H. vastatrix* in Kenya. It was therefore deduced that either these new races are not yet wide spread in all coffee growing areas in Kenya or that there are other major and minor genes conditioning the coffee-rust interactions besides the SH genes. However, for precautionary measures, pyramiding of the known resistant SH genes is recommended to ensure durability of resistance in the varieties being developed. In addition, search for new resistance genes against CLR is highly desirable. The study therefore provided additional knowledge about the pathogen variability and the status of CLR resistance in Kenyan coffee. This information will be useful in investigating the pathogen evolution and in designing strategies for developing new resistant varieties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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