academicJournals

Vol. 8(21), pp. 2450-2454, 6 June, 2013
DOI: 10.5897/AJAR2013.6717
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Methods for early evaluation for resistance to bacterial blight of coffee

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Accepted 24 May, 2013

Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* pv garcae has become of major concern in Kenya due to its increasing incidence and severity. For decades, the disease was confined within and to the west of the Great Rift Valley, but recently it has spread to reach other coffee growing areas. In order to minimize the chemical input in its management, which apart from polluting the environment have high cost implications, development of resistant/tolerant cultivars is highly recommended. This study aimed at developing an effective method(s) for early evaluation of resistance to BBC and to use the method(s) to evaluate the reaction of selected coffee genotypes to different isolates of *P. syringae* pv *garcae.* Three isolates from different coffee growing areas in Kenya were used to inoculate thirteen coffee genotypes using injection and cut methods. The two inoculation methods were found to be effective and can be recommended with slight modifications. However, it was not possible to clearly authenticate the reaction of the different genotypes to BBC since the genotypes responded differently to different isolates and inoculation methods.

Key words: Coffee, *Pseudomonas syringae* pv *garcae*, inoculation method, Kenya.

INTRODUCTION

Although the genus *Coffea* is diverse and reported to comprise about 130 species (Davis et al., 2006), only two species namely Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* Pierre) are under commercial cultivation (Lashermes et al., 1999; Anthony et al., 2002; Pearl et al., 2004). *Coffea arabica* L. is the most important species of the *Coffea* genus, followed by *C. canephora* (Silveira et al., 2003). Coffee production is fundamental for over 50 developing countries, for which it is the main foreign currency earner (Gichimu and Omondi, 2010). Its production is, however, constrained by a number of major diseases, including Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix*, Coffee Berry disease (CBD) caused by *Colletotrichum kahawae* and Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae pv. garcae* (Mugiira et al., 2011).

BBC has been described in Brazil, Kenya, Uganda and China where it is becoming of some concern due to its higher incidence and severity (Silva et al., 2006). In Kenya, the disease has been reported since the establishment of coffee plantations in 1893 but it was confined within and to the west of the Great Rift Valley. The symptoms include dark, water-soaked necrotic lesions on leaves, tips and nodes of vegetative and cropping branches culminating in a die-back (Mugiira et al., 2011). It can be a serious problem in high altitudes, where plants are injured from heavy winds (Jansen, 2005) and have a protracted bimodal pattern of rainfall and often experience storms accompanied by hail (Kairu et al., 1985). The disease was previously known as

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Table 1. Details of isolates used.

Isolates	Date sampled	Source	Host Genotype	Altitude (masl)
Kap-1/012	25-01-2012	Kapsabet	Batian	1983
Kap-2/012	16-04-2012	Kapsabet	Ruiru 11	1983
Nak-1/012	31-05-2012	Nakuru	SL28	2099

"Elgon dieback" and “Solai dieback”; names derived from the areas where the disease occurred (Kairu et al., 1985). The inherent growth and flowering rhythm of *C. arabica* trees governed by the annual rainfall pattern, greatly influence seasonal periodicity of BBC (Ramos and Kamid 1981). Although the disease does not affect more than 5% of the crop in Kenya, it can cause total crop loss in some areas and severely affected trees sometimes have to be destroyed.

Over the years, coffee growers have relied greatly on copper-based formulations to control BBC. However, excessive use of copper sprays has certain drawbacks which include environmental pollution and high costs of chemicals. Besides, increased soil concentration of available copper may have phytotoxic effects on coffee trees which cause shortening and hardening of internodes of young shoots, chlorotic and diminished leaf area with consequent yield reduction (Kairu et al., 1985). In addition, chemical control accounts for up to 30% of the total cost of production and is a major constraint to economic coffee production especially to the small-holders who find the use of pesticides beyond their financial and technical capabilities (Gichuru et al., 2008). There is a strong consensus that growing genetically resistant varieties is the most appropriate cost effective means of managing plant diseases and is one of the key components of crop improvement. It has also been recognized that a better knowledge of both the pathogen and the plant defense mechanisms will allow the development of novel approaches to enhance the durability of resistance (Silva et al., 2006). There is therefore, need to develop a breeding programme for the control of bacterial blight.

Crop improvement depends on the availability of adequate amounts of genetic diversity. It is recognized that the cultivated varieties, in particular *C. arabica,* have a very narrow genetic base (Van der Vossen, 1985; Anthony et al., 2002) that greatly limits the breeding programs especially for improvement of pest and disease resistance (Van der Vossen, 1985). Considerable success has been obtained in the use of classical breeding to control economically important plant diseases, such as the Coffee Leaf Rust and the Coffee Berry Disease (Silva et al., 2006). However, sources of resistance to Bacterial Blight of Coffee are not known. As a prerequisite to development of a breeding programme for BBC in *C. arabica*, there is need to develop a method for early selection of resistance to the disease and subsequently use the method to screen available accessions and developed hybrids for resistance/tolerance to the disease. These requirements formed the objectives of this study.

MATERIALS AND METHODS

Survey and sample collection

A BBC survey on occurrence of the bacterium was conducted between January and May 2012 and suspected diseased coffee samples collected for isolation and identification in the laboratory. Infected twigs or shoots were cut using sterilized pair of secateurs. The samples were put in well labeled paper bags and stored in an ice box. The samples were collected from diverse coffee agro-ecological zones in Kenya including Kisii, Kipkelion, Kapsabet, Nakuru, Ruiru and Nyeri. Nine out of twenty four isolates collected confirmed positive bacterial growth but only three isolates Kap-1/012, Kap-2/012, Nak-1/012 were used for inoculation (Table 1).

Test materials

A total of 13 coffee genotypes comprising of 1 Robusta accession and 12 Arabica varieties were used in this study. The Arabica genotypes included seven Kenyan commercial cultivars (Batian 1, Batian 2, Batian 3, Ruiru 11, K7, SL28 and SL34), two Indian commercial cultivars (Selection 5A and Selection 6) and three museum accessions (Rumesudan, Bourbon and Catimor 134). The reaction of all these genotypes to CBD and CLR is known but their reaction to BBC has not been documented.

Experimental layout and design

Four months old pre-germinated seedlings (with 2 pair of leaves) of the test genotypes were transplanted in black polythene bags measuring 9” x 5” with a potting media comprising of soil, river sand and well decomposed farm yard manure at a ratio of 3:2:1. Triple Super Phosphate (TSP) fertilizer (125 g/15 kg of potting mixture) was added in the media. The experiment was laid out in a temperature controlled room in an inoculation chamber and covered with a polythene sheet to ensure high relative humidity. They were arranged in a completely randomized design with three replications. Each genotype was represented by two seedlings per replicate. The inoculation room was maintained at 18°C.

Inoculation

Two inoculation methods were tested during this study. The first method was through injection where a 30 µl drop of *P. syringae* general inoculum suspension standardized to 108 cfu/ml was placed on each of the mature pair of leaves and a sharp sterile needle used to prick through each drop of the 30 µl of bacterial suspension inoculum. The second inoculation was conducted through a cut where mature leaf pair of the test genotypes was cut using a sharp sterilized blade and then 30 µl bacterial suspension was smeared on the cut edge.

Table 2. Means of disease score of the 13 coffee genotypes using both inoculation methods.

Injection method		Cut method		Both methods combined	
Genotype	**LS means**	**Genotype**	**LS means**	**Genotype**	**LS means**
Batian 1	2.167	Ruiru 11	1.500[a]	Ruiru11	1.875[a]
Sln 5A	2.167	Bourbon	1.583[a]	Bourbon	2.042[ab]
Ruiru 11	2.250	Batian 2	2.000[b]	Batian2	2.250[abc]
Bourbon	2.500	Rume Sudan	2.167[bc]	Robusta	2.375[abc]
Robusta	2.500	K7	2.250[bcd]	SL28	2.375[abc]
Batian 2	2.500	Robusta	2.250[bcd]	Catimor134	2.458[bcd]
SL28	2.500	SL28	2.250[bcd]	RumeSudan	2.458[bcd]
Catimor134	2.583	Catimor134	2.333[cde]	K7	2.478[bcd]
SL34	2.583	Batian 3	2.500[de]	Sln5A	2.500[bcd]
Rume Sudan	2.750	SL34	2.582[ef]	SL34	2.583[cd]
K7	2.792	Sln6	2.582[ef]	Batian1	2.667[cd]
Batian 3	2.917	Sln 5A	2.833[f]	Batian3	2.708[cd]
Sln6	3.250	Batian 1	3.167[g]	Sln6	2.917[d]
LSD (5%)	NS	LSD (5%)	0.292	LSD(5%)	0.427

Means marked with the same letter(s) are not significantly different at p=0.05; NS = not significant.

Data collection and analysis

Disease severity was recorded on a scale of 1 to 5, from the least to the most, based on the degree of necrosis reached after every 7 days, where: 1 = absence of the dark necrotic lesions, with yellow halo (bacterial blight); 2 = 1 to 15% diseased leaves; 3 = 16 to 30% leaves with bacterial blight; 4 = 31 to 45% leaves with bacterial blight; 5 = over 45% of leaves with dark necrotic lesions and dieback of some vegetative shoots (Ito et al., 2008). Recording of disease severity continued after every 7 days for 12 weeks. The peak data was subjected to analysis of variance (ANOVA) using XLSTAT version 2012 and effects declared significant at 5% level. Least significance difference ($LSD_{5\%}$) was used to separate the means. The seedlings that scored ≤2 were classified as resistant; those that scored >2 but ≤3 were classified as moderately susceptible, while the ones that scored >3 were classified as susceptible.

RESULTS AND DISCUSSION

The disease symptoms were observed from the first week after inoculation in all the genotypes except in the control. This was an indication of successful inoculation for both methods. In most cases, the symptoms (dark necrosis) started from the point of inoculation and spread to other parts though in some cases necrosis occurred away from the point of inoculation. Some genotypes reacted by shedding off the infected leaves as a way of managing the disease. The peak infection was achieved in the 7^{th} and 9^{th} week for injection and cut methods respectively. Using the injection method, a combined analysis of variance for all the three isolates conducted using the peak disease infection score that was reached at week 7 showed no significance difference ($p>0.05$) between the genotypes (Table 2). For the cut method, a combined analysis of variance for all the three isolates conducted using the peak disease infection score that was reached at week 9 showed highly significant ($p<0.001$) difference between the genotypes. Ruiru 11 recorded the lowest infection followed by Bourbon and Batian 2 with mean infection scores of 1.50, 1.58 and 2.0 respectively. Batian 1 was the most susceptible genotype with a mean score of 3.167 (Table 2). A combined analysis of variance for the two methods demonstrated highly significant differences between the genotypes ($p<0.001$), isolates ($p<0.0001$) and inoculation method ($p<0.01$).

For the injection method, there was no interaction between the genotypes and isolates indicating that the genotypes responded more or less the same to different isolates. However, for the cut method, there was high interaction between the genotypes and isolates indicating that the genotypes responded differently to different isolates. Following these contradicting results, it was not possible to clearly authenticate the reaction of the different genotypes to BBC. The injection method appeared to be more severe than the cut method representing a high disease score. The cut method however differentiated the varieties better in their level of resistance. Apparently the reaction of most of the genotypes tested ranged between moderately susceptible to susceptible except Ruiru 11 and Bourbon which portrayed appreciable tolerance to BBC for both methods of inoculation. Contrary to this finding, observations in the field especially in BBC prone areas in Kenya like in the Rift valley depict Ruiru 11 as being susceptible.

Although it was not possible to know the races in which the three isolates belonged, Ito et al. (2008) reported that SH_1 gene which confers resistance to some races of *Hemileia vastatrix* (causal agent of Coffee Leaf Rust) also confers resistance to some races of *P. s.* pv. *garcae*. The SH_1 gene is found in pure Arabicas of Ethiopian origin such as Dilla and Alghe. None of the 13 genotypes that were tested in this study is known to contain the SH_1

Table 3. Comparative effectiveness of the two methods.

Injection method		Cut method		Both methods combined	
Isolate	**LS means**	**Isolate**	**LS means**	**Isolate**	**LS means**
Control	1.385[a]	Control	1.231[a]	Control	1.308[a]
Kap-1/012	2.051[b]	Kap-1/012	1.385[a]	Kap-1/012	1.718[b]
Nak-1/012	3.128[c]	Kap-2/012	3.154[b]	Nak-1/012	3.295[c]
Kap-2/012	3.731[d]	Nak-1/012	3.462[c]	Kap-2/012	3.429[c]
LSD (5%)	0.446	LSD (5%)	0.162	LSD (5%)	0.233

Means marked with the same letter(s) are not significantly different at p=0.05.

gene. Previous studies conducted in Brazil also showed that apart from SH_1 gene, there are other resistance sources such as Catucaí (Petek et al., 2006), Hibrido de Timor (HDT) and Icatu (Sera et al., 1980). HDT is a derivative of *Coffea canephora* (Robusta coffee) while Catimor is a derivative of HDT. In addition, all improved Kenyan varieties, namely Ruiru 11, Batian 1, Batian 2 and Batian 3, also have HDT in their pedigree. Although these varieties recorded mixed reaction to BBC, it should be noted that none of them was selected for resistance to BBC and therefore the gene that confers resistance to BBC in HDT may have been lost during selection of the Kenyan varieties. Kenyan BBC isolates are also reportedly more virulent than Brazilian isolates (Kairu, 1997).

For both methods, the isolates depicted a highly significant difference (p>0.001) among themselves. The injection method portrayed isolate KAP-2/012 as the most virulent recording a score of 3.731, followed by isolate NAK-1/012 with a score of 3.128, and isolate KAP-1/012 with a score of 2.051. The control recorded the lowest infection score of 1.385 (Table 3). Unlike in the injection method, the cut method portrayed isolate NAK-1/012 as the most virulent recording a score of 3.46, followed by isolate BBC 18/012 with a score of 3.154, and isolate KAP-1/012 with a score of 1.385. The control recorded an infection score of 1.231 (Table 3). Possible contamination from the infected genotypes may have caused infection recorded in the control since all treatments were applied in the same environment. Differences in Kenyan isolates of *P. s.*pv*garcae* have also been reported by Kairu (1997) and Mugiira et al. (2011).

Conclusion

The two inoculation methods were found to be effective and can be recommended with slight improvements in the layout of the experiment e.g. use of many seedlings per replication. It was not possible to clearly authenticate the reaction of the different genotypes to BBC since the genotypes responded differently to different isolates and inoculation methods. Further study is therefore recommended using a wide range of genotypes and isolates under improved experimental set up. Field based evaluation studies can subsequently be conducted in BBC prone areas to determine whether there is any correlation between the laboratory results and those obtained under natural environment.

ACKNOWLEDGMENT

Thanks are due to the technical staff of CRF Breeding and Pathology sections who participated in this study. This work is published with the permission of the Director of Research, CRF, Kenya.

REFERENCES

Anthony F, Combes MC, Astorga C, Bertrand B, Graziosi G, Lashermes P (2002).The origin of cultivated *Coffeaarabica* L. varieties revealed by AFLP and SSR markers. Theor. Appl. Genet. 104:894-900.

Davis AP, Govaerts R, Bridson DM, Stoffelen P (2006). An annotated taxonomic conspectus of the genus *Coffea* (*Rubiaceae*). Bot. J. Linn. Soc. 152:465-512.

Gichimu BM, Omondi CO (2010). Early performance of five newly developed lines of Arabica Coffeeunder varying environment and spacing in Kenya. Agric. Biol. J. N. Am. 1(1):32-39.

Gichuru EK, Agwanda CO, Combes MC, Mutitu EW, Ngugi ECK, Bertrand B, Lashermes P (2008). Identification of molecular markers linked to a gene conferring resistance to Coffee Berry Disease (*Colletotrichum kahawae*) in *Coffea arabica.* Plant Pathol. 57:1117–1124

Ito DS, Sera T, Sera GH, Grossi LD, Kanayama FS (2008).Resistance to Bacterial Blight in Arabica coffee cultivars. Crop Breed Appl. Biotechnol. 8:99-103.

Jansen AE (2005). Plant protection in coffee: Recommendations for the common code for the coffee community-initiative. Deutsche Gesellschaft fur TechnischeZusammenarbeit, GmbH. Online Publication: http://www.evb.ch/cm_data/4C_Pesticide_Annex_final.pdf

Kairu GM (1997). Biochemical and pathogenic differences between Kenyan and Brazilian isolates of *Pseudomonas syringaepv. garcae.* Plant Pathol. 46:239-246.

Kairu GM, Nyangena CM, CrosseJE (1985).The effect of copper sprays on Bacterial Blight and Coffee Berry Disease in Kenya. Plant Pathol. 34:207-213

Lashermes P, Combes MC, Robert J, Trouslot P,Hont AD, Anthony F, CharrierA (1999). Molecular characterization and origin of the *Coffeaarabica* L. genome. Mol. Genet. 261:259-266

Mugiira RB, Arama PF, Macharia JM, Gichimu BM (2011). Antibacterial activity of foliar fertilizer formulations and their effect on ice nucleation activity of Pseudomonas syringarpvgarcae Van Hall; the causal agent of Bacterial Blight of Coffee. Int. J. Agric. Res. 6(7):550 – 561.

Pearl HM, Nagai C, Moore PH, Steiger DL, Osgood RV, Ming R (2004). Construction of a genetic map for Arabica coffee. Theor. Appl. Genet.

108:829-835.
Petek MR, Sera T, Sera GH, Fonseca IC de B, Ito DS (2006). Seleção de progênies de *Coffeaarabica*com resistênciasimultânea à manchaaureolada e à ferrugemalaranjada. Bragantia 65(1):65-73.
Ramos AH, Kamidi RE (1981) Seasonal periodicity and distribution of Bacterial Blight of coffee in Kenya. Plant Dis. 65:581–584.
Sera T, Cardoso RML, Mohan SK (1980) Resistênciaaocrestamentobacteriano (*Pseudomonas garcae*) emmateriaissegregantespararesistência à ferrugem (*Hemileiavastatrix*).In Proceedings of the 7th Congressobrasileirode pesquisascafeeiras, Araxá, Brazil, pp. 60-61. http://www.sbicafe.ufv.br/SBICafe/publicacao/frpublicacao.asp.
Silva MC, Várzea V, Guerra-Guimarães L, Azinheira HG, Fernandez D, Petitot AS, Bertrand B, LashermesP, Nicole M (2006). Coffee resistance to the main diseases: Leaf rust and Coffee Berry Disease. Braz. J. Plant Physiol. 18(1):119-147
Silveira SR, Ruas PM, Ruas CF, Sera T, Carvalho VP, Coelho ASG (2003). Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. Genet. Mol. Biol. 26:329-336.
Van der Vossen HAM (1985). Coffee selection and breeding. In: M.N. Clifford and K.C. Willson (Eds.), Coffee botany, biochemistry and production of beans and beverage, pp. 48-96. Croom Helm, London.