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Evaluation of Released Coffee varieties against Bacterial Blight of Coffee in South Ethiopia under Laboratory Condition

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Abstract

This study was carried out with the objective of evaluating the potential Resistance of released coffee Varieties for Bacterial Blight of Coffee (BBC) under laboratory conditions of the causative agent *(Pseudomonas syringae pv. garcae).* Bacteria isolates from four coffee growing areas in Sidama and Gedeo zones were characterized based on, biochemical and pathogen city characteristics to distinguish the pathogenic isolates of *P. syringae* from other phylloplane epiphytic bacteria. Isolates of bacteria from diseased coffee plants were categorized in three groups. Isolates that were pathogenic to coffee and produced typical symptoms of BBC fell under group 1 and were assumed to be of *P. syringae* pv. *garcae.* Other isolates that fell under groups 2 and 3 were considered to be saprophytic epiphytes. Nine commercially available and one promising coffee varieties were tested at Jimma plant pathological laboratory pathogenic bacteria isolates collected from the two zones. Inoculation was done with a drop of 9 approximately 10µl of the isolates suspension (10 CFU/ml) using injection method on 4 months old 0 seedlings from potting. Incubation temperature was set at 19-23 C and relative humidity maintained at 78%. The experiment was carried out in a Complete Randomized Design. Disease symptoms were scored using a scale of 1 to 5 and the data subjected to analysis of variance and effect declared significant at 5% level. The results revealed significant difference among the coffee genotypes of south and southwest with G-14/13 being the most resistant. Odicha coffee variety had scored the highest incidence bacterial blight while the promising (G-14/13) was showed least incidence of bacterial blight. It needs further Mass screening research because G-14/2013 from Wollega collection is an indication of the presence of resistant coffee genotypes at different coffee producing areas of Ethiopia.

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Keywords

Coffee, Evaluation, Disease intensity, Bacterial blight, *Pseudomonas syringae* pv. Garcae.

Introduction

Bacterial blight of coffee, caused by *Pseudomonas syringae* pv. *garcae* is responsible for losses in coffee crop, mainly in windy and cooler cultivation areas. In coffee at higher altitudes, without wind protection and in cold regions, the severity of this bacteriosis is more intense (Carvalho and Chalfoun1998). In the state of Paraná, Brazil, the highest Intensity of this disease was observed where the monthly averages of temperature and relative air humidity varied from, respectively, 13.1ºC to 20.5ºC and 57 to 73% and precipitation was 111.3 mm (Cardoso and Mohan 1980).

The disease causes damage mainly to young tissues, where the bacteria induce dark necrotic lesions, with

yellow halo, and dieback of vegetative shoots. The dark necrotic lesions with yellow halo are more frequent along the leaf margins, where the infection is facilitated by mechanical damages. On the branches, the disease provokes dieback with necrosis from the tip to the base. The infection in flowers and young fruits promotes necrosis. Young coffee plants are more affected, with defoliation, dieback, and excess of shoots and reduced plant development. In nurseries, infected seedlings loose leaves, the tip dies and can result in plant death (Godoy *et al.*, 1997). The use of resistant coffee cultivars is the most efficient and ecologically correct control method. The chemical control is, usually, uneconomical in adult plants and in nursery.

The SH1 gene found in the Arabic coffee genotypes Harar, Dilla and Alghe, S12 Kaffa and Geisha, originated from Ethiopia (Moraes *et al.*,1974), confers simultaneous resistance to some races of *Hemileia vastatrix* and to *P. s.* pv. *garcae*. Other resistance sources without gene SH1 were identified (Moraes *et al.*, 1974, Mohan *et al.*, 1978, Sera *et al.*, 1980. Ito *et al.*, 2004, Petek *et al.*, 2006), indicating the presence of other genes that confer qualitative and quantitative resistances. Few Arabic coffee cultivars with complete resistance to *P. s.* pv. *garcae* were identified (DS Ito *et al.*,2008).

Materials and Methods

The study was carried out at Jimma Agricultural Research Center (JARC). The test plant materials consisted of 9 coffee *arabica* varsities and one from Gidami promising that available at Jimma Agricultural Research Center coffee germplasm conservation fields (Table 1). BBC isolates obtained from hot spot areas of Sidama and Gedeo zones were used for screening. Coffee seeds from each selected genotypes were planted in germination boxes containing sterilized sand and watered regularly at room temperature. The germinated seedlings at the eighth week when they had attained the cotyledonous leaves were transplanted into black perforated polybags measuring 5" x 9" inches containing a potting medium composed of soil, sand and manure at a ratio of 3:2:1 respectively. The seedlings were watered regularly and relative humidity was maintained at 80-90% to enable the seedlings to produce a new pair of true leaves approximately every four weeks. The BBC Isolates were cultured in Sucrose Nutrient Agar (SNA) medium prepared by dissolving 8g of nutrient agar and 20g of sucrose in 400ml of distilled water. The medium was sterilized in an autoclave at 120oC, 15psi for 15 minutes, cooled to 45 C and approximately 15-20ml poured into sterile petri-dishes. The bacterial spores from actively growing lesions were allowed to ooze out into distilled water and films of the isolates were streaked on the medium using a nichrome wire loop and incubated for 48 hours at 25°C. Single colonies of the pathogen identified as opaque ash-white, levan forming and mucoid were sub-cultured to ensure purity of cultures. After 48 hours the culture was re-suspended in sterile water and standardized 9 to a concentration of 10 CFU/ml.

Arginine production

Arginine medium was stab-inoculated by the test strains and incubated for 24-48 hrs. The colour was changed from yellow to red-pink recorded as positive reaction to Arginine production (York *et al.*, 2004).The results were recorded and used for identification of isolates.

Gelatine liquefaction

Gelatine liquefaction was tested with 12% (w/v) gelatine containing test tube prepared and stab-inoculated by the test isolates and incubated at 20^{O}C for up to 15 days and then kept at 5^{O}C for 15 minutes before determining liquefaction. Positive reaction of liquefaction of gelatine was recorded when the test tube were tilted (Sands, 1999). The results were recorded and used for identification of isolates.

Oxidase

A filter-paper saturated with 1% Kovac's oxidase reagent (tetra-methyl-p-phenylenediaminedihydrochloride) and placed in a clean Petri dish to look for cytochrome enzymes. A suspected colony of bacteria from NA transferred with wooden stick to the filter paper and rubbed onto the reagent for 30 seconds.

Isolates developed blue or deep purple colours within 30 seconds will considered as positive for Cytochrome oxidase (York *et al.*, 2004).The results were recorded and used for identification of isolates.

Inoculation

Inoculation was done with a drop of approximately 10µl of the isolate suspension CFU/ml) using the injection method (Figure 1) described by Ithiru *et al.*, (2013) with a few modifications where the target was

the shoot primordium between the 1st and the 2nd nodes of 4 month old seedlings from potting which had approximately 4 pairs of true leaves.

A sterile needle was used to prick through each drop of the inoculum to create avenue for the pathogen to enter into the plant. The inoculated seedlings were put in a controlled room arranged in a Complete Randomized Design (CRD) with three replications each having 100 seedlings. Incubation temperature was set at 19-20 C with the relative humidity maintained at approximately 75% by misting with water twice every day.

Data Collection and Analysis

Disease symptoms were scored using a scale of 1 to 5, from the least to the most described by Ito *et al.*, (2008) with modifications where: 1= absence of the dark necrotic lesions, with yellow halo (bacterial blight); 2 = small black lesion; 3 = black lesion coalescing; 4 =black coalesced lesion with over 50% meristem girdling and 5 = complete girdling around the meristem based on the degree of necrosis reached from day 14 from inoculation date and after every 7days thereafter (Figure 2). Each seedling was scored individually for disease infection and mean for each replication was used for analysis. The data was subjected to analysis of variance (ANOVA) using COSTAT version 2012 and confidence levels set at 95%. Least significant difference (LSD 5%) was used to separate the means.

Results and Discussion

The experimental variation coefficient was 19.0%, indicating experimental accuracy for a laboratory experiment. By the test of means, it was possible to separate the cultivars in three resistance groups (a, b and c), statistically different at 0.05% probability (Table 1). Odich variety which was released CBD resistant for southern coffee producing of Ethiopia showed susceptible reaction both at field and laboratory conditions. Four coffee varieties which were released CBD resistant from southwestern Ethiopia and widely adaptable were moderately resistance reation. The G-14/13 cultivar (collected from Gidami area) was the only one classified as "resistance group a" at Laboratory. The resistance of G-14/13 was probably originated from germplasm Wollega, while the cultivars Odicha, Fayate, Angafa and Qoti were classified as susceptible were from South Ethiopia.

Previous studies also showed that aside from the SH 1gene of coffee genotypes originated from Ethiopia (Moraes *et al.,* 1974), there are other resistance sources such as Icatu (Mohan *et al.*, 1978, Sera *et al.*, 1980), Catucaí (Petek *et al.,* 2006) and Hibrido de Timor (Moraes *et al.*, 1974, Mohan *et al.,* 1978, Sera *et al.*, 1980). In our study, the varieties 7410, 7412 and74140 are confirmed results of a preliminary evaluation by Gabisa. (2018) in adult plants, where this varieties were also classified as resistant under field conditions in southern region, Sidama and Gedeo zones where as probably partially resistance at laboratory condition.

This partial resistance could be the effect of minor genes that would make the penetration and reproduction of *P. s.* pv. *garcae* difficult. Leaf thickness and shape and undulation of the leaf margins or even the plant architecture could be traits that would reduce disease severity. Other studies reported the possibility of the existence of quantitative resistance to *P. s.* pv. *garcae* (Petek *et al.,* 2006). The Southwest released varieties 7440, 7410, and 74112 were classified in resistance group b, indicating a possible quantitative resistance, because few plants of these varieties were scored with grades 4 and 5 in comparison with other varieties of resistance group c (susceptible like Qoti, Odicha, Fayate and Angafa) (Table 2).

Bacterial blight of coffee, caused by *Pseudomonas syringae* pv. *garcae* is responsible for losses in coffee crop, mainly in windy and cooler cultivation areas. In coffee at higher altitudes, without wind protection and in cold regions, the severity of this bacteriosis is more intense (Carvalho and Chalfoun1998). The study confirmed that there exists diversity among coffee genotypes in resistance to BBC.

Genotypes like G-14/2013 and 7410, 74112 that have been used in breeding resistant varieties have proved to be promising sources of resistance to BBC and are therefore preferable as donors for resistance against the disease. 7410, 74112 which is an improved breeders' line that combines high yield, good quality and resistance to the other two major fungal diseases in Ethiopia is a suitable candidate as a recurrent parent or for further selection in breeding for resistance towards BBC

Table.1 Coffee genotypes used in the study with a description of their known characteristics

SN	Genotype	Description
1	74110	Commercial variety Resistant to CBD and CLR
2	74112	Commercial variety Resistant to CBD and CLR
3	74140	Commercial variety Resistant to CBD and CLR
4	1377	Commercial variety tolerant to CBD and CLR
5	971 (Fayate)	Commercial variety Resistant to CBD and CLR
6	974 (Odicha)	Commercial variety Resistant tolerant to CBD and CLR
7	85257 (Koti)	Commercial variety resistant to CBD and CLR
8	279	Susceptible to CBD but resistant to CWD
9	G-/14/13	Moderately resistant to CBD and CLR ,tolerant to CWD
10	370	Commercial variety Susceptible to CBD but resistant to CWD

Table.2 Mean scores and percentage of susceptible plants to *Pseudomonas syringae* pv. *garcae* of coffee varieties evaluated under laboratory conditions for symptoms of bacterial blight severity,

Acc. no	Mean % at lab
Odicha	40.39^{A}
Qoti	31.20^{B}
279	31.21^{B}
Fayate	30.20^{B}
370	28.63^{CB}
Angafa	22.27^{CB}
74140	21.00^{D}
74110	20.06^{D}
74112	19.87^{D}
G-14 /13	7.68^{E}
mean	**25.00**
LSD(0.05)	**7.3**
CV (%)	**6.9**

1. Cultivars in increasing sequence based on the mean scores of P.Syringe pv. Garcae severity
2. Score 1 = absence of disease symptoms, 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 % leaves with dark necrotic lesions and dieback of some vegetative shoots

Fig.1 Inoculation of a coffee seedling at the underside of the Leaf using the Injection methods

Fig.2 Classification of BBC disease symptoms after inoculation.

Key: 1 –no infection (class 1), 2- signs of water soaking with small scab or tiny brown lesions (class 2), 3- small black lesions (early class 3), 4- black lesion becoming wider and starting to coalesce (class 3), 5- black coalesced lesion over 50% of meristem girdling (class 4), 6- complete girdling around the meristem (class 5).

Fig.3 Response of some coffee genotypes for *Pseudomonas syringae* pv. *Garcae.*

Recommendation

The possible existence of diverse strains of the *P.syringae* pv.*garcae* pose a threat to coffee farming and therefore a breeding programme to introgres resistance genes into the commercial varieties through pyramiding of resistance genes could offer durable resistance to the coffee. Further studies of the pathogen to confirm its diversity should be prioritized to ensure that a horizontal broad-spectrum breeding for resistance against the entire pathogen species is achieved. It needs further Mass screening research because G-14/2013 from Wollega collection is an indication of the presence of resistant coffee genotypes at different coffee producing areas of Ethiopia.

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