Distribution assessment and pathogenicity test of coffee berry disease (*Colletotrichum kahawae*) in Hararghe, Ethiopia

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Coffee berry disease causes about 30% national average crop losses every year in Ethiopia. This study was conducted to assess the distribution of coffee berry disease and test to know the pathogenicity of the disease on coffee landraces. Assessment of the disease was conducted in Bedeno, Boke, Habro and Darolebu districts from August to September 2011. Incidence and severity were recorded on 50 and 10 randomly selected coffee trees per farm; respectively. This disease was prevalent in all surveyed districts of Hararghe. The mean disease incidence was 51% at Darolebu and 75% at Bedeno and the mean disease severity was 26% at Boke and 50% at Bedeno. There was a highly significant difference among cultivar * isolate interactions. This indicated the presence of resistance sources in Hararghe coffee germplasms that may be exploited for coffee improvement purpose. Hence, it is important to conserve both in situ and ex situ and use sustainably the Hararghe coffee germplasms by conducting intensive selection from more diverse coffee populations and evaluations for resistance to coffee berry disease.

Key words: Hararghe coffee, Pathogenicity, Colletotrichum kahawae, germplasm

INTRODUCTION

Among the crops that Ethiopia has served as the source of germplasm for several economically important cultivated crops around the world, coffee (*Coffea arabica* L.) is the most important gift of Ethiopia to the world which had and still has a tremendous economic, social and spiritual impact on many people of different geographical locations, cultural backgrounds and psychological behaviors (Tefestewold, 1995). It is mainly produced in the South Western, Southern and Eastern parts of the country. Forests in Southwestern of Ethiopia are the primary center of origin and center of genetic diversity of *Coffea arabica* (Kimani et al., 2002). Coffee berry disease (CBD) causes economic crop losses. Van der Graaff (1981) reported that the average national yield loss was about 28% between 1974 and 1978. Merdasa (1986) estimated the average yield losses ranging from 51% to 81% from Wondo Genet, Gera and Melko experimental plots. In Hararghe, the loss was estimated to be as high as 100% (Tefestewold and Mengistu, 1989). The severity of CBD and the losses caused often under estimated annually because young coffee berries drop off the tree at an early stage of the disease (Tefestewold, 1995). The consensus is that CBD causes 30% national average crop losses to total harvestable coffee yield every year in Ethiopia (Eshetu et al., 2000).

The most aggressive species causing the coffee berry disease is present only in East and Central Africa. *Colletotrichum kahawae* is the only species, which is pathogenic to green coffee berries, which also colonizes berries of other stages, leaves and maturing bark of the branches. Other species, namely, *Colletotrichum gloesporioides* and in some instances *Colletotrichum acutatum* are nonpathogenic to green coffee berries...
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(Rodrigues et al., 1992). Tefesetewold (1995) distinguished the CBD pathogen from other Colletotrichum spp. associated with Arabica coffee in Ethiopia based on thorough analyses of cultural, morphological, biochemical, and physiological characteristics. However, he failed to support the new species name Colletotrichum kahawae introduced by Waller et al. (1993) that the first nomenclature of Colletotrichum Coffeanum Noack (SensuHindorf) was based on berry samples taken from Brazil where the disease is not existent.

No coffee berry disease resistant variety have been developed and distributed to coffee farmers in Hararghe, eastern part the country except some efforts which made to introduce those cultivars have been released for planting since 1977 that are especially suitable for the western parts of the country. However, these cultivars have not performed well in the coffee growing areas of the Hararghe districts. Recently, Jima Agricultural Research Center collected some coffee landraces from districts of Hararghe zones to see the resistance level of landraces. Therefore, this project was designed to assess the distribution of coffee berry disease and test the pathogenicity of the disease so as to plan the management options.

MATERIALS AND METHODS

Descriptions of study sites

The field studies were conducted in the major coffee producing districts of Hararghe: Bedeno (East Hararghe), Boke, Habro and Darolebu (West Hararghe). In each district disease assessment and sampling were conducted and laboratory works were done at Haramaya University.

Disease Survey

The survey of coffee berry disease was undertaken in major coffee producing districts of Hararghe. Based on the secondary information from the district Bureau of Agriculture, the surveyed coffee producing districts were divided into lowland, midland and highland. From each agro ecological zone three to five farms and a total nine to fifteen were surveyed from each district. Fields were sampled at intervals of about 5-10 km along the roads and the distance between sample fields was based on the topography and the relative importance of coffee production within each district. Two types of assessment methods (Incidence and severity) were conducted on the same tree following procedures used by Tesfaye and Ibrahim (2000). (a) Disease incidence: Fifty trees per farm were randomly observed and diagnosed for presence and absence of the disease on each tree and disease incidence was calculated as (number of diseased trees / total observed trees) x 100. (b) Disease severity: Ten trees / farm were randomly selected and each tree was divided into 3 strata of branches (top, middle and bottom). From each stratum one middle branch was randomly selected to determine severity.

Pathogenicity Test of Colletotrichum kahawae Isolates on Coffee Seedlings

The pathogenic variability in C. Kahawae population was studied by inoculating six representative isolates on coffee landraces, resistant and susceptible checks. Representative isolates from Bedeno (B1,B2, B3), Habro (H1, H2) and one isolate from Gera (G) were inoculated on seedlings of three widely grown Hararghe coffee landraces (H-05/02, H-568/02 and H-87/02) and, a resistant (code 741) and a susceptible (code 370) checks to investigate their interaction. The interaction of selected cultivars and coffee berry disease isolates was evaluated following the methods and procedures used by Bayetta (2001) and Arega et al (2008).

Colony aerial mycelial growth of Colletotrichum kahawae isolates

Vigor of aerial mycelial growth as dense, irregular (scarce) or very scarce type was observed on obverse side from 10 days cultures on potato dextrose agar and malt extract agar (Pda and Mea).

Data Analysis

All data were analyzed following following respective statistical procedures and treatment means of pathogenicity test were compared using Least Significance Difference (LSD) (Townend, 2002). SAS statistical package software was employed to perform analysis of variance (ANOVA) and mean comparison.

RESULTS AND DISCUSSION

Occurrence of CBD in Hararghe

The incidence and severity of coffee berry disease (CBD) varied among and within Hararghe coffee. The disease incidence ranged up to 95 %, 55 %, 65 % and 70 % was observed in Bedeno, Boke, Habro and Darolebu districts respectively. The mean incidence ranged between 45.7 % at Boke and 77.3 % at Bedeno whereas the severity of the disease varied between 26.7 and 55 % as indicated in Table 1 at Boke and Bedeno, respectively. Similarly, Bayetta (2001) explained high CBD occurrence related with high humidity with high altitude around Gera. High incidence of CBD may be explained in particular high rainfall found in relatively high altitudes of Bedeno and to
some extent in Habro. As Cook (1975) explained that high rainfall, high humidity or wetness and relatively low temperatures that persist for long periods favor CBD development and the disease is invariably severe at higher altitudes where these conditions generally prevail. The incidence and severity of the disease varied from one district to other. In 1994 crop season, prevalence of CBD was conducted in Oromiya Region and Southern Nations Nationalities and Peoples Region (SNNPR) and the result indicated 38.8 and 17.2 % of mean percent prevalence of the disease, respectively (IAR, 1997).

Differences in CBD levels were observed among Hararghe coffee landraces, resistant and susceptible checks. There existed highly significant ($P < 0.05$) differences in Hararghe coffee selections in seedling percent infection in reaction to CBD. Out of the tested selections from all Hararghe coffee landraces 33% revealed significantly ($P = 0.05$) low level of seedling infection rate as compared to the standard susceptible check (Table 2). Similarly, Arega et al (2008), Tefestewold (1995) and Bayetta (2001) also reported significant differences in seedling percent infection in reaction to CBD.
Table 2. Pathogenicity of 6 *Colletotrichum Kahawae* Isolates on Seedlings of 4 Coffee Selections

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Colletotrichum kahawae isolates</th>
<th>B2</th>
<th>B3</th>
<th>B1</th>
<th>H3</th>
<th>H1</th>
<th>G1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0 g</td>
<td>0.0 g</td>
<td>0.0 g</td>
<td>0.0 g</td>
<td>0.0 g</td>
<td>0.0 g</td>
</tr>
<tr>
<td>741</td>
<td>H-05/02</td>
<td>35.2e</td>
<td>25.9 ef</td>
<td>0.0 g</td>
<td>13.6 fg</td>
<td>0.0 g</td>
<td>0.0 g</td>
</tr>
<tr>
<td></td>
<td>H-568/02</td>
<td>94.5ab</td>
<td>97.0 ab</td>
<td>81.0bc</td>
<td>87abc</td>
<td>95.0 ab</td>
<td>89.6 abc</td>
</tr>
<tr>
<td></td>
<td>H-87/02</td>
<td>92.8ab</td>
<td>96ab</td>
<td>79bc</td>
<td>90.4abc</td>
<td>99 ab</td>
<td>90 abc</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>100 a</td>
<td>95a</td>
<td>98.2 a</td>
<td>79.2 bc</td>
<td>60.0 d</td>
<td>83.3 abc</td>
</tr>
</tbody>
</table>

Table 3. Comparisons of tendency to form sector and aerial mycelial growth of *C. kahawae* isolates on potato dextrose agar and malt extract agar (PDA and MEA)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sectoring or Saltation</th>
<th>PDA</th>
<th>MEA</th>
<th>Areal mycelial growth (vigor)</th>
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</thead>
<tbody>
<tr>
<td>Ge1</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ge2</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ge3</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>H1</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>H2</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H3</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>H4</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>DL1</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DL2</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>DL3</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DL4</td>
<td>Ab</td>
<td>P</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Bo1</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Bo2</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Bo3</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Bo4</td>
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<td>Bo5</td>
<td>Ab</td>
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<td>+</td>
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<tr>
<td>Be1</td>
<td>Ab</td>
<td>P</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Be2</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Be3</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Be4</td>
<td>Ab</td>
<td>P</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Three (Ge1, Ge2, Ge3), Four (H1, H2, H3, H4), Four (DL1, DL2, DL3, DL4), Five (Bo1, Bo2, Bo3, Bo4, Bo5) and Four (Be1, Be2, Be3, Be4) *Colletotrichum kahawae* isolates collected from Gera, Habro, DaroLebu, Boke and Bedeno respectively.

*Areal mycelial growth (vigor): +=Dense, ++=irregular (scarce), +++=very scarce
-Ab: absent, P: present*

Differences in CBD levels were observed among Hararghe coffee landraces, resistant and susceptible checks. There existed highly significant (P < 0.05) differences in Hararghe coffee selections in seedling
percent infection in reaction to CBD. Out of the tested selections from all Hararghe coffee landraces 33% revealed significantly (P = 0.05) low level of seedling infection rate as compared to the standard susceptible check (Table 2). Similarly, Arega et al (2008), Tefestewold (1995) and Bayetta (2001) also reported significant differences in seedling percent infection in reaction to CBD.

Coffee cultivar 741(resistant check), H-05/02, H-568/02, H-87/02 and 370 (susceptible check)

Pure culture of Colletotrichum kahawae isolates from Hararghe districts and representative isolate from Gera as a check were examined for colony (mycelia) aerial growth and sectoring. The isolates were categorized into 3 classes to study variability of the isolates on the basis of aerial mycelial growth (vigor): dense, irregular (scarce) and very scarce colony types (Table 3). Out of 20 C. kahawaeisolates tested for their aerial mycelial growth (vigor) 65 % and 90 % showed consistently dense aerial mycelial growth on both potato dextrose agar and malt extract agar (Pda and Mea) media, respectively; whereas 30 and 5 % isolates revealed irregular (scarce) and 5 % revealed very scarce aerial mycelial growth on both potato dextrose agar and malt extract agar (Pda and Mea) media, respectively. Similarly, Tefestewold (1995) and Arega et al (2008) also reported differences in aerial mycelia growth among C. kahawaeisolates from Kaffa and Illubabor on PDA.

CONCLUSIONS

Coffee berry disease was present in all assessed districts but the incidence and severity varied from one district to other depending on environmental condition and diversity of coffee selections. Pathogenicity test of 6 isolates on seedlings of 5 Coffea arabica L. cultivars indicated that there was a highly significant difference among cultivar x isolate interactions. The seedling inoculation test result confirmed that high aggressive in colletotrichum kahawae population associated with high mean percent infection of inoculated coffee hypocotyls. The difference in virulence and aggressiveness implies care should be taken in that to develop resistant varieties aggressive isolates should be used for successful screening of coffee germplasms before the release of the new developed cultivar(s). Isolates could be grouped into 3 based on their colony color manifestation on the obverse side of potato dextrose agar (pda) and Malt extract agar (mea) culture Petriplates viz., light gray, darkgray and gray mycelial form.

RECOMMENDATION

It is important to conserve both in situ and ex situ and use sustainably the Hararghe coffee germplasms by conducting intensive collection and selection from more diverse coffee population and evaluation for resistance to coffee berry disease.

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Accepted 05 January, 2015.

Citation: Emana BT (2014). Distribution assessment and
pathogenicity test of coffee berry disease (Colletotrichum
kahawae) in Hararghe, Ethiopia. International Journal of
Plant Breeding and Crop Science, 2(1): 038-042.

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