

Evaluation of released arabica coffee varieties (*coffea arabica* L) for major coffee diseases with especial emphasis to coffee wilt disease (*Gibberella xylarioides*) at jimma, Ethiopia

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Abstract

Seedling inoculation test is important for rapid assessment of resistance of young coffee seedlings and may also be used for pre screening of large number of progenies for major coffee disease such as CBD and CWD. Seedlings of released coffee varieties were inoculated with *C. kahawae* and *Gibberella xylarioides* isolate in the laboratory, where environmental conditions are best regulated. The result showed highly significant ($p < 0.01$) difference in CBD and CWD infection among released coffee varieties. The mean percent infection varies from 2.93 - 85.92% and 0.0-90 for CBD and CWD respectively. Low disease severity levels were recorded on released coffee variety 741, 754 and 75227, 744 and Feyate, Odicha for CBD and CWD respectively. Higher CBD severities were recorded on coffee released variety 74165, 74148, 74140, 74110, 74112, 74158 and 7487 under laboratory condition however; no disease outbreak was reported under field condition as the varieties are field resistant. On the other hand except Feyate, Odicha, Catimor J-19 and Mechara-1 most pure line and all hybrids released coffee varieties exhibit susceptible reaction. In areas where there is enough CWD inoculum load pathogen and environmental condition is highly suitable Varieties which express susceptible reaction under laboratory also express susceptible reaction under field condition. Understanding the gene(s) that govern resistance to CWD and mode of inheritance mechanisms and large CWD screening program is of paramount importance to implement disease management options. More research work on biochemical and morphological defense mechanism which contributes to CBD resistant and Host defence mechanisms needs to be investigated.

Keywords: Released coffee varieties, Arabica Coffee, Seedling test, Coffee berry disease, Coffee wilt disease.

Introduction

Coffee is the most important cash crop for Africa as a whole, contributing some 10% of the total foreign exchange earnings in the continent. A number of coffee-producing countries in sub-Saharan Africa, including Uganda, Ethiopia, Rwanda and Burundi, depend on the export of this commodity for more than half of their foreign exchange earnings (Phiri *et al.* 2010). Arabica coffee has become a major global commodity which accounts for 66 per cent of the world coffee market. More importantly, majority of the coffee farmers in the producing countries are small scale growers who primarily depend on coffee for their livelihood. Its cultivation, processing and transportation provide employment for millions of people.

The average yield in Ethiopia is low (about 700kg /ha per year) which is half of that achieved in Latin America and almost one third of Asia's productivity. This is partly due to continued reliance on unproductive coffee varieties, the widespread and prevalence of pests and diseases (Girma *et al.*, 2009a). However, coffee suffers from a range of co-evolved diseases including coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR) caused by *Colletotrichum kahawae*, *Gibberella xylarioides* and *Hemileia vastatrix*, respectively.

There are many research findings documented on status major coffee diseases. Survey conducted in 1997 and 1998 in six major coffee growing zones (in 32 woredas) of Oromiya region showed an average of 31% and 32% disease CBD severity for the respective years (Melaku Jirata and Samuel Assefa, 2000). CBD incidence and severity assessment in 10 zones and 31 woredas of Southern Nations Nationalities and Peoples Region (SNNPR), conducted in September 1998, resulted with 40% and 22.8% mean incidence and severity of the disease, respectively (Tsfaye Negash and Sinedu Abate, 2000). Mean percent severity 17.9, 4.0, 5.4 and 2% was reported for forest production system of for Bonga, Yayu, Harena and Sheko, respectively (Arega 2006). However the overall national average loss due to coffee berry disease is estimated to range 25-30%, (Eshetu Derso, 1997; Eshetu Derso *et al.*, 2000). Coffee wilt disease is one of the three economically important disease which is caused by fungal pathogen called *Gibberella xylarioides*. The disease incidence ranged from 3.6-15.5 % in semi forest and 18.6-25.4 % in some garden fields (Girma, 2004). However the national incidence and severity of CWD in Ethiopia was 27.9 and 3 % respectively (CAB international, 2003).

Arabica coffee breeding principles and methods applied in different coffee growing countries are generally similar. However, in Ethiopia, pure-line selection and intra-specific hybridization are commonly used (Bayeta, 2001). The fact that Ethiopia is the center of origin and diversity for arabica coffee there is great potential for improvement of the crop. A comprehensive variety development research work on arabica coffee resulted 34 pure lines and 3 hybrids. Major coffee diseases screening protocol was established and refined by

different researchers which make realize the development of commercial coffee varieties (Vander graff, 1981, Eshetu, 2000 and Girma, 2004).

Thus reaction of released arabica coffee (*Coffea Arabica* L.) varieties against coffee berry disease (*C.kahawae*) and coffee wilt (*Gibberella xylarioides*) resistance under laboratory condition is presented in this article.

MATERIALS AND METHODS

2.1 Description of the study site

The study was conducted in the laboratory and greenhouse at Jimma Agricultural Research Center. The center is found in Oromiya Regional State in Jimma zone, Ethiopia, located around 07°46'N latitude and 36°47'E longitude coordinate and at an elevation of 1753 m.a.s.l. It represents the medium agro ecological zones which receive annual rainfall of 1572mm, with mean minimum and maximum temperatures of 11.6^oc and 26.3^oc, respectively. The major soil type of the area is Eutric Nitosol and Cambiosl (reddish brown) of upland and fluvisol of bottom land with pH around 5.2 (IAR, 1997).

Experimental Materials

Seeds were prepared from 36 coffee varieties (including resistant and susceptible checks to coffee berry disease and coffee wilt disease). Two sets of experiments were independently conducted in completely randomized design with three replication in two batches. The seeds were sown into heat sterilized and moistened sandy soil in disinfected plastic pots (10 % sodium hypochlorite). Sterile water was regularly applied every two days, to maintain adequate moisture for seed germination, emergence and growth of the plants throughout the experimental period.

Experiment I. Testing for CBD Resistance

Seedling hypocotyls inoculation test

Thirteen coffee varieties and one check were tested for their reaction to coffee berry disease. Seedling hypocotyl inoculation technique was executed following the methods of Vander Graff (1981), and Bayetta (2001). Spore suspension (2x10⁶ conidia ml⁻¹) was prepared from 10days old colonies of a representative *C.kahawae* isolate on PDA. Inoculation was effected by dipping small and soft camel hairbrush in the spore suspension in a beaker and gently brushing individual seedlings (4-6 weeks old). The inoculated seedlings were arranged on the bench in a growth room and covered with plastic sheet for 48 hours to maintain 100% relative humidity and temperature was adjusted to 21 °C that favour infection. In order to ensure infection, the seedlings were re-inoculated following the same procedure and maintained under the same conditions for further 48 hours. After three weeks incubation, the number of infected seedlings per box examined and recorded using (Van der Graff, 1981; Tefestewold, 1995).

Table 1: Arabica coffee cultivars their origin and year of release

Coffee cultivar	District/Zone	Year of Released
741	Gera / Jimma	1977/78
744	Washi, Kaffa	1979/80
7440	Washi, Kaffa	1979/80
7454	Washi, Kaffa	1979/80
7487	Washi, Kaffa	1980/81
74110	Metu / Illubabor	1978/79
74112	Metu / Illubabor	1978/79
74140	Metu / Illubabor	1978/79
74148	Metu / Illubabor	1978/79
74158	Metu / Illubabor	1978/79
74165	Metu / Illubabor	1978/79
754	Wush Wush, /Kaffa	1980/81
75227	Gera, /Jimma	1980/81
Ababuna*	Gera & Gimbo / Jimma & Illuababor	1998
Melko-CH2*	Gera & Gimbo /Jimma & Kaffa	1998
Gawe*	Metu & Yayo/ Illubabor	2002
Bunowashi	Washi/ Kaffa	2006
Wush-wush	Wush wush/ Kaffa	2006
Yachi	Yachi/ Jimma	2006
Merdacheriko	Gera/ Jimma	2006
Catimor J-19	International collection	1998
Gesha	International collection	2002
Manasibu	Menesibu/West Wollega	2010
Sende	Haru/ West Wollega	2010
Challa	Haru/ West Wollega	2010
HaruI	Haru/ West Wollega	2010
Bultum	Habro /West Hararghe	2010
Harusa	Chiro/ West Hararghe	2010
Mocha	Mesela/ West Hararghe	2010
MecharaI	Habro/ West Hararghe	2010
Odicha	Gelana Abaya/ Borena	2010
Fayate	Gelana Abaya/ Norena	2010
Koti	Yirga Cheffe/GEdeO	2010
Dessu	Gimbo/Kaffa	1998
3/70**	Seka Chekorsa/ Jimma	

*hybrid coffee variety

**Resistant check

Experiment II. Testing for resistance to Coffee Wilt Disease Inoculum multiplication and Inoculation of Coffee Seedlings

Thirty four coffee varieties and one resistant check were tested for their reaction to coffee wilt disease. Standard *Gibberella xyliaroides* isolate were multiplied on sterile coffee twigs placed in test tubes. After 14 days incubation, conidia were harvested by scratching and rinsing from branches with distilled sterile water and the concentration of spore suspension was counted with haemocytometer and then adjusted to 2×10^6 conidia ml^{-1} (Girma and Mengistu, 2000; Girma et al., 2009b). The seedling of each coffee variety were inoculated with viable conidial suspension by stem nicking technique described by pieters and Van der Graaf, (1980) and Girma et al., (2009b). All the treated plants were then placed on experimental benches and immediately covered with plastic sheet in a growth room with high humidity and temperature of about 23°C to favour infection (Girma et al; 2009b). After 10 days, the inoculated seedlings were transferred into green house.

Data collection and analysis

Based on the typical wilting symptoms and death of coffee seedlings, the number of wilted/ dead and healthy seedlings were counted and recorded per pot every two weeks for six months starting a month after inoculation. Isolation from samples were inoculated seedlings were computed from cumulative number of dead seedlings (during 6 months period) over the total number of inoculated seedlings. The percentage of data was transformed to angular values before statistical analysis with SAS system for windows (9.2 version) (SAS, 2008).

RESULTS AND DISCUSSION

There were significant differences among the coffee varieties tested for resistance to coffee berry and coffee wilt diseases. In seedling hypocotyl test for CBD resistance, Seedlings of 13 released cultivars and one susceptible check were inoculated with *C. kahawae* isolate under controlled laboratory conditions where mean average relative humidity and temperature was 76% and 18.8 °C, respectively. The results of this study revealed a highly significant ($p < 0.01$) differences in CBD severity among treatments in seedling inoculation test. The actual mean percent infection varied from 2.93 - 85.92%. Very lower disease severity level of 2.93, 7.33, and 8.71% were observed on coffee cultivar 741, 754, and 75227, respectively (Table 2).

Lower disease level of 22.41% was observed on coffee cultivar 744. Moderate level of CBD infection of 32.4 and 35.26 % were observed on coffee cultivar 7454 and 7440, respectively. Coffee cultivar 74165, 74148, 74140, 74110, 74112, 74158 and 7487 showed higher disease level ranging from mean 67.37 - 85.92 %.

Coffee type 370 showed significantly ($p < 0.01$) high mean percent CBD infection. The resistant cultivar 741 reacted in classes 0-1 (resistant reaction) on the other hand coffee type 370 reacted in class 4 which is susceptible reaction.

The result of this study is in consistent with the conclusions that have been drawn by Tefsetewold, (1995), Arega, (2006). The report of Tefsetewold (1995), point out macroscopic symptomless lesions (classified as zero) were observed on resistant coffee selection 741, where as coffee selection 74110 manifested high susceptibility to all isolates, 744 showed resistant reaction except to Hararghe isolate. Arega (2006) also reports susceptible check 370 and released cultivar 74110 showed 96.7 and 78 % seedling infection respectively, on the other hand 741, 754 showed resistant reaction to all isolates tested.

In the second experiment there existed highly significant ($P < 0.05$) difference among coffee varieties both in percent dead (wilt) seedlings and incubation period (Table 3). Disease severity and incubation period varied between 0.0-90 and 60.7 -160 days. Coffee variety fayate showed no seedling death. Coffee variety Odicha resulted significantly ($p = 0.05$) low percentage (<20%) of dead seedlings (resistant i.e. 16.5 % with incubation period of 160 days. Coffee varieties such as Merdacheriko, Catimor J-19, and Mechara- 1 showed moderate coffee wilt disease infection. Coffee accession 370 which is highly susceptible to CBD expressed resistant reaction with longer incubation period. Coffee variety 7440 showed moderately susceptible reaction with percent seedling death of 51.9 % with incubation period of 109.67 days. On the other hand most pure line and all hybrids released coffee varieties exhibit susceptible reaction.

The present finding is in agreement with the findings of Jefuka et al., (2012) and Kifle et al., (2014). Jefuka et al., (2012) reported best performance of coffee varieties such as Fayate and Odicha for their resistance to coffee wilt disease. Kifle et al., (2014) also described the genetic variability against coffee wilt disease (Longest incubation period and lowest disease severity) on Bale 2004 collection.

Table 2: Percentage of CBD infection among 13 released CBD cultivars and one susceptible check inoculated with *C. kahawae* isolate

Variety	Actual Value (in %)*	Transformed Value (in %)
741	2.9	9.67 ^a
744	22.4	28.09 ^b
7454	32.4	34.50 ^c
7487	85.9	68.65 ^c
7440	35.3	36.40 ^c
74110	71.1	57.47 ^d
74112	73.8	59.30 ^d
74140	71.1	57.45 ^d
74148	70.7	57.28 ^d
74158	74.1	59.45 ^d
74165	68.4	55.79 ^d
754	7.3	15.19 ^a
75227	8.7	16.98 ^a
**370	100.0	88.19 ^c
LSD (at 1%)		2.84

C.V= 7.85%

*Average of three replications.

**Susceptible check

Means followed by a common letter are not significantly different at the 1% level of significance.

Table 3. Resistance levels of released coffee varieties to coffee wilt disease in the green house at Jimma Agricultural Research Center

Variety/ Accession	Severity (%)		Incubation period (days)
	Actual value	Transformed value	
741	78.46	66.94	75
744	96.67	83.86	87
7454	92.98	77.78	87
7487	84.71	67.57	89
7440	61.67	51.90	109.67
74110	86.17	71.94	89
74112	88.25	74.51	89
74140	90	78.93	87
74148	86.58	73.49	87
74158	90	71.95	80.330
74165	88.33	70.11	81.67
754	88.33	71.39	77.33
75227	92.57	77.00	78.67
Ababuna*	92.6	80.63	76.67
Melko-CH2*	89.07	75.09	83.67
Gawe*	90.28	75.07	87
Bunowashi	90.34	72.78	75.67
Wush-wush	78.07	62.85	81.67
Yachi	86	68.83	80.33
Merdacheriko	50.79	45.45	80.33
Catimor J-19	43.53	41.23	67.67
Gesha****	97.23	84.42	60.67
Manasibu	84.17	66.88	90
Sende	88.5	70.31	98
Challa	89.77	71.56	78
Haru 1	91.49	76.15	90
Bultum	84	66.83	79.67
Harusa	76.8	61.23	100.67
Mocha	60.3	50.96	123.67
Mechara 1	33.23	34.93	130.67
Odicha	9.33	16.55	160
Fayate	0	0.00	0
Koti	83.1	70.03	86
Dessu	100	90.00	70.67
3/70***	15.13	22.79	141.67
Mean		62.26	90
CV		16.99	10.6
LSD _{0.05}		17.20	15.6

*Hybrid coffee varieties

* * Coffee Robusta

*** Resistant check

**** Susceptible check

Incubation periods indicate the number of days between inoculation and the first date of symptom appearance.

0 (zero) values indicate no incubation period, i.e.; there was no infection symptom until termination of the experiment.

Means followed with the same letter are not different according to Tukey test.

SUMMARY AND CONCLUSION

Lower disease CBD severity was observed on coffee variety 741, 754, and 75227 and 744. Moderate CBD infection was observed Coffee variety 7454 and 7440. Coffee variety 74165, 74148, 74140, 74110, 74112, 74158 and 7487 showed higher disease level ranging from mean 67.37 - 85.92 %. In General the current CBD seedling test reveals the presence of field, laboratory resistant and laboratory susceptible and field resistant. In Ethiopia no CBD outbreak was reported on the released coffee varieties under field condition . Concerning CWD fayate and odicha were found resistant & Merdacheriko, Catimor J-19, showed moderately resistant to CWD

under laboratory condition. Some coffee varieties fayate and Odicha had higher resistance to at least two of the diseases evidencing multiple resistances. These resistant cultivars can be utilized in CBD and CWD hot spot areas of southern Ethiopia (Sidama and Gedeo), coffee accession 370 which is highly susceptible to CBD is found resistant to CWD, and such type of accessions can be tested further in low altitude whether CBD pressure is low. Furthermore CWD resistant accessions can be used in breeding programmes and as a root stock for grafting. The released coffee variety Gesha becoming highly susceptible in low land areas of Southern West Ethiopia (Guraferda wereda) particularly where low in put less cultural control of the disease is practiced, small scale growers are advised to use Desu, Mioftu and 7440 by integrating strict phytosanitary measures. More research work on biochemical and morphological defense mechanism which contributes to CBD resistant and Host defence and CWD inheritance mechanism needs to be investigated.

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