Bioprocess Engineering
2025, Vol. 9, No. 2, pp. 58-69
https://doi.org/10.11648/j.be.20250902.11

Research Article

Reaction of Southern Coffee Germplasms to Coffee Berry Disease (*Colletotrichum kahawae* Waller and Bridge), in Southern Ethiopia

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Abstract

Coffee berry disease (CBD) still remains a limiting factor in the production of Arabica coffee in Ethiopia. This current study was carried out to evaluate *Coffea arabica* accessions from southern Ethiopia for their reaction to the disease under field and laboratory conditions. The evaluation of seventy six *C. arabica* accessions and four CBD resistant varieties as checks was conducted in 2021 on coffee plants already established in 2015. Disease average infection, percent severity index (PSI) and area under disease progress curve (AUDPC) were calculated. *C. arabica* accessions significantly differed in their resistance to CBD both at field and laboratory conditions. From seventy six accessions, fourteen were selected in visual assessment for attached berry test, detached berry and seedling hypocotyls tests. In attached berry test in the field, eight accessions showed low level (<30%) of CBD infection (relatively resistant to CBD), whereas six accessions were susceptible and showed higher levels of CBD infection. Five accessions were found to be resistant, whereas nine were susceptible under laboratory conditions. The present study demonstrated the role of host resistance in combating CBD in the study areas. The future research work should focus on evaluating the promising coffee accessions in multi-locations and multi-years field trials as well as further studies on the resistance mechanisms of these accessions to the CBD causal pathogen.

Keywords

Accession, AUDPC, PSI, Resistance, Susceptible

1. Introduction

Coffee (*Coffea* spp.) is a means of livelihood and has tremendous economic, political, social and spiritual impacts in many countries [1, 2]. Coffee is a very vital crop to the Ethiopian economy accounting for 4 to 5% of the gross domestic product, 10% of total government revenue, 10% of total agricultural production, 40% of total exports and 25 to 30% of total export earnings [3].

However, the average coffee productivity (0.71 t ha^{-1}) of Ethiopia is relatively lower than that of other coffee producing countries, such as Brazil (0.78 t ha^{-1}), Vietnam (1.31 t ha^{-1})

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Received: 17 January 2025; **Accepted:** 16 June 2025; **Published:** 7 July 2025

and Colombia (0.76 t ha^{-1}) [4]. This might be due to a number of factors, like biotic factors (e.g., plant diseases, insect pests and weeds), abiotic factors (e.g., recurrent drought and rainfall fluctuation, low soil fertility) [5, 6] and traditional coffee management practices (lack and slow adoption of improved coffee varieties and agronomic practices) [7, 8]. Plant diseases are the major constraints, of which coffee berry disease (CBD), coffee wilt disease (CWD) (tracheomycosis), and coffee leaf rust (CLR) are the most impactful diseases ravaging coffee crops and have limited their production to a fairly high extent of coffee production in the country [9].

Coffee berry disease (*Colletotrichum kahawae* Waller and Bridge) is one of the most limiting factors of Arabica coffee production in Ethiopia [10] and highly aggressive and specialized pathogen of coffee [11]. It is a typical anthracnose of green and ripe coffee berries which attacks all stages of the crop from flower to ripe fruits and occasionally leaves, but the maximum crop loss occurs following the infection from green pinhead berries to red cherries [12]. The occurrence and distribution of CBD in Ethiopia has been reported and documented at different times of cropping seasons [10]. For instance, previously, mean incidences of 38.8 and 17.2% were reported in Oromia and Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) [13], while recently Alemu *et al*. [10] reported mean incidences of 52.2 and 56.1% in Oromia and SNNPRS, respectively. Negash and Abate [14] previously reported a mean severity of 22 and 32.5%, while Alemu *et al*. [10] reported recently 33.2 and 52.7% in Sidama and Gedeo Zones, respectively, of SNNPRS.

Coffee berry disease can be managed by the use of resistant coffee varieties, spraying fungicides or by cultural practices [15] or by integration of the various disease management tactics. Plant breeding for resistance to CBD may provide a sustainable long term management of CBD [15, 16]. Promising sources of CBD resistance in coffee germplasm have been developed in different countries like Ethiopia, Kenya and Tanzania [17, 18]. The exhaustive testing of selected materials for resistance to CBD in the mother trees and their progenies in the laboratory and under field conditions/locations, where the epidemic is not only severe but also regularly present, is a very vital pivot for the development of resistant coffee varieties to CBD [19]. The nature of resistance to CBD is believed to be quantitative and highly influenced by environmental conditions [19, 20].

Apart from the release of forty two resistant coffee varieties in different years, efforts have been made to the improvement of the genetic base of resistance, but this has faced the problem of possible and frequent pathogen variation [21, 22]. Good understanding of the biology of CBD causing pathogen's genetic diversity could lead to the development of varieties with sufficient disease resistance [23]. The use of locally adapted and high yielding coffee cultivars (agro ecological based local landraces development) with high level of resistance to CBD is of paramount importance in Arabica coffee production in Ethiopia [23]. However, only four improved varieties have been developed recently for the agro ecologies of southern region of the coffee production areas in the period from 2006 and 2010. Therefore, to boost economic development program of the coffee growers in the region, such genetically very limited number of improved varieties is not sufficient as compared to the diverse agro ecological niches, enormous available coffee genetic resources and the high coffee production potential of the southern areas of Ethiopia [22]. Thus, the present study was carried out to evaluate coffee germplasms collected from southern Ethiopia for their reaction to CBD.

2. Materials and Methods

2.1. Description of the Study Areas

A field experiment was conducted at Wonago Agricultural Research Sub-station (WARSs) in 2021 cropping season to evaluate reaction of *C. arabica* germplasms to CBD (*Colletotrichum kahawae*) by superimposing on already established coffee accessions in August 2015 cropping season. The sub-station is located at 6.56 ° N latitude, and 38.46 ° E, longitude and at an altitude of 1850 m. a. s. l. and classified into the highland of the coffee growing agro ecologies of the country [24]. The area is known to be suitable (hot spot) for CBD development [25]. However, the laboratory and greenhouse experiments were conducted at Jimma Agricultural Research Center (JARC).

2.2. Source and Preparation of Inoculum

The most aggressive isolate previously reported from Yirgacheffe district (around Fisagenet) was used for the screening purpose [26]. A conidial suspension of *C. kahawae* isolate was prepared from 10 days old culture [19]. The isolate cultured on potato dextrose agar (PDA) plate was washed by flooding with 10 mL sterile distilled water (SDW), rubbed with sterilized scalpel and transferred to 50 mL sterilized beaker, thoroughly stirred for 15 minutes with magnetic stirrer and then filtered through double layers of sterile cheesecloth. The spore concentration was prepared using haemocytometer to standard suspensions (2×10^6 conidia mL^{-1}) for the inoculations purposes in attached berry test (ABT) (in Field), detached berry test (DBT) and hypocotyl tests.

2.3. Materials, Treatments and Experimental Design

Coffee germplasms, i.e. accessions used as treatments, collected from southern Ethiopia and that were planted at Wonago, were evaluated for their reaction to CBD. The treatments consisted of seventy-six *C. arabica* accessions and four CBD resistant varieties as checks for screening of *C. arabica* accessions in the field trial (Table 1). The treatments

were arranged in an augmented design with four blocks each with nineteen accessions and four checks and replicated four times. Similarly, each accession within each block consisted of six coffee trees having 2 x 2 m spacing between rows and plants. All agronomic management practices were applied according to standard procedures.

The promising accessions that showed lower (<5% threshold level) CBD infection according to Yonas *et al*. [27] in visual scoring were used for the next step screening (ABT, DBT and hypocotyl inoculation test) for resistance to CBD. The selected accessions (treatments) were arranged in randomized complete block design with three replications (individual trees of the accession were used as replications) for field inoculation on ABT as employed by Zeru [15] and randomized complete design with three replications for DBT and hypocotyl inoculation test.

2.4. Evaluation Procedures for Resistance to CBD

2.4.1. Resistance Test Under Natural Field Conditions

Visual disease assessment test: The visual assessment was conducted on seventy six accessions and four checks at WARSs. For early discrimination of the susceptible accessions, the overall disease pressure (%CBD infection) was assessed on each individual accession (three trees per accession) by counting the number of diseased berries over total assessed berries multiplied by hundred [19].

Attached berry test: The ABT was performed by applying selected *C. kahawae* inoculum on the branches of growing green coffee berries following methods and procedures used by Van der Graaff [19] at WARSs. Inoculation was done by random sampling of three trees per plot and then three strata per tree, followed by one branch per stratum (from top, middle and bottom layers), total of nine branches per plot. The marked strata at the expanding stage of berries (14 weeks after flowering) were sprayed with (≈25 µL per berry) *C. kahawae* spore suspension using hand sprayer [19]. Immediately after inoculation, branches were covered with paper bags to maintain humidity and favor disease development. The bags were removed 24 hrs. after inoculation. Disease index data were scored 3 weeks after inoculation and computed as the ratio of diseased to total berries multiplied by hundred.

2.4.2. Resistance Test Under Laboratory Conditions

Detached berry test: The detached berry test was conducted in the laboratory following the procedure employed by Van der Graaff [19]. The expanding green coffee berries that were 14 weeks old after flowering [28], including the well-known reference varieties (the resistant variety 741 and susceptible 370) were collected during the mid-July in 2021 cropping season. The berries were picked randomly from bottom, middle and top of the coffee tree to have a representative sample. The berries were surface sterilized with sodium hypochlorite (NaOCl 0.05% W/V) solutions for 2 minutes and rinsed 3 times with SDW for 1 minute each and dried using sterile cotton cloth. A total of 12 berries per accession were placed in 2 rows in each box.

A drop, 25 µL, of conidial suspension was deposited on the berries using a sterilized pipette, while shaking from time to time when drawing the inoculum [28]. The inoculated green coffee berries were incubated at 25 ℃ for fourteen days and were observed for CBD symptom development. Regular opening after every three days was carried out for ten minutes to allow for aeration of the berries. Data collection was carried out at four time points starting from 4th day post inoculation when the 1st visible symptoms appeared [28].

Then, CBD severity was estimated using a disease assessment key 0 to 6 disease score scale via critical observation of the lesion size and its extent on the diseased berry parts using the field guide [29], where, 0= Healthy green berries without symptoms; 1= Black sunken lesions cover < 2% of the green berries surface; 2= Black sunken lesions cover 2-5% of the green berries surface, approximately 3 mm in diameter; 3= Black sunken lesions cover 6-10% of the green berries surface, approximately 5 mm in diameter; 4= Black sunken lesions cover 11-50% of the green berries surface, approximately 7 mm in diameter; 5= Black sunken lesions cover 51-99% of the green berries surface, approximately 15 mm in diameter; and 6= >99% or the whole surface of green berries covered with black sunken lesions, mummified berries. The scores were changed into Percentage Severity Index (PSI) for the analysis using the formula of Wheeler [30]:

$$PSI = \frac{\text{Sum of numerical rating x 100}}{\text{Total number of rated plant x maximum score of the scale}}$$

A score range of ≤2 was considered resistant, while that of ≥3 was regarded to be susceptible. After scoring each coffee berry individually, average infections (AI) on each accession across the replicates were calculated as follows:

$AI = \Sigma\,[Ir1 + Ir2 + Ir3 + \cdots Irn]/N$, where, I is the sum of disease scores; n is the number of replications; Irn is the sum of disease score in replication n; N is the total number of berries scored in the replications.

Area under disease progress curve (AUDPC) was also calculated from PSI for each treatment using the formula:

$$AUDPC = \sum_{i=1}^{n-1}(0.5(xi + xi + 1)\ (ti + 1 - ti))$$

Where n is total number of assessment times, t_i is time of the ith assessment in days from the first assessment date, x_i is percentage of disease severity at ith assessment. AUDPC is expressed in per cent-days because severity (x) was expressed in per cent and time (t) in days [31].

Seedling hypocotyl inoculation tests: Coffee seedlings were raised in growth room from freshly picked seeds of all treatments and two well-known reference varieties (i.e. re-

sistant 741 and susceptible 370). Ripened cherries were picked from mother trees in the field and dried under shade after removing the pulp by hand. After removing the parchment, the seeds were soaked in SDW and kept for 48 hrs. Thereafter, seeds were sown (30 to 40 seeds per box) in heat sterilized and moistened sandy soil in disinfected plastic boxes each with 2295 cm^3 capacity arranged on benches and covered with chip wood in growth room.

Two days before inoculating the hypocotyls at unfolding stage (i.e. 2 weeks after sowing), the temperature was adjusted to 20 ^oC and seedlings were sprayed with SDW and covered with plastic sheet for 48 hrs. to obtain 100% relative humidity. Then, the coffee hypocotyls were inoculated with conidial suspension by stem brushing procedure with fine camel hairbrush as described by Van der Graaff [19]. The second re-inoculation was conducted 48 hrs. after the first inoculation and following the same procedures. All the treated hypocotyls were immediately covered with transplant plastic sheet to create humid condition for infection and maintained in cool place with a mean temperature of 20 ±2 ℃ in a growth room.

The reaction of each hypocotyl was assessed at 7, 14 and 21 days after inoculation using the 0-4 disease scale symptom classifications and assessment key based on measured percentage of affected area employed by Van der Graaff [19], where: 0 = no symptom; 1 = from very tiny to 1 or 2 narrow brown lesion up to 0.5 mm wide; 2 = more than 2 brown lesions or brown coalescing lesions exceeding 0.5 mm, black dots if present are rare; 3 = wide brown lesions with numerous black dots and/or black lesion may completely surrounded the stem but the top remain alive and 4 = black lesion girdling the stem and top killed.

A disease index reaction for each accession was described as a percentage of the maximum possible infection using the following formula [19]:

$$DIR = \frac{(w+2x+3y+4z) \times 100}{4(v+w+x+y+z)}$$

Where, v = number of hypocotyls in class 0; w = number of hypocotyls in class 1; x = number of hypocotyls in class 2; y = number of hypocotyls in class 3 and z = number of hypocotyls in class 4. Resistance categories were classified as: resistant (DIR 0 – 25), moderately resistant (DIR 26 – 50), moderately susceptible (DIR 51 – 75), and susceptible (DIR 76 – 100). The area under disease progress curve was also computed from DIR for each treatment using the formula described under DBT.

2.5. Data Analysis

The disease data were subjected to analysis of variance using Statistical Analysis System (SAS) Version 9.4 software package [32]. Treatment means for a mean comparison was made using the Duncan's Multiple Range Test (DMRT) at 5% probability level.

3. Results and Discussion

3.1. Resistance Test Under Natural Field Conditions

Visual assessment test: Visual estimation in%CBD infection was assessed and significant variation was observed among the coffee accessions at Wonago (hot spot for CBD infection) (Table 1). The mean of%CBD infection was 23.5% and mean ranged from 0.8 to 67.9%. In this study, most (62 or 81.6%) of the accessions were susceptible that showed higher (above the tolerable threshold level of 5%) of CBD infection (Table 1). These accessions may not be used for the production purpose at higher elevation areas due to their susceptibility to CBD and need to be studied further at lower and medium elevation areas over location and time. A related result was reported by Yonas *et al*. [27] that the genotypes exhibited higher or above tolerable threshold level of 5% CBD infection may not be used for production purpose at higher elevation areas like Gera (hotspot) due to higher CBD infection and deteriorates quality; hence considered to be susceptible. On the other hand, 14 (18.4%) coffee accessions showed lower levels (below the threshold level of 5%) of CBD infection, which indicated higher CBD resistance at this hot spot area (Tables 1 and 2). These better performed accessions need to be confirmed further for resistance to CBD using artificial inoculation methods (ABT, DBT and seedling hypocotyl inoculation test) for future use in breeding and pathology programs or to recommend a reliable source of CBD resistance (Table 2).

Table 1. *The mean CBD severity on C. arabica accessions/varieties evaluated under field conditions without inoculation in southern Ethiopia in 2021 cropping season.*

Accession	% CBD	Accession	% CBD	Accession	% CBD	Accession	% CBD
69/661	26.72	11/74	34.78	74112*	0.67	9647	41.53
23/94	4.72	944	28.47	77/94	31.75	9306	42.72
7/94	40.94	19/83	25.95	125/05	2.50	17/06	3.89
3/94	32.78	85220	36.72	67/94	7.72	9654	47.31

Accession	% CBD	Accession	% CBD	Accession	% CBD	Accession	% CBD
963	23.20	971*	2.17	65/83	16.47	7440*	0.58
85257*	0.16	26/96	42.53	7440*	0.33	9636	30.17
852117	32.08	965	45.68	85292	64.86	11/95	16.55
85178	57.81	9613	8.60	85254	39.31	964	4.91
16/77	42.22	85176	2.91	22/83	2.22	21/94	3.10
45/94	32.75	9664	38.89	23/83	22.54	28/95	6.67
9643	29.18	85257*	0.93	38/83	23.33	74112*	0.42
971*	0.75	70/94	50.11	27/83	23.17	25/295	38.83
21/70	22.44	85282	31.43	85174	53.20	14/74	4.96
45/94	32.75	14/83	8.33	31/95	67.92	85284	49.17
9651	33.19	9646	4.44	85214	54.44	9629	33.33
9657	6.74	9663	11.33	85278	48.17	85171	55.30
9620	9.79	7440*	0.70	85170	41.03	85257*	2.15
7440*	0.26	95286	33.25	971*	0.17	33/94	25.87
9659	1.73	9635	3.11	13/94	44.58	26/94	2.67
85186	2.23	954	0.83	397/72	23.33	16/95	26.27
72/94	35.19	74112*	0.67	9653	65.28	2/94	32.86
966	35.14	9635	12.78	9652	55.47	9649	44.94
74112*	0.33	85211	48.99	85257*	1.23	971*	3.83
Mean	23.74						
Stdev	19.64						

'*'Resistant varieties.

Table 2. *Test results on C. arabica accessions/varieties used for their reactions to CBD under field and laboratory conditions.*

Accession name	PSI	Accession name	PSI
23/94	4.72	22/83	2.22
9659	1.73	85257*	4.47
85/186	2.23	17/06	3.89
85/176	2.91	964	4.91
9646	4.44	21/94	3.10
9635	3.11	14/74	4.96
954	0.83	26/94	2.67
74112*	2.09	971*	1.81
125/05	2.50	7440*	1.88

<5% CBD severity, '*'Resistant varieties.

Attached berry test: The results of ABT test revealed that there was a significant ($p<0.01$) variation in%CBD infection

among tested accessions (Table 3). Accordingly, the highest (57.3%)%CBD infection was recorded from accession 964, which was statistically at par with accession 125/05 (56.6%), while the lowest (4.6%) was from 85/176 and statistically similar with accession 9659 (6.0%). The mean severity ranged from 4.6 to 57.3% and, out of the tested accessions, eight showed low (<30%) level of infection, statistically similar with the known reference varieties 74112 (15.4%), 971 (21.5%), 85257 (18.7%) and 7440 (23.0%) (Table 3). The result in this study clearly indicated the presence of certain accessions that have been better or comparable with CBD resistant to the reference varieties. These known reference varieties were highly resistant to CBD under field conditions and better performed in highland areas (hot spot areas), like Wonago and Yirgacheffe) of southern Ethiopia [33]. This current finding is in agreement with the investigation by Van der Graaff [19], who reported that ABT was used to estimate the difference in natural infestation and further to verify the resistance level of coffee accessions with artificial inoculation under natural field conditions in that it gives a satisfactory indication of susceptibility under the field conditions. Similarly, Zeru [15] and Jefuka *et al*. [34] reported that the genotypes showed low (<30%) level of CBD infection in ABT could appear as resistant to CBD under field conditions.

Table 3. *Per cent infection of attached berries inoculated with C. kahawae at Wonago, in southern Ethiopia in 2021 cropping season.*

Accession name	PSI	Accession name	PSI
23/94	28.90^{cdef}	22/83	10.67^{hij}
9659	5.96^{ij}	85257*	18.65^{fghij}
85186	44.93^{ab}	17/06	39.00^{bcd}
85176	4.59^{j}	964	57.27^{a}
9646	41.44^{bc}	21/94	24.53^{defgh}
9635	16.13^{fghij}	14/74	37.66^{bcde}
954	12.65^{ghij}	26/94	27.50^{cdefg}
74112*	15.35^{fghij}	971*	21.48^{fghi}
12/505	56.64^{a}	7440*	23.04^{efgh}
Mean	-	27.02	-
CV (%)	-	30.71	-

Means followed with the same letters are not significantly different from each other (DMRT; at p<0.05). ''Reference varieties.

3.2. Resistance Test Under Laboratory Conditions

Detached berry test: The analysis of variance indicated that the effect of accessions was a significant (p<0.01) in PSI, AI and AUDPC in DBT (Table 4). Accordingly, the highest (99.1%) PSI of CBD was recorded from coffee accession 125/05, which was at par with accession 85/186 (96.8%). The resistant reference variety 741 showed the lowest (12.2%) PSI of CBD which was statistically similar with accessions 9659 (21.1%). While, accessions from 125/05 and 85/186 showed higher CBD PSI than the well-known susceptible reference variety 370 in magnitude, while accession 96/4 showed statistically similar result (Table 4). On the other hand, lower CBD infection percentage was recorded from accessions 9659, 85176, 954, 9635 and 22/83, which showed lower CBD PSI than susceptible variety 370 (Table 4). The mean PSI CBD ranged between 21.1 and 99.1% and out of the tested accessions, five showed low (21.1 to 57.7%) level of CBD infection and AI (≤2 in the resistant class), while nine showed higher (67.3 to 99.1%) level of infection and AI (≥3 in susceptible class) (Table 4). A comparable report was presented by Pinard *et al*. [28] where the lower of%CBD infection (in resistant class) was recorded with mean score of AI ≤2, while the higher was (in susceptible class) ≥3 to CBD in DBT. Detached berry test technique is the possible means of relative ranking of cultivar resistance starting from early time, which is still useful for a differential interaction analysis and varietal characterization [28].

In addition, the highest (608.3%) AUDPC was observed in coffee accession 85/186, which was statistically at par with accession 125/05 (603.5%), while the lowest (91.2%) was in accession 9659 and statistically similar with 85/176 (93.8%) (Table 4). Overall, higher AUDPC was observed in accessions 85/186, 125/05, 964, 14/74, 21/94, 26/94, 96/46, 17/06 and 23/94 whereas the lower was in accessions 9659, 85/176, 954, 22/83 and 9635 (Table 4).

Table 4. *Responses of C. arabica accessions/varieties to C. kahawae in DBT.*

Accession name	PSI	AI	Reaction category	AUDPC
23/94	67.33^{ef}	3.91^{cd}	S	370.22^{f}
9659	21.11^{hi}	1.17^{f}	R	91.23^{i}
85186	96.76^{a}	5.81^{ab}	S	608.33^{a}
85176	22.62^{hi}	1.17^{f}	R	93.79^{i}
9646	78.61^{bcd}	3.93^{cd}	S	408.76^{ef}
9635	55.56^{g}	2.08^{e}	R	275.67^{g}
954	26.47^{h}	1.97^{e}	R	150.09^{h}
125/05	99.07^{a}	5.95^{a}	S	603.47^{a}
22/83	57.66^{fg}	2.08^{e}	R	241.42^{g}
17/06	76.86^{cde}	4.17^{cd}	S	387.83^{ef}
964	88.95^{ab}	5.35^{b}	S	526.79^{b}
21/94	69.21^{de}	3.67^{d}	S	430.98^{de}
14/74	81.96^{bc}	4.48^{c}	S	466.91^{cd}
26/94	78.37^{bcd}	5.32^{b}	S	430de
741	12.22^{i}	0.14^{g}	R	45.87^{j}
370	88.72^{ab}	5.72^{ab}	S	482.53^{c}
Mean	63.84	3.56	-	350.87
CV (%)	9.50	9.06	-	7.26

*Mean values followed with the same letter(s) are not significantly ($p<0.05$) different from each other according to DMRT. AI= average infection, R= resistant and S= susceptible at mean score range (AI) ≤2 CBD infection (in resistant class) and ≥3 CBD infection (in susceptible class).

Seedling Hypocotyl Inoculation Test: The research results revealed that there was a significant ($p<0.01$) variation among accessions in their%CBD infection in hypocotyl inoculation test (Table 5). Accordingly, the highest (100%) mean%CBD infection was recorded from coffee type 370, which was statistically at par with accessions 125/05 (98.1%) and 85186 (96.6%); whereas the lowest (9.4%) was recorded from the resistant variety 741 followed by accessions 85176 (16.9%) and 9659 (17.2%) (Table 5).

Moreover, the percentage of coffee hypocotyl laboratory tests per accession resistant or susceptible to CBD reaction differed significantly (Table 5). Accordingly, coffee accessions 85176, 9659 and 954 had resistant reactions to CBD, while 9635 and 22/83 were moderately resistant. Likewise, coffee accessions 21/94, 23/94 and 26/94 showed moderately susceptible, while accessions 125/05, 85186, 964, 9646, 14/74 and 17/06 exhibited susceptible reaction to CBD (Table 5). The results of this current study also revealed highly significant ($p<0.01$) differences in AUDPC values among the tested coffee accessions (Table 5). The highest (1284.3% days) AUDPC value was calculated for the susceptible coffee cultivar 370, which was statistically at par with accession 125/05 (1239.6% days), while the lowest (95.1% days) was computed for the reference or control resistant cultivar 741 (Table 5). Besides, accessions 9659 (172.8% days) and 85176 (181.8% days) also showed the lowest AUDPC value with no significant difference with each other (Table 5). The results of the present study in%CBD infection are in line with previous findings [15] who reported that four coffee selections from Harena (HA21, 23.3%) and HB29, 27.3%), Bonga (BA25, 69.0%), and Yayu (YB, 57.5%) forests showed very low level of CBD infection as compared to the susceptible check (coffee type 370). Similarly, Demelash and Kifle [25] reported very low level of CBD infection on the cultivars 741 (2.9%), 754 (7.3%), 75227 (8.7%) and 744 (22.4%).

Table 5. *Responses of C. arabica accessions/varieties to C. kahawae in hypocotyl laboratory test at JARC in 2021 cropping season.*

Accession name	DIR (%)	Reaction category	AUDPC
23/94	56.43^{e}	MS	695.97^{f}
9659	17.17^{h}	R	172.84^{j}
85186	96.63^{ab}	S	1176.25^{b}
85176	16.94^{h}	R	181.81^{j}
9646	82.50^{c}	S	1035.42^{c}
9635	35.83^{f}	MR	371.39^{h}
954	22.78^{g}	R	246.94^{i}
125/05	98.06^{a}	S	1239.58^{a}
22/83	36.67^{f}	MR	434.58^{g}
17/06	77.50^{c}	S	980.97^{d}
964	91.76^{b}	S	1178.68^{b}
21/94	55.28^{e}	MS	671.81^{f}
14/74	78.89^{c}	S	1001.39^{cd}
26/94	65.56^{d}	MS	800.14^{e}
741	9.38^{i}	R	95.07^{k}
370	100^{a}	S	1284.31^{a}
Mean	58.84	-	722.95
CV (%)	5.33	-	4.04

*Mean values followed with the same letter(s) are not significantly ($p<0.05$) different from each other according to DMRT. DIR= Disease index reaction, R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= Susceptible; Resistance category: Resistant (DIR 0 – 25); Moderately resistant (DIR 26 – 50); Moderately susceptible (DIR 51 – 75); and Susceptible (DIR 76 – 100).

The present study clearly showed that considerable variations among coffee accessions in reaction to CBD were detected from the results of visual assessment, ABT, DBT and seedling inoculation test. The probable reason for the variation in CBD infection among these accessions might be the effect of genetic make-up of the accessions. This current research finding is in agreement with the investigation by Van der Vossen *et al*. [23], who explained that the variation in resistance to CBD and host pathogen reaction within varieties can be due to natural out cross in pollination with susceptible varieties in neighbouring plots since even though *C. arabica* is a self-fertile crop; there is chance outcross of up to 10%. Similarly, Van der Graaff [19] reported that the resistance to CBD is quantitatively expressed and variation in levels of disease is continuous among coffee types in the field, field inoculation tests, detached berry, and seedling inoculation tests.

The other probable reasons could be attributed to the variations in these coffee accessions defence mechanisms (structural and chemical) as soon as challenged by the *C. kahawae* pathogen. Interestingly, Hoglund *et al*. [35] and Singh and Upadhyay, [36] explained that fungal growth can be restricted with a series of hypersensitive reaction in the resistant coffee genotypes that leads to a localized plant cell death in response to invasion by a pathogen and is characterized by a rapid loss of membrane integrity in the infected host cells. According to Gichuru [37], the coffee berry cuticle and wax layer could act as a physical barrier to the penetrating pathogen. Likewise, Lampard and Carter [38] and Chen *et al*. [39] reported the presence of antifungal compounds in the cuticular wax layers of green berries of coffee counteracting infection of coffee by *C. kahawae* strains. Such antifungal compounds may exist in these coffee accessions that contributed to CBD resistance. Thus, more studies are needed to confirm the role of antifungal compounds for these resistant coffee accessions to CBD.

Moreover, Silva *et al*. [40] and Van der Vossen and Walyaro [41] explained that the occurrence of host resistance appears to be largely based on the rapid formation of a cork barrier in the pericarp of the developing fruit distal from the initial infection site (initial attachment). Resistant varieties are inherently able to do this but susceptible ones are not, unless assisted by the environment [42]. The brown crust formed on the berry surface could restrict further infection (eliminate

bio-trophic associations with pathogen) and leads to starvation of the pathogen (block nutrient transfer to the infected area) [37, 43]. Masaba and van der Vossen [42] reported that scab formation on the green coffee berry surface is due to cork barrier formation and limits fungal hyphal growth inside the plant tissue. These cork barriers corresponded macroscopically to the scab lesion [37]. Such a resistance mechanism is likely to be stable (race-nonspecific) and depends apparently on actively metabolizing plant tissues [42, 44]. The scab lesions are common expression of CBD resistance (resistant host response) at distinct stages of pathogenesis that is more common on the coffee cultivars having resistance nature [37, 44]. These resistance mechanisms may exist in these resistant coffee accessions and absent in susceptible accessions which may needs further studies in the future research works.

In this current study, the variations of these accessions in reaction to CBD could be an opportunity for the next better resistant varietal development through breeding future line over locations and time since resistance in perennial crops like coffee is observed and screened during the late stage of development [23].

3.3. Relationship Between CBD Intensity Under Field and Laboratory Conditions

The correlation analysis revealed similar trends among the three sets of tests in the level of CBD infection. A positive highly significant ($p<0.01$ correlation) of disease severity was observed between ABT and DBT ($r = 0.76$), ABT and hypocotyl test ($r= 0.80$) and DBT and hypocotyl test ($r= 0.94$) (Table 6). The results in the current tests are comparable to the ones that were reported by Van der Vossen *et al*. [45] who found a good correlation between the seedling inoculation tests (hypocotyl laboratory test) and field inoculation, and detached berry test and field inoculation test in resistance test to CBD [19]. The authors also suggested that the combination of one year's field observation after selection, field inoculation test, and seedling inoculation test is very effective to eliminate unsatisfactory mother trees.

The result revealed that coffee accessions that were susceptible in the laboratory were susceptible under field conditions (ABT) too, whereas resistant reactions in the laboratory also became resistant under field conditions. However, some accessions were susceptible in the laboratory, but were resistant under field conditions. Accordingly, five accessions were laboratory and field resistant, while other three accessions were susceptible under laboratory conditions and but were resistant under field conditions. This finding is in agreement with the observation by Teferi [46], who reported that coffee varieties 741, 754, 75227 and 744 are laboratory and field resistant, whereas coffee varieties 74165, 74148, 74140, 74110, 74112, 74158 and 7487 are laboratory susceptible and field resistant. According to Bellachew [20], the genotypes susceptible under field conditions could be susceptible under controlled conditions too if there is no change in pathogen strains. Interestingly, Van der Vossen *et al*. [45] noted that the mechanism of CBD resistance in the field conditions is similar to the reaction of *C. kahawae* inoculum in the hypocotyl of six-week old seedlings.

Thus, in the present study the three resistance tests (ABT, DBT and hypocotyl laboratory test) after selections of accessions from one year's field observation to CBD were done to recommend a reliable source of CBD resistance accessions. This implies that these promising accessions are reliable sources of CBD resistance, and can provide the base line or foundation for breeding programs in the future works.

Table 6. *Person correlation analysis between methods of resistance tests in infection%CBD.*

Variable	PIDBT	PIHT	PIABT
PIDBT	1.00	0.94**	0.76**
PIHT	-	1.00	0.80**
PIABT	-	-	1.00

PIDBT= Per cent infection in detached berry test; PIHT= Per cent infection in seedling hypocotyl tests in the laboratory; PIABT= Per cent infection in attached berry test. '**'indicated, highly significant at $p<0.01$.

4. Conclusions

Generally, *Coffea arabica* L. is a very vital crop to the Ethiopian economy. However, the economic production of the crop in the country is greatly hampered by coffee berry disease (CBD). Plant breeding for resistance to CBD may provide a sustainable long term management of CBD. In this current study, the identification of resistance in *C. arabica* accessions through different methods of evaluation, i.e. field observations, ABT, DBT and seedling hypocotyl test indicated the potential use of these accessions in *C. arabica* breeding programs for CBD resistance. Those *C. arabica* accessions that showed low level of infection in CBD resistance evaluation test under field and laboratory conditions offer opportunities for further breeding work and could be the best options for management of CBD in different southern agro ecologies of coffee growing areas. For full recommendation, additional research works should be done on testing these promising accessions across multi-locations over multi-years and genetic resistance mechanisms identification with up to advanced molecular methods.

Abbreviations

ABT	Attached Berry Test
AI	Average Infection
AUDPC	Area Under Disease Progress Curve

CBD	Coffee Berry Disease
CLR	Coffee Leaf Rust
CWD	Coffee Wilt Disease
DBT	Detached Berry Test
DIR	Disease Index Reaction
DMRT	Duncan's Multiple Range Test
JARC	Jimma Agricultural Research Center
m a. s. l.	Meters Above Sea Level
PDA	Potato Dextrose Agar
PSI	Percentage Severity Index
SAS	Statistical Analysis System
SDW	Sterilized Distilled Water
SNNPRS	Southern Nations, Nationalities and Peoples' Regional State
WARSs	Wonago Agricultural Research Sub-station

Acknowledgments

The authors are extremely grateful to the Ethiopian Institute of Agricultural Research (EIAR) for funding this research study. In addition, the authors would like to thank Dr. Mohammed Yusuf the EIAR, Plant Protection Directorate and Mr Muluken Philipos the EIAR, Wondo genet Agricultural Research Center Directorate for their vehement collaboration and encouragement throughout the whole research work. The acknowledgment also goes to all staff members of plant pathology at Wondo genet (Awada sub-center) and Jimma Agricultural Research Centers, EIAR, Ethiopia, who contributed directly or indirectly to this study.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Chauhan R, Hooda MS, Tanga AA. Coffee: the backbone of Ethiopian economy. International journal of economic plants. 2015; 1(2): 82-6.

[2] Pham Y, Reardon-Smith K, Mushtaq S, Cockfield G. The impact of climate change and variability on coffee production: a systematic review. Climatic Change. 2019 Oct; 156: 609-30. https://doi.org/10.1007/s10584-019 02538-y

[3] Adem MW. *Quality control, quality determinants and indication of geographic origin of Ethiopian coffee* (Doctoral dissertation, Ghent University).

[4] Pawlak K, Kołodziejczak M. The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production. Sustainability. 2020 Jul 7; 12(13): 5488. https://doi.org/10.3390/su12135488

[5] Tolera FG, Gebermedin GA. Opportunities and constraints of coffee production in West Hararghe, Ethiopia. Journal of Agricultural Economics and Rural Development. 2015; 2(4): 054-9.

[6] Degaga J, Melka T, Angasu B, Alemu G, Zewdu A, Amin M. Constraints and Opportunities of Coffee Production in Arsi Zone: The Case of Chole and Gololcha Districts. Constraints. 2017; 9(10).

[7] Amarasinghe UA, Hoanh CT, D'haeze D, Hung TQ. Toward sustainable coffee production in Vietnam: More coffee with less water. Agricultural Systems. 2015 Jun 1; 136: 96-105. https://doi.org/10.1016/j.agsy.2015.02.008

[8] Jezeer RE, Santos MJ, Boot RG, Junginger M, Verweij PA. Effects of shade and input management on economic performance of small-scale Peruvian coffee systems. Agricultural systems. 2018 May 1; 162: 179-90. https://doi.org/10.1016/j.agsy.2018.01.014

[9] Bellachew K, Teferi D, Gidisa G. Screening of some coffee arabica genotypes against coffee wilt diseases (Gibberella xylarioides heim and saccus) at jimma, southwest Ethiopia. International Journal of Sustainable Agricultural Research. 2015; 2(3): 66-76. https://doi.org/10.18488/journal.70/2015.2.3/70.3.66.76

[10] Alemu K, Adugna G, Lemessa F, Muleta D. Current status of coffee berry disease (Colletotrichum kahawae Waller & Bridge) in Ethiopia. Archives of Phytopathology and Plant Protection. 2016 Nov 7; 49(17-18): 421-33. https://doi.org/10.1080/03235408.2016.1228736

[11] Vieira A, Diniz I, Loureiro A, Pereira AP, Silva MC, Várzea V, Batista D. Aggressiveness profiling of the coffee pathogen Colletotrichum kahawae. Plant pathology. 2019 Feb; 68(2): 358-68. https://doi.org/10.1111/ppa.12950

[12] Garedew W, Lemessa F, Pinard F. Assessment of berry drop due to coffee berry disease and non-CBD factors in Arabica coffee under farmers fields of Southwestern Ethiopia. Crop protection. 2017 Aug 1; 98: 276-82. https://doi.org/10.1016/j.cropro.2017.04.012

[13] Zeru A, Assefa F, Adugna G, Hindorf H. Occurrence of fungal diseases of Coffea arabica L. in montane rainforests of Ethiopia. Journal of applied Botany and food quality. 2012 Nov 29; 82(2): 148-51. https://doi.org/10.5073/JABFQ.2008.082.024

[14] Negash T, Abate S. The status of coffee berry disease in the southern nation's nationalities and peoples region (SNNPR).

[15] Zeru A. *Diversity of Arabica coffee populations in afromontane rainforests of Ethiopia in relation to Colletotrichum kahawae and Gibberella xylarioides* (Doctoral dissertation, Addis Ababa University).

[16] Alemu K, Adugna G, Lemessa F, Muleta D. Induction of systemic resistance in Arabica coffee (Coffea arabica L.) against coffee berry disease (Colletotrichum kahawae Waller & Bridge) mediated through plant defense activator. International Journal of Pest Management. 2019 Oct 2; 65(4): 313-23. https://doi.org/10.1080/09670874.2018.1506190

[17] Mtenga DJ, Reuben SO. Variation in resistance to coffee berry disease (Colletotrichum kahawae) among germplasm progenitors at Tanzania coffee research institute (TaCRI). International Journal of Agricultural Sciences. 2012 Jun 1; 2(6): iv+-198.

[18] Derso E, Adugna G. Teame G/Ezgi (2000). Control of CBD by fungicides in Ethiopia. Inproceedings of the workshop on control of coffee berry Disease (CBD) in Ethiopia 1999 Aug 13 (pp. 35-46).

[19] Van der Graaff NA. Selection of arabica coffee types resistant to coffee berry disease in Ethiopia. Wageningen University and Research; 1981.

[20] Bellachew B. *ARABIC A COFFEE BREEDING FOR YIELD AND RESISTANCE TO COFFEE BERRY DISEASE (Colletotrichum kahawae sp. nov.)* (Doctoral dissertation, University of London).

[21] Benti T. Progress in Arabica coffee breeding in Ethiopia: achievements, challenges and prospects. Int J Sci Basic Appl Res. 2017; 33(2): 15-25.

[22] Demelash T, Kifle B. A review of coffee diseases research in Ethiopia. International Journal of Agriculture and Biosciences. 2018; 7(2): 65-70.

[23] Van der Vossen H, Bertrand B, Charrier A. Next generation variety development for sustainable production of arabica coffee (Coffea arabica L.): a review. Euphytica. 2015 Jul; 204(2): 243-56. https://doi.org/10.1007/s10681-015-1398-z

[24] Degaga J. Review on coffee production and marketing in Ethiopia. Journal of Marketing and Consumer Research. 2020 Apr 30; 67: 7-15. https://doi.org/10.7176/JMCR/67-02

[25] Demelash T, Kifle B. 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. Journal of Biology, Agriculture and Healthcare. 2015; 5(15): 81-6.

[26] Derso E, Waller JM. Variation among Colletotrichum isolates from diseased coffee berries in Ethiopia. Crop protection. 2003 Apr 1; 22(3): 561-5.

[27] Yonas B, Bayetta B, Chemeda F. Assessments of resistances of indigenous Arabica coffee genotypes for multiple diseases. Greener journal of plant breeding and crop science. 2014; 2(3): 054-61.

[28] Pinard F, Omondi CO, Cilas C. Detached berries inoculation for characterization of coffee resistance to coffee berry disease. Journal of Plant Pathology. 2012 Nov 1: 517-23.

[29] Kamau AP. *Characterization of coffee genotypes derived from crossing Rume Sudan and SL 28 coffee varieties against coffee berry disease (CBD) causal pathogen (Colletotrichum kahawae)* (Doctoral dissertation).

[30] Wheeler BE. An introduction to plant diseases. An introduction to plant diseases. 1969.

[31] Campbell CL, Madden LV. Introduction to plant disease epidemiology. 1990.

[32] Soloviev AG, Solovjeva TM, Ivankov OI, Soloviov DV, Rogachev AV, Kuklin AI. SAS program for two-detector system: Seamless curve from both detectors. InJournal of Physics: Conference Series 2017 May 1 (Vol. 848, No. 1, p. 012020). IOP Publishing.

[33] Gessese MK. Decade of South Ethiopian Coffee Improvement Activities at Awada Coffee Research Center. Adv Crop Sci Tech. 2019; 7(420): 2. https://doi.org/10.4172/2329-8863.1000420

[34] Jefuka C, Adugna G, Teferi D, Zeru A, Bogale S, Adem A. Development and release of coffee berry disease resistant varieties to specialty coffee producing regions in Ethiopia. Inthe Proceedings of the 24th International Conference on Coffee Science San José (Costa Rica), 12th-16th November 2012.

[35] Höglund S, Larsson S, Wingsle G. Both hypersensitive and non-hypersensitive responses are associated with resistance in Salix viminalis against the gall midge Dasineura marginemtorquens. Journal of Experimental Botany. 2005 Dec 1; 56(422): 3215-22. https://doi.org/10.1093/jxb/eri318

[36] Singh VK, Upadhyay RS. The hypersensitive response: A case of cell death induction in plants. Int. J. Eng. Res. Technol. 2013; 2: 1828-32.

[37] Gichuru EK. *Characterization of genetic resistance to Coffee Berry Disease (Colletotrichum kahawae Waller and Bridge) in Arabica coffee (Coffea arabica L.) that is introgressed from Coffea canephora Pierre* (Doctoral dissertation, University of Nairobi).

[38] Lampard JF, Carter GA. Chemical investigations on resistance to coffee berry disease in Coffea arabica. An antifungal compound in coffee cuticular wax. Annals of Applied Biology. 1973 Jan; 73(1): 31-7. https://doi.org/10.1111/j.1744-7348.1973.tb01306.x

[39] Chen Z, Nunes MA, Silva MC, Rodrigues Jr CJ. Appressorium turgor pressure of Colletotrichum kahawae might have a role in coffee cuticle penetration. Mycologia. 2004 Nov 1; 96(6): 1199-208.

[40] Silva MD, Várzea V, Guerra-Guimarães L, Azinheira HG, Fernandez D, Petitot AS, Bertrand B, Lashermes P, Nicole M. Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian journal of plant physiology. 2006; 18: 119-47.

[41] Van der Vossen HA, Walyaro DJ. Additional evidence for oligogenic inheritance of durable host resistance to coffee berry disease (Colletotrichum kahawae) in arabica coffee (Coffea arabica L.). Euphytica. 2009 Jan; 165(1): 105-11. https://doi.org/10.1007/s10681-008-9769-3

[42] Masaba DM, Van Der Vossen HA. Evidence of cork barrier formation as a resistance mechanism to berry disease (Colletotrichum coffeanum) in arabica coffee. Netherlands Journal of Plant Pathology. 1982 Jan; 88: 19-32. https://doi.org/10.1007/BF01976423

[43] Waller JM, Bigger M, Hillocks RJ, editors. Coffee pests, diseases and their management. CABI; 2007.

[44] Masaba D, Waller JM. Coffee berry disease: the current status. Coffee berry disease: the current status. 1992: 237-49.

[45] Van der Vossen HA, Cook RT, Murakaru GN. Breeding for resistance to coffee berry disease caused by Colletrotrichum coffeanum Noack (sensu Hindorf) in Coffea arabica LI Methods of preselection for resistance. Euphytica. 1976 Jan; 25(1): 733-45. https://doi.org/10.1007/BF00041613

[46] Teferi D. Status of released Coffea arabicavarities for their resistance to Colletotrichum kahawae (coffee berry disease) under laboratory condition. Discourse Journal of Agriculture and Food Sciences. 2014; 2(6): 197-202.