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Research Article
Gidami *Coffea arabica* Collections Against Coffee Berry Disease (*Colletotrichum kahawae*), Western Ethiopia

[1]Zenebe Wubshet Hordofa, [2]Daniel Teshome Lopisso and [2]Weyessa Garedew Terefe

[1]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, P.O. Box 192, Jimma, Ethiopia
[2]Jimma University College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia

Abstract

Background and Objective: Ethiopia is the centre of origin and diversity of Arabica coffee which serves as a driving force for the country's economy. However, fungal pathogens especially *C. kahawae* induce coffee berry disease challenges coffee production widely. Hence, the objective/s/ of this study was to evaluate the reaction of local *C. arabica* accessions against coffee berry disease under field and laboratory conditions. **Materials and Methods:** CBD was assessed on a total of 100 coffee accessions (92 accessions plus 8 checks) under field condition visually (0-100% disease scale) and further evaluation was undertaken on the best performed promising accessions via attached (field) and detached berry test (lab.) conditions. **Results:** the result indicated significant differences ($p<0.001$) among treatments at both conditions. Six accessions namely G63, G65, G57, G72, G15 and G70 revealed the lowest disease severity score (<10%) at field ABT and G65, G63 and G15 showed 24-28% infection percentage in the lab DBT, hence, relatively resistant for CBD. While, four coffee accessions i.e. G50, G89, G92 and G67 showed a susceptible reaction (>25% berry infection). Here, the present study not only directed the impact of CBD rather demonstrates the role of host resistance in combating this disease. **Conclusion:** Therefore, future research should focus on the evaluation of these promising coffee accessions across multi-location field trials several years, diversity/identity verification of *C. kahawae* isolates using more other methods and further studies on the resistance mechanism of CBD as a priority research topic for full understanding about *C. arabica*-*C. kahawae* pathosystem.

Key words: *Coffea arabica*, coffee berry disease, *Colletotrichum kahawae*, disease protection, host-resistance

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Corresponding Author: Zenebe Wubshet Hordofa, Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, P.O. Box 192, Jimma, Ethiopia Tel: +251924138583

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coffee (*Coffea arabica* L.) is a nonalcoholic beverage crop that serves as the major currency holder for different countries and the communities using livelihood as economic, social and spiritual impact and diverse cultural and/or psychological backgrounds mainly for Africa, Asia and Latin America[1]. Truthfully, it is the backbone of the Ethiopian economy and contributes about 27% of the foreign exchange earnings and more than 25% of rural and urban employment[2].

Ethiopia is the origin and diversity centre of *Coffea arabica* and placed in 1 and 5th production level from Africa and World, respectively[3]. The crop has also diversified in over 80 countries with10 M ha of land coverage today[4]. The availability of divers' agro-ecological zones and different production systems (forest, semi forest, garden and plantation) is a great opportunity for its production in the country[5]. Among these, the highest coffee production potential (about 69.5%) is found in the Oromia Regional State of Ethiopia. Besides, the West Wollega zone of Oromiya also covers around 90,626 t of products that contribute a lot to the national export market[6].

Moreover, germplasm diversity in the Gidami district (Western part of Oromiya) provides an immense opportunity for the local landrace development plan of the research. According to the Gidami Agricultural Office report in 2017/2018, the district is the place where the highest germplasm exists[7]. Yet, the production potential and economic importance of the crop in the area have been affected by several biotic and abiotic factors. Coffee berry disease (*C. kahawae*), coffee wilt disease (*G. xylarioides*) and coffee leaf rust (*H. vastatrix*) are the most important issues that seriously challenged coffee production today[8].

The significance of CBD in *C. arabica* growing areas of Ethiopia has been reported from different parts of the country. For instance, Oromiya and Southern Nation Nationality and People (SNNP) regional states have reported 38.8 and 17.2% mean incidence, respectively[9]. Alemu *et al*.[10] have reported 45, 70, 50 and 60% mean incidence in Borena, Gedio and Hararghe, Illubabor, Jimma and Sidama areas, respectively. Studies[10,11] have reported 22 and 30-80% a disease incidence, respectively. This indicates the increment of disease importance and will cause total yield loss when susceptible landraces are cultivated[12]. Similar author, Alemu *et al*.[10] reported 52.5 and 29.9% national average CBD incidence and severity, respectively.

Agroecological-based local landrace development is quite important to utilize the available genetic resources from anywhere[13,14]. The wide uses of resistant varieties provide ample opportunities for disease control sustainably and safely. With this regard, promising sources of disease resistance in coffee germplasm have been developed from different countries like Ethiopia, Kenya and Tanzania[15]. However, the challenges of the long breeding cycle associated with the growth period of coffee have slowed down the progress of further varietal improvement works[16].

With these existing challenges, Jimma Agricultural Research Center (JARC) played a crucial role by developing and releasing improved coffee verities adapted across different agro-ecologies. The centre has developed 31 CBD-resistant varieties for different coffee-producing areas of the countries till now[17,18]. Since 2010, only four varieties have been developed for Western coffee growing areas. As compared to the diverse agro-ecological niches, enormous available coffee genetic resources and the high coffee production potential of the zone, achieving the apparent economic development program of the producers as a whole and the region, in particular, such a very small number of improved varieties are not sufficient.

So, increasing the genetic base of improved coffee varieties preferred by farmers brings physical change to their economy by increasing productivity through effective control of CBD by minimizing production costs and reducing potential consequences on human health and the environment is crucial. This goal can achieve via assessing the extent of CBD and further understanding the potential of local plant materials in combating this disease. With this background, the present study was aimed to evaluate local *C. arabica* germplasm collections from Gidame for resistance to *C. kahawae*.

MATERIALS AND METHODS

Description of the study area: The greenhouse experiments (resistance evaluation) were conducted at JARC and the field experiment was also conducted in 2014/2015 Gidami coffee collections planted at Gera Agricultural Research Sub-Center (GARSc). GARSc is located at Jimma zone of South-Western Ethiopia (latitude: 7.1170 N, longitude: 36.00 E) at 1900 m.a.s.l elevation. The area represents cool to subhumid, low to high altitudes of coffee growing agro-ecologies and receives an average annual rainfall of 1877.8 mm. The minimum and maximum temperatures of the area are 10.4 and 24°C, respectively[19].

Evaluation of gidami coffee collections for CBD at the field attached berry test: The experiment was conducted on the 4th years old coffee trees planted at GARSc during the 2017/18 cropping year. The trial was laid out in a 10×10 simple lattice design with two replications. It consists of

92 *C. arabica* accessions and 8 CBD resistant varieties namely W92, W76, W66, 8136, W78, 7514, 7416 and 7576[20] as control at field level. Each replication contains 10 incomplete blocks with 10 germplasm. Each plot in the incomplete blocks consisted of 6 coffee trees with 2m spacing between rows and plants. All the agronomic practices were applied according to standard procedures as usual. For early discrimination of susceptible accessions, overall disease pressure was assessed on each accession following the procedure used by Mohammed and Jambo[21]. Each tree was monitored for the absence or presence of CBD symptoms (like scab and dark sunken lesion on the berry, berry rot, depressed or dried berry and fruit fall before harvest) and then percent disease incidence was computed using (Eq. 1):

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected trees}}{\text{Total number of trees assessed in the farm}} \times 100 \quad (1)$$

After data analysis, promising accessions showed lower CBD percentage in visual scoring in Table 1 were promoted to the next step resistance screening via attached and detached berry test i.e., ABT and DBT, respectively.

ABT was conducted by applying *C. kahawae* inoculum on branches of growing green berries following the procedures of Kilambo *et al.*[22] at Gera. This study aimed to estimate the difference in natural infestation and further verify the level of resistance in coffee accessions with artificial inoculation. With this truth, inoculation was done by random sampling of 3 trees/plot and then 3 strata/tree followed by 1 branch/strata (from the top, middle and bottom layers), resulting in a total of 9 branches per plot using *C. kahawae* pathogen isolated from Gera as a source of inoculum.

For inoculum preparation, green berries with black active lesions from infested fields were collected in plastic boxes, slightly wetted with sterile distilled water (SDW) and stored at room temperature (RT) for 48 hrs. After sporulation, spores were harvested from berries by rinsing with distilled water and filtered using sterile cheesecloth. Then, conidial density was counted using a haemocytometer adjusted to 2×10^6 conidia mL^{-1} and the marked strata were sprayed with (≈25 µL per berry) of *C. kahawae* spore suspension using a hand sprayer. Immediately after inoculation, branches were covered with a paper bag to favour disease development. The bags were removed 24 hrs after inoculation (HAI). Three weeks after inoculation, disease data have scored following the standard procedures using a 0-6 disease score scale in Table 2 via critical observation of the lesion size and its extent (spread) on the diseased berry parts[23]. Finally, the disease percent severity index (PSI) is calculated as follows (Eq. 2):

$$\text{Percent severity index (PSI)} = \frac{\text{Sum of numerical rating}}{\text{Total number of rated plant} \times \text{X max. score of the scale}} \times 100 \quad (2)$$

Evaluation under greenhouse condition

Detached berry test: The total of 36 genotypes, 30 best-performed accessions/varieties/ from ABT study (Table 1), 4 highly susceptible *C. arabica* accessions identified under visual disease score at the field and 2 reference varieties {laboratorial resistant 741 and susceptible 370 varsities} and one virulence *C. kahawae* isolate (GC) from Gera were used for this study. The experiment was laid out in CRD design in three replications containing 6 berries per replication.

Inoculum production and inoculation procedures: The conidial suspension was prepared from 10 days old culture via washing the mycelial spore from the cultured isolate by flooding with 10 mL of sterilized distilled water. Then, rubbed with sterilized scalpel and transferred to 50 mL sterilized beaker, thoroughly stirred for 15 min with a magnetic stirrer and then filtered through double layers of sterile cheesecloth. Spore concentration was determined with a hemocytometer and adjusted to 2×106 mL^{-1} [23,24]. Then, at the centre of each berry, a drop (≈25 µL) of pathogen inoculum was sited using a micropipette. Sterilized distilled was used for the control boxes and to facilitate pathogen infection and symptom development with high relative humidity, the treated boxes were tightly closed and incubated at 25°C for 14 days.

Data collection: Data collection was started on the 7th day after inculcation (DAI) at the time of the first CBD symptom appeared and taken three times[24]. After counting the number of damaged and healthy berries, disease incidence was calculated. Likewise, CBD severity was computed using a 0-6 scale score as described above (Table 2). Finally, the average infection percentage (AIP) was calculated as (Eq. 3):

$$\text{AIP} = \frac{\Sigma\,(\text{Ir1} + \text{Ir2} + \text{Ir3})}{\text{N}} \quad (3)$$

Where:

I = Sum of disease score

r = Replication

N = Total number of berries in the replication

Table 1: Promising *Coffea arabica* accessions (varieties) used for attached berry test

Numbers	Accessions codes	Numbers	Accessions codes	Numbers	Accessions codes	Numbers	Accessions codes
1	G-42	13	G-19	25	G-50	37	G-66
2	G-47	14	G-13	26	G-31	38	G-52
3	G-48	15	7514^{R}	27	G-85	39	W-92^{R}
4	G-49	16	G-16	28	G-87	40	G-57
5	G-92	17	G-82	29	G-89	41	G-51
6	G-72	18	G-91	30	G-67	42	G-56
7	G-73	19	G-10	31	G-65	43	G-55
8	G-71	20	W-78^{R}	32	G-69	44	7416^{R}
9	G-77	21	G-54	33	G-68	45	G-37
10	G-83	22	G-84	34	8136^{R}	46	G-21
11	G-15	23	G-40	35	G-70	47	G-63
12	W-76^{R}	24	W-66^{R}	36	G-64	48	7576^{R}

R: Resistance varieties used as reference under field condition

Table 2: Scales for coffee berry disease severity assessment ([7])

Disease indexs	Descriptions
0	Healthy green berries/berries without disease symptoms/
1	Black sunken lesions cover <2% of the green berries surface
2	Black sunken lesions cover 2-5% of the berries surface, approximately 3mm in diameter
3	Black sunken lesions cover 6-10% of the berries surface shows black lesions approximately 5 mm in diameter
4	Black sunken lesions cover 11-50% of the berries surface, approximately 7mm in diameter
5	Black sunken lesions cover 51-99% of the berries surface, approximately15 mm in diameter
6	>99% or the whole surface of berries covered with black sunken lesions, mummified berries

Statistical analysis: The collected disease data were summarized and subjected to ANOVA using SAS software (version 9.3). Before analysis of variance, all data sets were tested for normal distribution using the normality test, the data from field evaluation was transformed with Arcsine (score data). Means were separated using Duncan's Multiple Range Test (DMRT) at ($p = 0.05$)

RESULTS AND DISCUSSION

Field result/attached berry test: The results obtained from the analysis of variance showed a highly significant difference ($p<0.001$) among accessions for CBD resistance. The highest CBD severity (33.4%) was recorded from accession G50 which showed high significance compared to all other treatments. Interestingly, the lowest (2.3%) disease severity (DS) was recorded from G63 followed by W76, G65, G72, 7416, G66, G57, G15 and G70 which did not statistically differ from G63. On the other hand, G84 and G85 showed the intermediate resistance similar to the known reference varieties (W66, 8136 and 7576) which were not significantly different from each other. Hence, means ranged between 2.3 to 33.4% for the attached berries in Table 3 and indicated the presence of certain accessions that have better or comparable resistance for CBD rather than the checks.

As resistance in *Coffea arabica* is controlled by the recessive genes, it is considered horizontal[18]. Besides, the variations among *C. arabica* accessions against CBD can be associated with the genetic makeup of each accession in this study. For a long time, *C. arabica* is known to be a self-fertile crop. But the recent study[25] on the unmanaged forest trees, has reported as *C. arabica* yields 76% of out crossing from neighbouring plots of coffee accessions. This leads to a change in the genetic make of the genotypes and variations against the pathogen. On the other hand, Silva *et al.*[26] and van der Vossen and Walyaro[27] found that cork barrier development on the pericarp limits additional pathogen invasion of host plants. This implies that fungal growth can be restricted with a series of hypersensitive reaction (HR) responses of resistant genotypes.

Host resistance implies the active and proficient means of host plant response which restricts plant cell death due to pathogen attack that causes quick membrane integrity loss in the damaged cells[28,29]. The earlier studies illustrated that *C. arabica* resistance for CBD (*C. kahawae*) can be from constitutive and incite means of operating at different stages of disease development (pathogenesis)[32]. The difference in response of the genotypes will depend on the lifestyle of the pathogen and the genetic constituent of the host[31,32]. Also, variation could exist within individual coffee selections of each coffee population (locality) in reaction to CBD shown in this experiment. Likewise, Zeru *et al.*[16], Gichuru *et al.*[33] and Kilambo *et al.*[22] have reported that genotypic variation in pathogen infection under field conditions could be examined by ABT via artificial inoculation but better knowledge of both the pathogens and crop diversity allowed to identify durable resistant which are novel and economical approaches against CBD.

Table 3: Disease percentage of *Coffea arabica* selections inoculated with *Colletotrichum kahawae* in attached berry test

Accessions codes	Severity	Accessions codes	Severity
G63	2.3^{v}	G51	15.8^{h-o}
W76^{R}	2.6^{v}	G71	16.1^{g-o}
G65	3.7^{uv}	G21	16.8^{f-n}
G72	4.7^{t-v}	G77	17.0^{e-m}
7416^{R}	6.3^{s-v}	G16	17.7^{e-m}
G66^{R}	7.3^{r-v}	G47	17.8^{d-m}
G57	8.7^{q-v}	G37	17.8^{d-m}
G15	8.6^{q-v}	G54	17.9^{d-k}
G70	9.3^{o-t}	G69	18.4^{c-k}
W78^{R}	9.4^{o-t}	G48	19.3^{c-j}
W66^{R}	10.3^{n-t}	7514^{R}	19.3^{c-j}
8136^{R}	10.4^{n-t}	G49	19.4^{c-j}
G84	10.9^{n-t}	G42	20.6^{b-i}
G85	11.0^{m-t}	G55	20.6^{b-i}
7576^{R}	11.0^{l-t}	G19	20.7^{b-i}
G10	11.9^{d-k}	G73	21.6^{b-g}
G91	12.7^{k-s}	G88	22.7^{b-g}
W92^{R}	12.7^{j-s}	G83	23.2^{b-f}
G82	12.9^{j-s}	G31	23.7^{b-e}
G56	13.2^{j-s}	G13	24.6^{bc}
G40	13.3^{j-r}	G92	24.8^{bcd}
G64	13.4^{r}	G67	26.3^{b}
G87	14.4^{i-q}	G89	27.0^{b}
G52	15.1^{h-p}	G50	33.4^{a}
Mean			15.4
CV (%)			22.5

Means followed with the same letters are not significantly different (DMRT, 5.6-7.1 at $p<0.05$) and R: Reference verities

Indeed, plants have different ways of defence mechanisms that recognize potentially dangerous pathogens and rapidly respond before serious pathogen damage[34,35]. Once the pathogen attacks plant tissue, the host plant challenges the advancement of the infection in a series of defence reactions. Among these, basal resistance is the first line of pre-formed and inducible defence response that protects plants against various groups of pathogens[34]. Successful pathogens use effectors that would deceive basal defence for further infection and colonization. This chain of effectors-resistance gene co-evolution can be attributed to mutation and horizontal transfer of genes of the pathogen and selection pressure on the plant for resistance[36]. Variations within *C. arabica* collections are a basic opportunity for resistance development via breeding. Yet, resistance in perennial crops like coffee is observed and screened during the late stage of development[14], it needs great efforts of the researchers' infrequent evaluation of genotypes in multi-location over time.

Detached berry test: The result indicated that there was a highly significant difference ($p<0.001$) among *C. arabica* accessions. The lowest CBD infection (6.7%) was recorded from the resistant check 741 which significantly varied from all other accessions and/or varieties. On the other hand, the relatively lowest percent CBD infection (24.0%) was recorded from G65 followed by G63, W76, G15, G72, 7416 and G66 which did not differ from G65 statistically. While, W78, 8136, G70, G57, G85 revealed intermediate infection percentages. The highest infection percentage (87.3%) was from G78 accession which was also susceptible at the field in infested naturally followed by G04, G92, G71 370, 71 and G91 which did not differ statistically in Table 4. Remarkably, the highest infection percentage /in magnitude/ was recorded from those three coffee accessions namely G78, G04 and G71. Also, G78 and G04 taken from early discarded accessions during visual evaluation under natural field infestation repeated their susceptibility under the laboratory condition again. Similarly, Bayetta[17] reported that genotypes susceptible under field conditions could be susceptible under controlled conditions if no change in pathogen strains.

Clear variations between susceptible and resistant accessions were observed with the detached berry inoculation test in this study. Unlike resistant accessions in the areas of successful pathogen infection, continuous and entirely covered berry surface with a black lesion on the susceptible accessions. While restricted scab lesions that limit further pathogen penetration into intercellular parts of the berries were observed on the resistant accessions and Waller *et al.*[12] also noticed that scab lesion formation is a resistant host response which is more common on the coffee cultivars possessing resistance.

Table 4: Response of *Coffea arabica* accessions (varieties) to *Colletotrichum kahawae* in detached berry test

Code of accessions	Infection (%)	Code of accessions	Infection (%)
741^{R}	6.7^{k}	G10	55.5^{fg}
G65	24.0^{j}	G82	56.8^{efg}
G63	25.5^{j}	G31	62.2^{d-g}
W76	28.2^{j}	G51	63.3^{d-g}
G15	31.9^{j}	G56	63.7^{c-g}
G72	34.0^{j}	G02	64.4^{c-g}
7516	36.6^{ij}	**W92**	65.1^{c-g}
G66	38.1^{hij}	**W66**	66.1^{c-g}
8136	51.3^{ghi}	G03	66.1^{c-g}
W78	51.3^{ghi}	G89	67.8^{c-g}
G84	51.5^{ghi}	G67	69.6^{b-f}
G57	51.6^{ghi}	G91	72.4^{a-e}
G85	52.4^{f-i}	G70	75.1^{a-d}
7514	52.7^{f-i}	**370^{S}**	76.5^{a-d}
G47	54.0^{fgh}	G71	80.5^{abc}
G83	54.1^{fgh}	G92	84.9^{ab}
G55	55.0^{fg}	G-04	85.1^{ab}
7576	55.2^{fg}	G78	87.2^{a}
Mean			78.9
CV (%)			15.5

Means followed the same letters are not significantly different (DMRT; 14.3-17.9 at p<0.05). The reference varieties had shown in bold. (R) Resistant and (S) Susceptible varieties under laboratory condition

Plant pathogens infection and resistance strategies vary depending on the host and environmental (internal and external) conditions. Similarly, several factors can facilitate coffee susceptibility when in contact with C*. kahawae* in all stages of pathogen development (from conidial germination to sporulation)[23]. Moreover, coffee genotypes which resist CBD attack be able to reduce the pathogen infection sites via restricting the conidial germination and formation of appressoria and offers extra advantages for the genotypes in favouring of movable resistance factors concerted in limited areas even whilst initial capacities are similar to those in susceptible accessions[24].

The existence of fungi toxic compounds in coffee could be another resistance mechanism. Brown crust formed on the berry surface restricts further infection and leads to starvation of the pathogen. Furthermore, the formation of cork barriers at the periphery of the infected area also leads to cell death. The scab lesions are a common expression of CBD resistance at distinct stages of pathogenesis[33]. All these mechanisms can eliminate biotrophic associations with a pathogen and block nutrient transfer to the infected area[12] and the occurrence of responses to infection ahead of hyphae demonstrated the existence of elicitors[26]. Gill *et al.*[37] have reported that the inherent antifungal compounds in green coffee berries can hamper infection due to *C. kahawae* strains.

The disease is initiated mainly from diseased berries (green, ripen and mummified) and infected plant parts (flowers, barks, twigs and leaves) and appears every year again on previously infected coffee trees. Detached berry technique is the possible means relative ranking of cultivar resistance starting from early time which is still useful for a differential interaction analysis and varietal characterization[24]. Generally, resistant varieties have the potential to reduce the cost of production and are the safe ways of disease management approach and need great focus in the sustainable use[38]. These promising accessions in this study exhibited better results can be the baseline of breeding programs in future work.

CONCLUSION

The result from resistance evaluation activities under field (ABT) and laboratory (DBT) conditions showed considerable variations among coffee accessions. The mean percent of berry infection ranged from 2.31-33.4% in field ABT and 6.7-87.3% in the laboratory DBT tests. In comparison to all accessions/varieties/*C. arabica* accessions namely G65, G66, G63, G72 and G15 revealed low CBD infection at both conditions. Various contributing aspects like the genetic makeup, outcrossing, production of fungi toxic compounds and physical barriers existed can be considered as the factors of variation in resistance among genotypes. Likewise, the result in this study confirmed that G78 and G04 revealed susceptible reactions for CBD under both field and laboratory conditions indicating, resistance is genetically inherited and cannot be reversed unless changing the nature of the pathogen virulence.

As a whole, the present study displayed the importance of resistant varieties and demonstrates the role of host

resistance in combating diseases as well. Those *C. arabica* accessions that showed low CBD infection under field and laboratory conditions of this study can be a respectable opportunity for further breading work in the future and as alternatives to limit the impact of CBD in the country particularly for Western parts of Ethiopia.

SIGNIFICANCE STATEMENT

This study discovered some promising resistant coffee varsities that can be beneficial for the producers and or coffee breeders to help the researchers to uncover the critical areas of coffee berry disease that many researchers were not able to explore. Thus a new theory on host resistance may be arrived at.

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