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SCREENING OF SOME *COFFEE ARABICA* GENOTYPES AGAINST COFFEE WILT DISEASES (*GIBBERELLA XYLARIOIDES* HEIM AND SACCUS) AT JIMMA, SOUTHWEST ETHIOPIA

Kifle Belachew[1†] --- Demelash Teferi[2] --- Gabisa Gidisa[3]

[1,2,3]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Plant Pathology Research Section, Jimma, Ethiopia

ABSTRACT

It is essential to reduce coffee yield losses due to coffee wilt disease (CWD) in the country through the development and use of genetically resistant coffee varieties to increase and consistently supply Arabica coffee to the fast growing coffee industry. The soil-borne nature of the pathogen and perennial character of coffee have made management of coffee wilt disease difficult through the conventional control approach of 'uproot and burn infected trees at the spot'. Therefore, longer-term prospects of successful management of coffee wilt disease depend principally upon employing resistant coffee cultivars. With this objective laboratory and field evaluations were conducted to screen some coffee genotypes against coffee wilt disease. Disease severity or mean percent seedling death ranged from 0.00 to 89.96 %. The result showed in lowest seedling death rate, long incubation period and high field survival rate of most accessions indicating resistant reaction to coffee wilt disease. Thus present experiment implied that the potential of obtaining coffee wilt disease resistant coffee variety from these accessions provided that they have other desirable traits like resistance to major coffee diseases, high yield and improved quality.

Keywords: Coffee arabica, Coffee wilt diseases, Disease resistant, Gibberella xylarioides, High yield, Incubation period, Seedling death rate.

Contribution/ Originality

This study documents coffee genotypes found in coffee gene pool of Ethiopia may hold key to sustianable survival providing traits needed to cope with new pest outbreak and climate change; this underline the importance of systematic evaluation of coffee accessions and utilization of best performing having high yielding, good quality.

† Corresponding author

1. INTRODUCTION

Coffee is the most important agricultural commodity upon which more than 50 countries, including Ethiopia, are dependent for their economy, especially foreign exchange earnings. Unlike Carl Linnaeus, contemporary scholars and writers about coffee authentically confirm that the primary centre of origin and genetic diversity of *Coffea arabica* L. (arabica coffee) is Ethiopia, and some of them wish to rename this species as *Coffea abyssinica*. Wild forest coffee is still found today in the south-western parts of the country (Flood, 2009). Coffee is directly or indirectly a source of livelihood for more than about 25 million people who engaged in production, processing and marketing of the crop in Ethiopia (CABI, 2003; CSA, 2008).

There are however, many research findings documented that coffee diseases and insect pests situation in coffee production pose great treat. Among three major coffee diseases namely coffee berry diseases (*Colletotrichum kahawae*), coffee wilt disease (*Gibberella xylarioides*) and coffee leaf rust (*Hemileia vastatrix*); coffee wilt disease is the second distractive disease dramatically limiting coffee production in Ethiopia (Eshetu *et al*., 2000; CABI, 2003; Flood, 2009). The disease has been a serious problem to the production of coffee in central and eastern Africa like DR Congo, Uganda, Tanzania and Ethiopia since the 1990s killing hundreds of trees and the disease attacks all commercial coffee species including *Coffee arabica* and *Coffee canifora* at any growth stage (Rutherford, 2006; Flood, 2009; Girma *et al*., 2009).

Different studies indicate that the prevalence of coffee wilt diseases at different coffee growing regions and production systems of Ethiopia was very high. Coffee Wilt Disease (tracheomycosis) is a systemic vascular disease caused by the fungal pathogen, *Gybbrella xylarioides* Heim and Saccus (Fussarium *xylarioides* Steyaert) that totally kills coffee plant at any growth stage in all production sytem and agroecollogy. Coffee wilt diseases is more prevalent in plantation and garden coffee than forest and semi-forest coffee (Girma *et al*., 2001; 2009; Sihen *et al*., 2012). CWD was more prevalent in fields of garden production system like Harar and Bale area with severity range of between 27.2% and 43.5% which is high compared to that of the semi-forest coffee production system (Girma, 2004). The incidence of CWD was above 35% in garden coffee of West Gojam zone of Amhara regional state. CWD is prevalent in almost all coffee-growing regions, with national average incidence and severity of 28% and 5%, respectively. The average annual national crop losses attributed to CWD was 3360t amounting to US$3,750,976 in Ethiopia. This economic loss coupled with difficulty to manage the disease indicates that CWD is the second leading disease of coffee, after CBD in Ethiopia (CABI, 2003; Flood, 2009).

However, a number of control methods are available for the management of CWD, which include use of resistant varieties, chemical control and cultural methods. Most of these management options are not practical and unavailable in Ethiopia. However cultural method 'uproot and burn infected trees at the spot' was in place and other control methods are not yet implemented. The soil-borne nature of the pathogen and perennial character of coffee have made management of the disease difficult through the conventional control approach of 'uproot and

burn infected trees at the spot. More over this approach is labor intensive and impractical at smallholder coffee producers.

In management of coffee wilt diseases the uses of resistant varieties is the most appropriate, efficient, environmentally friendly and economically sound method. Ethiopia is known for its center of *Coffee arebica* genetic variability thus this was good opportunity to develop CWD resistant genotype in the country. Jimma agricultural research center released 37 CBD resistant commercial coffee varieties (Jimma Agricultural Research Center (JARC), 2011). Coffee varieties (971 and 974) had the best performance with moderate to high levels of multiple resistances to both CBD and CWD infections (< 15%). Thus, the present study was undertaken to determine the reaction of some coffee genotypes to coffee wilt disease (CWD) pathogen pertaining to pathological investigations as a potential of obtaining CWD resistant coffee genotypes.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

Field, laboratory and greenhouse studies were undertaken at Jimma Agricultural Research Center (JARC) research field and pathological laboratory from 2014 to 2013. JARC is located at 07°46'N latitude and 36°47'E longitude coordinate and at an elevation of 1753m.a.s.l. The site is 360 kilometer away from Addis Ababa and 12 kilometers west of Jimma town. It is situated in the tepid to cool humid-mid highlands of South Western Ethiopia which receives annual rainfall of 1572mm. Its mean minimum and maximum temperature is 11.6^{0}C and 26.3^{0}C, respectively. The major soil type of the area is Eutric Nitosol and Cambiosl (~~R~~ reddish brown) of upland and fluvisol of bottom land with pH around 5.2 (IAR, 1997).

2.2. Treatments and Design Used

The study was conducted using 44 coffee accessions, which were collected from Bale area, South-eastern part of Ethiopia, in 2004, along with four controls (catimor J-19, catimor J-21, Gesha and 370). The treatments were outlined using single plot design in which six trees were planted per plot at 2m x 2m spacing. The laboratory and greenhouse studies were conducted using randomized complete block design (RCBD).

2.3. Coffee Seedling Rising

Seeds were collected from 44 Bale coffee accessions and four controls (i.e. coffee cultivar 370 as resistant, catimor J-19 and catimor J-21 as moderately resistant and cultivar Gesha as susceptible). The seed lots of each germplasm were first soaked in distilled sterile water for about 24 hours after removing the parchment in order to facilitate early germination. The soaked seeds (30-40 seeds/pot) of each germplasm were sown into heat sterilized and moistened sandy soil in disinfected plastic pots (each has 5652 cm^3 capacity). Sterile water was applied every two days to maintain adequate moisture for seed germination, emergence and growth of the plants throughout the experimental period.

2.4. Inoculum Preparation

Inoculation in green house started when the seedlings attained the full expanded cotyledon stage (seventy days after sowing). A representative isolate of *Gibberella xylarioides* ,the causative agent of coffee wilt disease, obtained from plant pathology section of JARC were multiplied for inoculation following method employed by Girma and Mengistu (2000). The stock culture of the representative isolate were used to initiate colony growth by sprinkling grains of sand on to Petri-dishes with SNA followed by further sub-culturing on the same medium for about a week. At the same time fresh coffee branches (twigs) were collected from healthy trees, cut into small pieces of 15 cm and the bark were slightly scratched off to expose the wood. The branches were placed in a test tube (3.75 cm^3) having a small roll of well-moistened cotton wool underneath and then sterilized in an autoclave. A batch of twigs were inoculated with two to three milliliter of conidia suspension of the isolate and incubated for ten days under standard conditions. The conidia used for inoculation were obtained by thoroughly rinsing off the branches with good colony growth with sterile water in a sterile beaker. The suspension of the isolate were stirred up with magnetic stirrer and filtered through double layers of cheese clothes. The spore concentration of the inoculums suspension was adjusted at about 2 x 10^6 conidia per milliliter.

2.5. Inoculation of Coffee Seedlings

Inoculation of the test seedlings were done with a viable conidial suspension of the isolate by stem nicking or stem wounding procedures (Pieters and Van Der Graaff, 1980; Girma and Mengistu, 2000). A sterile scalpel were first immersed into the suspension, then the stem of each seedling was nicked at about two centemeter from the soil level and drop of nearly one milliliter was placed in the notch. The treated plants were immediately kept in air conditioned growth room with high relative humidity (> 90%) and optimum temperature (23-25^{0}C) for facilitation of infection. After ten days, the inoculated seedling were transferred and arranged in green house benches. In each inoculation stage the experiment was laid out in randomized complete design (CRD) with three replications.

The inoculum was applied on 3 to 4 month old seedlings of *C. arabica* genotypes (Girma, 2004). This method was, therefore, adopted for large-scale germplasm screening by scientists from CIRAD and Ethiopia, as it is considered to be less expensive and effective (Girma and Mengistu, 2000; Girma, 2004).

2.6. Data Collection and Analysais

The number of seedlings which showed wilting symptom was recorded at fourteen days interval for 6 months in greenhouse, starting a month after inoculation. In addition incubation period or the dates from inoculation to first symptoms was periodically noted. Incubation periods indicate the number of days between inoculation and the first date of symptom appearance. Field survival rate of coffee tree was recorded. Field survival rate was calculated by counting the number of survived ones from the total number of initial stands per plot in the field.

The percentages of wilt or dead seedlings were calculated from cumulative number of dead over total number of seedlings (dead plus healthy) for total period of six months. The percentage of wilt for all treatments was transformed to arcsine-square root (angular values) to normalize the data as necessary before statistical analysis. Similarly, the percentages of dead seedlings were calculated by counting the number of wilted ones from the total number of initial stands per plot in the pots.

The analysis of variance (ANOVA) and means comparisons were performed employing general linear model (GLM) of SAS statistical software version 9.0 (SAS Institute, 2001).

3. RESULTS

3.1. Mean Percent Seedling Death

There existed highly significant difference ($p < 0.001$) among Bale coffee accessions tested for coffee wilt diseases with artificial inoculation in mean percent of seedling death at this experiment (Table 1). Disease severity or mean percent seedling death ranged from 0.00 to 89.96 %. Out of tested coffee accessions half of them showed less diseases reaction (< 10 %) against CWD under laboratory conditions.

Table-1. Reaction of Bale coffee accessions to CWD (*Gebbrelia xylariodes*) in seedling inoculation test under green house condition at Jimma research center

Accessions	Mean Seedling Death (%)	Accessions	Mean Seedling Death (%)	Accessions	Mean Seedling Death (%)
B-2/04	1.52^{qp}	B-45/04	85.33^{bc}	B-139/04	6.60^{nop}
B-3/04	1.11^{qp}	B-51/04	80.92^{cd}	B-140/04	5.59^{nop}
B-4/04	20.00^{kl}	B-53/04	3.41^{nopq}	B-143/04	0.00^{q}
B-5/04	64.35g	B-64/04	0.00^{q}	B-148/04	1.45^{qp}
B-6/04	13.67^{no}	B-70/04	0.00^{q}	B-156/04	2.30^{opq}
B-10/04	76.23^{de}	B-85/04	2.22^{opq}	B-157/04	2.26^{opq}
B-11/04	73.18^{ef}	B-89/04	52.02^{h}	B-159/04	17.32^{no}
B-12/04	89.96^{a}	B-92/04	23.32^{kj}	B-161/04	8.02^{nop}
B-20/04	25.83^{kj}	B-103/04	27.86^{j}	B-164/04	2.22^{opq}
B-27/04	72.68^{ef}	B-104/04	0.00^{q}	B-167/04	15.43^{no}
B-28/04	1.15^{qp}	B-110/04	4.52^{nop}	B-156/04	23.16^{kj}
B-29/04	1.15^{qp}	B-118/04	23.34^{kj}	B-169/04	1.75^{qp}
B-31/04	73.89^{ef}	B-124/04	0.00^{q}	J-19*	54.21^{h}
B-32/04	70.83^{f}	B-130/04	26.75^{j}	J-21*	38.81^{i}
B-37/04	86.56^{ab}	B-133/04	1.11^{qp}	Gesha**	88.65^{a}
B-38/04	84.14^{bc}	B-137/04	2.52^{opq}	370***	0.00^{q}
Mean					25.63
LSD (P<0.01)					5.09
CV (%)					12.26

Means followed by a common letter(s) are not significantly different at t 1% level of significance; LSD, Least significant difference; CV, coefficient of variation; *, Moderately resistant; **, Susceptible; ***, resistant Arabica coffee varieties to CWD used as check;

Coffee accessions B02/04, B03/04, B28/04, B29/04, B53/04, B64/04, B70/04, B85/04, B104/04, B110/04, B124/04, B133/04, B137/04, B139/04, B140/04, B143/04, B148/04, B156/04, B157/04, B161/04, B164/04 and B169/04 expressed resistant reaction against CWD

pathogen. Moreover, these accessions showed significantly different (P<0.05) reaction from two moderately resistant control cultivars 'Catimor J-19' and 'Catimor J-21' (Table 1). The result of this investigation is in agreement with the work of Sihen *et al.* (2012) where southeast Ethiopia forest coffee collections from Harena area exhibited lower seedling death rate under laboratory conditions. However, coffee accessions B10/04, B11/04, B12/04, B27/04, B31/04, B32/04, B37/04, B38/04, B45/04, B51/04 and B89/04 exhibited high degree of susceptible reaction to CWD with seedling death rate values of 76.23, 73.18, 89.96, 72.68, 73.89, 70.83, 86.56, 84.14, 85.33, 80.92 and 52.02, respectively (Table 1) .

3.2. Incubation Period (IP date)

Incubation period, the time period from inoculation to first symptom expression, exhibited highly significant (P<0.01) variation among Bale coffee accessions (Table 2). The mean incubation period among the tested accessions in days ranged from 0 to 77 days. Diseases symptom expression wasn't recorded on five accessions, namely, B64/04, B70/04, B104/04, B124/04 and B143/04. However, the least incubation period (77 days) was recorded for accession B-37/04 indicating that it was highly sensitive for coffee wilt diseases and the symptom appeared on it was earlier than susceptible check 'Gesha' cultivar (Table 2). Following B-37/04 seven accessions, viz, B05/04, B06/04, B10/04, B11/04, B12/04, B31/04, B32/04 , B51/04 and B89/04 that exhibited shorter (< 100 days) incubation period with values 91, 95, 95, 81, 86, 95, 86, 91 and 86 days, respectively, which were similar to Gesha cultivar (susceptible check) (Table 2).

Table-2. Incubation period (dates) for coffee wilt diseases on Bale coffee accessions under green house condition at Jimma research center

Accession	IP (date)	Accession	IP (date)	Accession	IP (date)
B-2/04	173^{a}	B-45/04	100^{cde}	B-139/04	178^{a}
B-3/04	173^{a}	B-51/04	91^{efgh}	B-140/04	134^{b}
B-4/04	109^{cde}	B-53/04	165^{a}	B-143/04	+
B-5/04	91^{efgh}	B-64/04	+	B-148/04	172^{a}
B-6/04	95^{defg}	B-70/04	+	B-156/04	175^{a}
B-10/04	95^{defg}	B-85/04	173^{a}	B-157/04	167^{a}
B-11/04	81^{gh}	B-89/04	86^{fgh}	B-159/04	133^{b}
B-12/04	86^{fgh}	B-92/04	105^{cde}	B-161/04	133^{b}
B-20/04	114^{bcd}	B-103/04	119^{b}	B-164/04	167^{a}
B-27/04	100^{cde}	B-104/04	+	B-167/04	105^{cde}
B-28/04	173^{a}	B-110/04	163^{ab}	B-156/04	105^{cde}
B-29/04	178^{a}	B-118/04	109^{cde}	B-169/04	178^{a}
B-31/04	95^{defg}	B-124/04	+	J-19*	86^{fgh}
B-32/04	86^{fgh}	B-130/04	119^{b}	J-21*	91^{efgh}
B-37/04	77^{h}	B-133/04	173^{a}	Gesha**	91^{efgh}
B-38/04	105^{cde}	B-137/04	170^{a}	370***	+
Mean					126.54
CV (%)					9.94
LSD (0.05)					20.43

+ indicate no external symptom was observed; Means followed by the same letters are not significantly different at 5% level of significance; LSD, Least significant difference; CV, coefficient of variation; IP, Incubation Period; * Moderately resistant; ** Susceptible; *** resistant Arabica coffee varieties to CWD used as check.

In this experiment, those accessions which showed susceptible reaction also showed earlier coffee wilt symptom development while those with resistant reaction had longer incubation period. In both of the cases, i.e., mean seedling death rate/severity and incubation period, the results indicating variation among accessions in terms of resistance reaction and time for disease symptom development implies the existence of variation among Bale coffee collection against coffee wilt disease.

3.3. Study of Field Survival Rate

The record of field survival rate of the coffee accessions revealed that there was variation among accessions. The mean survival rate of coffee accessions ranged from 16.67% to 100% with overall mean of 87.18 %. The majority of coffee accessions (28) survived 100% at field level (Table 3). However, the least survival rate (16.67%) was recorded for accession B27/04, B156/04 and B156/04 which indicates that these accessions are highly susceptible under field condition too (Table 3).

Table-3. Field Survival rate of Bale coffee accessions at Jimma agriculture research center

Accession	Survival Rate (%)	Accession	Survival Rate (%)	Accession	Survival Rate (%)
B-2/04	100.00	B-45/04	83.33	B-139/04	100.00
B-3/04	83.33	B-51/04	100.00	B-140/04	100.00
B-4/04	50.00	B-53/04	100.00	B-143/04	100.00
B-5/04	100.00	B-64/04	100.00	B-148/04	100.00
B-6/04	100.00	B-70/04	100.00	B-156/04	16.67
B-10/04	100.00	B-85/04	100.00	B-157/04	100.00
B-11/04	83.33	B-89/04	100.00	B-159/04	66.67
B-12/04	100.00	B-92/04	83.33	B-161/04	100.00
B-20/04	100.00	B-103/04	83.33	B-164/04	100.00
B-27/04	16.67	B-104/04	100.00	B-167/04	83.33
B-28/04	100.00	B-110/04	83.33	B-156/04	100.00
B-29/04	100.00	B-118/04	83.33	B-169/04	16.67
B-31/04	83.33	B-124/04	100.00	J-19*	60.00
B-32/04	100.00	B-130/04	100.00	J-21*	70.00
B-37/04	100.00	B-133/04	83.33	Gesha**	40.00
B-38/04	66.67	B-137/04	100.00	370***	100.00
Mean					86.18
Sdv					23.19

* Moderately resistant; ** Susceptible; *** resistant Arabica coffee varieties to CWD used as Check; Sdv=standard deviation

4. DISCUSION

Coffee Wilt Disease (tracheomycosis) is a systemic vascular disease caused by the fungal pathogen, *Gibberella xylarioides*, causes a total death of infected coffee trees. The disease has been a serious problem to coffee production in Ethiopia (CABI, 2003; Flood, 2009). Coffee wilt disease is currently restricted to coffee producing regions of central and eastern Africa, however, the disease can be potential threat to the world (Rutherford, 2006; Flood, 2009; Girma *et al*., 2009). CWD on

Coffee arabica was first observed in Ethiopia (Keffa province) by Stewart (1957), who described the wilting symptom and also identified the causal organism to be *Fusarium oxysporum* f.sp. *coffeae*. Lejeune. Based on comparative studies of the isolates collected from dying Arabica coffee trees from different origins and different *Coffea spp*., the causal agent was confirmed to be *Gibberella xylarioides* Heim & Saccas, of which *Fusarium xylarioides* Steyaert is the imperfect (conidial) state (Kranz and Mogk, 1973). Van Der Graaff and Pieters (1978) reported that this pathogen caused a typical vascular wilt disease and was the main factor of coffee tree death in Ethiopia. Subsequent surveys accompanied by isolation and identification demonstrated occurrence of *G. xylarioides* (*F. xylarioides*) in major coffee-growing regions of south and south-west Ethiopia (Van Der Graaff and Pieters, 1978; Merdassa, 1986; Girma, 1997; Eshetu *et al*., 2000; Sihen *et al*., 2012). During recent years, the prevalence and importance of CWD have been markedly increasing throughout coffee producing areas of the country (Girma and Hindorf, 2001; Girma *et al*., 2001; CABI, 2003; Girma, 2004; Oduor *et al*., 2005; Flood, 2009; Sihen *et al*., 2012).

The soil-borne nature of the pathogen and perennial character of coffee have made management of the disease difficult through the conventional control approach of 'uproot and burn infected trees at the spot'. Therefore, management of coffee wilt diseases using resistant varieties is the most appropriate, efficient, environmentally friendly and economical method. Several authors have reported varietal differences in resistance to CWD and suggested the use of resistant varieties as a means of controlling CWD (Fraselle, 1950; Deassus, 1954; Porteres, 1959).

The result of present experiment proved that there is important diversity in Bale coffee accessions in reaction to coffee wilt disease pathogen *Gibbrella xyralioides* infection. Coffee accessions like B02/04, B03/04, B28/04, B29/04, B53/04, B64/04, B70/04, B85/04, B104/04, B110/04, B124/04, B133/04, B137/04, B139/04, B140/04, B143/04, B148/04, B156/04, B157/04, B161/04, B164/04 and B169/04 had exhibited resistant reaction similar to the standard resistant control cultivar '370'. A number of researchers have reported existence of marked differences in resistance levels in Arabica coffee populations to CWD in Ethiopia (Van Der Graaff and Pieters, 1978; Merdassa, 1986; Girma and Hindorf, 2001; Girma, 2004; Sihen *et al*., 2012). Merdassa (1986) assessed the incidence of the disease in single-tree progenies of different coffee accessions for 6 years (1979–1984) at Gera and obtained tree loss ranging from 0.3% to 87%.

Symptom expression wasn't recorded on five coffee accessions in this experiment namely, B64/04, B70/04, B104/04, B124/04 and B143/04 which indicating that throughout study period they didn't show any wilt symptom and considered to be highly resistant for coffee wilt diseases. Lowest percentage seedling deaths accompanied by long incubation periods indicating resistant nature of genotype, while, high percentage of seedling death accompanied by short incubation period implies susceptibility of genotype (Girma and Chala, 2008). Accordingly, most of Bale coffee accession showed lowest value of seedling death rate and long incubation period implying their resistance reaction to CWD infection.

Significant variation was also observed in field survival rate .among coffee accessions. Number of researchers reported that, in fields affected by CWD, it is possible to observe considerable variation in disease severity among coffee cultivars (Van Der Graaff and Pieters, 1978; Merdassa, 1986; Girma, 1997; Girma and Hindorf, 2001; Girma *et al*., 2001; Girma, 2004). In previous study, cultivar SN-5, F-51/53 and 248/71 showed 100% tree loss, whereas F-35 and F-51 had significantly ($P < 0.05$) lower mortality rates of 9.3% and 27.9%, respectively, at Gera (Girma, 1997; Girma *et al*., 2001). Also some preliminary observations indicate that external factors such as temperature, rainfall, topography, coffee tree age, shade, soil type and weeding methods have significant effects on CWD (CABI, 2003; Flood, 2009).

5. CONCLUSION

The long-term prospects of successful management of CWD depend principally upon employing resistant coffee cultivars. A use of resistant varieties for management of CWD is the most appropriate, efficient, environmentally friendly and economically sounds method. In this regard, the genetic variability of Bale coffee accessions presents opportunity to develop CWD tolerant coffee varieties. Most of Bale coffee accessions, such as B02/04, B03/04, B28/04, B29/04, B53/04, B64/04, B70/04, B85/04, B104/04, B110/04, B124/04, B133/04, B137/04, B139/04, B140/04, B143/04, B148/04, B156/04, B157/04, B161/04, B164/04 and B169/04 resulted lowest seedling death rate (< 10 %), long incubation period and high field survival rate.

Further evaluation of promising coffee accessions in sick plot and detail field evaluation for major diseases, yield and quality is recommended. This study paper revealed alleles found in the coffee gene pool of Ethiopia may hold the key to the species long term survival providing the traits needed to cope with new diseases and climate change; this underline the importance of systematic evaluation of the coffee accessions and utilization of best performing accessions having high yielding, typical quality and disease resistant.

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