EVALUATION OF ARABICA COFFEE (*Coffea arabica* L) GERMPLASM FOR MAJOR COFFEE DISEASES WITH ESPECIAL EMPHASIS TO COFFEE WILT DISEASE (*Gibberella xylarioides)* AT JIMMA, ETHIOPIA

M.Sc. Thesis

DEMELASH TEFERI

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COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

MSc THESIS APPROVAL SHEET

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A Thesis

Submitted to the Department of Horticulture, School of Graduate Studies of College of Agriculture and Veterinary Medicines JIMMA UNIVERSITY

In partial Fulfillment of the requirements for the degree of MASTER OF SCIENCE IN AGRICULTURE (HORTICULTURE)

By

Demelash Teferi

June 2013

Jimma University

Jimma

DEDICATION

To My Parents, Teferi Busun **and** Wubayehu Demeke

STATEMENT OF THE AUTHOR

I, the undersigned, declare that this thesis is my original work and has not been presented for any institute or anywhere for the awards of any academic degree; diploma or certificate and all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of requirement for an MSc degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University of Library to be made available to borrowers under rule of the library.

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BIOGRAPHICAL SKETCH

The author was born from his father Teferi Busun and his mother Wubayehu Demeke in a small town called Sheboka which is found in Oromiya region, West Shewa Zone, Bako Tibe district in September 28, 1963. He attended elementary and junior school at Sheboka and Bako schools. He had his high school education at Ambo Comprehensive Secondary School. He joined Debrezeit Junior College of Agriculture and Jimma University College of Agriculture and Veterinary Medicine in 1982 and 2007 and graduated with Diploma in Crop Production and Protection Technology and BSC in Horticulture, respectively. He has been with coffee research working as senior technical assistant up to April 1983 and from May, 2007 promoted as a Researcher and currently working in Plant Protection Research Division stationed at Jimma Agricultural Research Center. He joined the School of Graduate Studies of Jimma University in February 2010 to pursue his MSc in Horticulture.

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LIST OF ABBREVIATIONS

ASIC	Colloque Scientifique International sur le Café
AUDPC	Area Under Disease Progress Curve
CABI	CAB International
CAR	Central African Republic
CBD	Coffee berry disease
CORI	Coffee Research Institute
DRC	Democratic Republic of Congo
Ha	Hectare
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
JARC	Jimma Agricultural Research Center
PSA	Potato sucrose agar
PDA	Potato sucrose agar
SNA	Synthetic low nutrient agar
SAS	Stastical analysis system, soft ware
RCWP	Regional Coffee Wilt Program
TaCRI	Tanzania Coffee Research Institute

ABSTRACT

Among diseases affecting coffee production, coffee berry disease (Colletotrichum kahawae), coffee wilt disease (Gibberella xylarioides) and coffee leaf rust (Hemileia vastatrix) are the most important ones. In Ethiopia, coffee wilt disease (CWD) is increasingly becoming more important, especially in garden semiforest and plantation coffees. The national incidence and severity of CWD in the country were 29.9 % and 3% respectively, with financial loss of more than 3.7 million USD, justifying all possible control options including use of resistant coffee varieties. This study was, therefore conducted under both laboratory and field conditions at Jimma Agricultural Research Center with objective of evaluating Arabica coffee germplasm and identifying resistant genotypes to coffee wilt disease. The experiment was conducted on one hundred coffee accessions (each 30 seedlings per box) grouped in two batches in a randomized complete block design with three replications. Stem nicking inoculation was employed with 2.3x 10^6 conidia of G. xylarioides isolate at cotyledon stage of the seedlings. In addition, the tested accessions were further assessed for coffee wilt, coffee berry disease (CBD) and coffee leaf rust (CLR) under field conditions at Jimma. In the seedling test, significant variations were recorded among coffee accessions in reaction to CWD. Mean wilt (dead) percent seedlings varied between 0 - 98.8% and 2.3 - 97.5 % in batch I and II, respectively. Nine accessions from batch I namely 279/71, 226/71, 259/71, 244/71, 120/71, 3/70, 16/70, 245/71, and 30/70; and 10 accessions from batch II 27/77, 12/74, 26/77, B3/06, B2/06, 44/83, 48/83, B1/06, 11/77 and 13/74 showed low percentages of seedling death expressing high to moderate resistance reactions as compared to resistant standard check catimor J-19. however, coffee accessions from batch I 1/70, 18/84, 5/84, 5/72, F-31, Geisha, 54/70,250/71, 4/84 13/79 199/71 and coffee accessions 9/78, 26/84, 20/84, 24/84, 22/84 from batch II were highly susceptible. Correlation between mortality of coffee trees in the field and wilt severity of seedlings in artificial inoculation were not significant, while incubation period and AUDPC was found significant. *The clustering patterns of 55 and 51 coffee accessions were generated from means of three CWD parameters grouped coffee accessions into three clusters. In both set of experiments, the first cluster was characterized by grouping all susceptible reaction to CWD, cluster three in batch one express resistant reaction, where as cluster two in batch one and cluster two and three in batch two express moderately resistant reaction to CWD. The disease assessment result indicated that coffee*

berry disease varied between 0-95.6 and 0-83.1 in batch I and batch II respectively. The mean coffee leaf rust varied from 0-34.4 in batch I and 0-13% in batch II. The results of the study, implicated that there is important diversity in conservation block of Jimma Agricultural Reserch Center in reaction to G. xylarioides infection. However, susceptible reaction was observed in coffee accessions collected from Kaffa, Iluababor, West Gojam and West Wellega. Further evaluation of moderately resistant CWD accessions in different agroecological areas in wilt devastated gardens or farms (sick plot) so as to validate their performance and adaptation to different localities (multilocation trials) are recommended.

Key Words: coffee wilt disease, disease severity, incubation period, Area Under Disease Progress Curve (AUDPC)

1. INTRODUCTION

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

Coffee has for centuries played an important role in the Ethiopian economy and represents the main cash crop cultivated by small scale farmers for social, economic, political and ecological sustainability. Historically, Ethiopia is the oldest exporter of coffee in the world (Girma, 2004). Ethiopia is currently producing an estimated 9.8 million bags that would rank the country as the third largest coffee producer in the world after Brazil and Vietnam, beating out Columbia (ICO, 2012).

The estimated coffee production area in Ethiopia is in the range of 450,000-600,000 ha (Alemayehu *et a*l. 2008). Although there are potential six million hectares of cultivable land suitable for coffee production (Mekuria *et al*. 2004), however production supply is seasonal and fluctuate due to climatic variation and so does with prices (ECX, 2011).

In general, all Ethiopian coffee production systems appear to be under four cultivation technique. The major production systems include: forest coffee (10%), semi-forest coffee (35%), garden coffee (50%) and plantation coffee (5%). More than 90% of the production is from the garden, semi-forest and forest coffee systems of small-scale farmers; whereas the rest comes from large-scale plantation coffee (Alemayehu *et al*. 2008, MoARD, 2008).

The average yield in the country is generally low (about 500kg /ha per year) which is half of that achieved in Latin America and almost one third of Asia's productivity. This is partly due to continued reliance on unproductive coffee varieties, the widespread and prevalence of pests and diseases (Girma *et al*., 2009a, Phiri *et al*., 2009). However, coffee suffers from a range of co-evolved diseases including coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR) caused by *Colletotrichum kahawae, Gibberella xylarioides* and *Hemileia vastatrix*, respectively.

The CBD resistance selection and screening national program released 31 CBD resistant varieties for different coffee growing agroecologies. Out of these, the application of landrace development program with special emphasis to maintain the typical quality of each specific area, viz, Hararghe, Sidama/Yirgachefe, Wollega (Gimbi) resulted in 11 varieties (Bayetta *et al*. 2000, Bayetta 2001, Chala *et al*. 2011). CLR existed in Ethiopia since centuries, but never eradicated trees like for instance in Asia. The diversity of susceptible, tolerant and resistant plants and the availability of hyperparasites, *Verticillum hemileiae,* protect coffee against coffee leaf rust under prevailing conditions (Hindorf and Arega, 2006).

Coffee wilt disease (CWD) or tracheomycosis become the principal production constraint for Robusta and Arabica coffee in Uganda, DRC, Tanzania and Ethiopia. CWD is a special significance because unlike other major disease such as coffee leaf rust (CLR) and coffee berry disease (CBD) it totally kills the tree. Once the tree is infected there is no remedy other than to up root the tree and burn it *in situ* (Phiri *et al.* 2009). In Ethiopia tracheomycosis is increasingly becoming more and more important, especially in garden, semi forest, and forest and plantations coffees. The national incidence and severity of CWD in the country were 29.9 % and 3% respectively, and in monetary terms it causes financial loss of more than 3.7 million USD (CABI, 2003).

Current control method of CWD in Ethiopia includes eradication of diseased trees, mulching, the use of cover crops and protection by stem painting as a preventive measure. However these methods are expensive, impractical to implement and does not provide effective control measure (Muleta *et al*. 2007). Searching for host resistance can serve as long term solution in managing coffee wilt disease.

Jimma Agricultural Research Center (JARC) having the national mandate for coffee research in the country has collected large number of Arabica germplasm from different parts of the country and from abroad. Exploration and germplasm collection are the major component of coffee genetic improvement program which are the basic raw materials to meet the current and future requirements of coffee improvement programs which offers good source of genes with potential resistance to major diseases and making them important for modern plant breeding (Bayetta and Labouisse, 2006).

So far at JARC with 51 collection missions about 5820 coffee accessions were collected and maintained in seven *ex-situ* sites (Fekadu *et al*. 2008).The Biodiversity Conservation Institute of Ethiopia preserved more than 5,000 accessions in *ex-situ* coffee gene bank at Choche biodiversity unit in Jimma Zone, south west Ethiopia. As an *in-situ* conservation about 25,000 ha of land have been preserved by the Ethiopian Government with support from EU (Paulos and Demel 2000, MoARD, 2008). Currently, the *C. canephora* and *C. arabica* gene pools in some Eastern and Central African countries are being threatened at varying rates by CWD. It is therefore necessary to design germplasm collection and utilization programmes (Musoli *et al.* 2009).

At Gemadro Coffee Plantation Project of Ethio Agr-iceft alone, 91.2 ha of coffee was up rooted due to CWD, coffee varieties 7454, 744 and Geisha were considered field susceptible, at Guraferda Woreda Betrework Alemu private farm out 340 ha planted with Geisha low land varitiey 200 ha was lost due to CWD (JARC, Crop protection unpublished data). In Ethiopia even though the current status of CWD is not updated by national survey, information obtained from different corner of coffee sector reveals that the disease is becoming serious especially in plantation and semi plantation coffee. In order to control coffee wilt disease effectively it is better to search and develop resistant varities of coffee. Therefore this study was initiated to evaluate some coffee germplasm maintained at JARC for for their resistance to CWD in green house at Jimma Agricultural Reserch Center (Jimma Zone).

Therefore the current study was designed with the following objectives.

General objective

- To evaluate some coffee germplasm maintained at Jima Agricultural Rearch Center for their resistance to coffee wilt disease in greenhouse and assess their performance for coffee berry disease, coffee leaf rust under field conditions.

Specific objectives

- To evaluate and identify resistant accessions against coffee wilt disease
- To assess the performance of moderately resistant CWD accessions for their resistance coffee berry disease and coffee leaf rust under field conditions.

2. LITERATURE REVIEW

2.1. Taxonomy and Genetic Diversity of *Coffea arabica* L.

2.1.1. Taxonomy

The genus *Coffea* belongs to the family Rubiaceae, which has around 500 genera and over 6000 species. Rubiaceae is one of the largest families of flowering plants, mostly trees and shrubs and more rarely herbs. Most species of the family occur in the tropics, particularly in the lower story of tropical rain forests.

New species of the genus *Coffea* are continually being discovered. Many new species have been discovered in recent years in the forests of Eastern Africa (Bridson, 1982) and Madagascar (Davis 2001, Davis and Rakotonasola 2000). In total around 92 species of *Coffea* are currently recognized, of which 45 species occur in Madagascar, 44 in Africa and three in the Mascareness (Dullo 1998a, Davis and Rakotonasolo 2001).

Several phenotypically deviating populations and lines of cultivars have been formally recognized as varieties of *C.arabica* (var.bourbon Choussy, var.typica Cramer). Sylvain (1958), attempted to systematically classify the coffee cultivars growing in Ethiopia. In the classification, 12 major types based on tree habit, leaf color and size, calyx characteristics, size and shape of fruits, seed and yielding ability has been recognized. The naming used was the locality where the types were found. The 12 Ethiopian coffee types of Sylvain (1955, 1958) are the Enarea, Jimma, or Kaffa, Agaro, Chochie, Zeghie, Walkite, Wallayita, Irgalem, Dilla, Arba Gougou, Harar and Loulo. Over 130 traditional land races or farmers' cultivars were recorded from different coffee growing regions of Ethiopia (Admasu *et al.* 1989, Demel and Assefa 1994, Tadesse *et al.* 2001). Known cultivars of *C. arabica* growing outside of Ethiopia include 'Blue mountain' in Jamica, 'French Mission' in Kenya and Tanzania 'Kent's' in India 'Mundo Novo' in Brazil, 'San Ramon ' and its segregate 'Villalobos ' in Costa Rica, which are derived from two main lines of cultivars (Anthony et al. 2002).

2.1.2. Genetic diversity and approaches in coffee genetic conservation

Coffea arabica differs from other species in the genus in that it is the only tetraploid (2n=44) and self fertile species. All others are diploid (2n=22) and self infertile (Charrier and Berthahud, 1985). Botanist and genetist who visited Ethiopia in the 1960s and before observed the presence of high phenotypic variability among the wild coffee populations and land races (Sylvain 1955, 1958). Many important characteristics were identified in the Ethiopian Arabica Coffee such as resistance to coffee leaf rust, (Meseret Wondimu, 1996) Cofee berry disease (Robinson, 1974; Van der Graaf, 1981) and Coffee wilt disease (Girma, 2004; Arega Zeru, 2006; Girma *et al*. 2008b) .The distinct attributes such as resistant to coffee diseases, adaptable to diverse environmental conditions also indicates the existence of diverse *C.arabica* genetic resource in the country. However this gene pool is under serious threat mainly because of deforestation of its natural habitat for timber and food crop production and replacement of land races by few high yielding and disease resistant improved varieties (Tadesse *et al*. 2001, Yigzaw, 2006).

Approaches in plant genetic conservation can be broadly categorized in to two: *Ex-situ* and *in-situ*. *In-situ* conservation is defined as conservation of ecosystems and natural habitats and the maintenance and recovery of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties. *Ex-situ* conservation is defined as the conservation of component of biological diversity outside their natural habitats. Seed storage, *in vitro* storage, DNA storage, pollen storage and storage of living plants and botanical gardens are the major techniques employed of *ex-situ* conservation. Among these two methods namely *in vitro* conservation and field gene banks are the only methods for conservation of coffee genetic resources (Dullo *et al*. 1998b).

2.1.3. Field gene banks

Field gene banks are commonly used for such plants as coffee, cocoa, coconut, mango and yam. The advantages of field gene banks are that the material is easily accessible, for utilization, and that evaluations can be undertaken while the material is being conserved. The disadvantages are that the material is restricted in terms of genetic diversity, is susceptible to pests, diseases and involves large area of land.

Coffee collection and *ex-situ* conservation in Ethiopia starts since 1960s, many of these genetic resources are conserved as *ex-situ* at Jimma Agricultural Research Center (JARC) its sub centers and Institute of Biodiversity at Chochie in Jimma Zone (Labouisse *et al*. 2008; MoARD, 2008). As *ex-situ* conservation , so far 5,820 Arabica coffee accessions are collected from many areas and are being maintained at Jimma Agricultural Research Center of Ethiopian Institute of Agricultural Research (Table 1). Also more than 5,000 coffee accessions are being maintained by the Institute of Biodiversity at Chochie. In order to serve as world heritage and global arabica coffee reserves about 25,000 ha of land have been preserved as *in-situ* conservation by the Ethiopian Government with support from EU (Fekadu et al. 2008; MoARD, 2008). This gene banks are important because coffee seeds cannot be stored for long period of time in seed banks as they are recalcitrant (Aga *et al*. 2003). The world *C.arabica* production is largely based on a very small number of cultivars with low genetic diversity, making them vulnerable to climatic and biotic hazards. The 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 germplasm collection and utilization programmes (Labouisse *et al*. 2008).

Table 1: Summary of indigenous and exotic coffee collections maintained at Jimma Agricultural Reaserch Center, Jimma Ethiopia.

Type of collection	Year collection	No of collection mission	No of accessions				Total
			Indiginious collections**			Exoitic Collection*	
			Oromiya (54)	SNNP (28)	Other regions (10)		
National collection Different regions	1966, 1970-2004	24	622	424	139		1185
CBD resistant Selection program	1973-75,1980-87	7	721	320	38		1041
Regional collection	1994-2005	20	2748	609	9		3404
Sub total		51	4091	1353	186		**5630**
Exotic collections*	1967-84	6				190	190
Total		57	4091	1353	186	190	5820

Figures in parenthesis indicate the number of weredas where collections were made.
Source: Fekadu et al. 2008.
* Introduced from India, Tanzania, Cuba and Portugal.
** Amhara and Gambella.

2.2. Constraints to Coffee Production

Although there has been a considerable increase in coffee yields and overall production, the average coffee productivity in Ethiopia remains low (about 500 kg/ha per year) as compared to the world standard and to other coffee producing countries, namely, Brazil, Vietnam, India and Kenya. Coffee is largely produced by small-scale subsistence farmers and is a low input– output crop in Ethiopia. It grows under age-old traditional production systems (forest, semi-forest and garden) (Girma *et al*. 2009a).

Agronomic practices and crop husbandry remain conventional, and the adoption and diffusion of improved technologies, e.g. high-yielding cultivars and better pre- and postharvest management activities, have been slow. The production of Arabica coffee in Ethiopia is to a great extent limited by diseases and pests. Coffee diseases such as CBD, CWD and CLR cause severe crop losses. Coffee insect pests, mainly antestia, leaf miners and coffee berry borer, cause damage in most coffee growing regions of the country. Volatility and fluctuation in coffee prices, be it at local or international markets, can have enormous impacts to farmers' livelihoods and to investment decisions in the coffee industry as a whole (Muleta *et al*. 2007, Girma *et al*. 2009a).

Fourteen diseases (Demelash *et al*. 2008) and more than 47 insect pests (Esaiyas *et al*. 2008) have been recorded on *C. arabica* in Ethiopia, and among others, CBD, CWD, CLR and antestia are foremost in reducing the quantity and quality of coffee in the country. Coffee berry disease (*Colletotrichum kahawae*) can cause up to 100% yield loss, with national average losses varying between 25% and 30% (Van der Graaff 1983, Merdassa Ejeta, 1986 and Eshetu *et al*. 2000). Coffee wilt disease is prevalent in almost all coffee-growing regions, with national average incidence and severity of 29.9 % and 3%, respectively (CABI, 2003). Coffee leaf rust (*Hemileia vastatrix*) is widely distributed all over coffee growing regions of the country with varying intensities. Highest incidence with mean percent 42.5 in Keffa, 41.9 in Illubabor, and 39.6 in Hararghe. The average national infected trees were estimated to 12.9%, in 1980 and increased by three fold 36.3% after ten years in 1990 (Mesret,1996). Eshetu *et al*. (2000) reported as high as 27% CLR severity in Hararghe and this might be attributed to the distribution of susceptible host, occurrence of virulent races and the type of coffee production systems. Antestia

(*Antestiopsis intricata*) is the major coffee pest inflicting considerable damage, amounting 9% berry fall and 48% darkened coffee beans (Million, 2000).

2.3. Coffee berry disease (CBD)

In Ethiopia CBD first reported in 1971 (Van der Vossen and Walyaro, 1980). Then spread to all Major coffee producing regions within very short period except to the lower altitude. Big plantations, garden and forest coffee, with and with out shade all were infested alike (Tefestewold Biratu, 1995). So environmental issues except low altitudes did not make much difference.

Merdassa Ejetta (1986) reported yield losses of 51 % at Melko and 81% at Wondo Genet due to CBD. In 1994 crop season prevalence of CBD was conducted in Oromiya Region and Southern Nations Nationalities and peoples region (SNNPR) and the result indicated 38.8 and 17.2 % of mean incidence and severity of the disease respectively (IAR, 1997). Accoding to the result CBD pressure was very high at higher altitudes in the South-West region, while severe disease was recorded in valleys of Gedeo Zone. According to to Tefestewold Biratu (1995) CBD severity varied from yeat to year among Woredas and regions. In Amhara region where CBD ocuurs, survey result showed that an average CBD severity for 1996/97 crop season was 38% (Tesfaye Alemu and Ibrahim Sokar, 2000).

Survey conducted in 1997 and 1998 in six major coffee growing zones (in 32 woredas) of Oromiya region showed an average of 31 % and 32% disease severity for the respective years (Melaku Jirata and Samuel Assefa, 2000). CBD incidence and severity assessment in 10 zones and 31 woredas of Southern Nations Nationalities and peoples region (SNNPR), conducted in September 1998, resulted with 40% and 22.8 % mean incidence and severity of the disease respectively (Tesfaye Negash and Sindu Abate, 2000).

The results of three independent studies evidenced no host specialization (physiologic races) in the CBD pathogen population in Ethiopia (Tefestewold 1995, Eshetu and Waller, 2003, Arega, 2006).Twelve C.kahawae isolates sampled from four montane rainforests coffee areas in Harena, Bonga, Sheko, and Yayu: inoculated with seedlings of three widely grown CBD resistant cultivars and susceptible check. The result emphasizes that

horizontal resistance in host populations practically advantageous to deploy resistant coffee varieties to CBD management.

In Ethiopia, pure-line variety development approach remains to be a short-term breeding strategy in Arabica coffee improvement program aimed for developing improved variety for different agro-ecologies so, far 31 CBD resistant varieties have been developed for different coffee growing agro ecologies/areas. Out of this, the application of land race development program with special emphasis, to maintain the typical quality of each specific area, viz, Hararge, Sidama/Yirgachefe, Wellega (Gimbi) resulted 11 varitiies. Three hybrids namely Ababuna, Melko CH2, and Gawe with yield of 24-26 q/ha clean coffee have been released for mid altitude (Behailu et al., 2008. Chala *et al*.2013).

The result of a recent in vitro and invivo study conducted by Amsalu (2011) on antifungal activity of some medicinal plants against coffee berry disease caused by *Colletotrichum kahawae* were promising. Of 8 medicinal plants 2 significantly inhibited the mycelial spread of *Colletotrichum kahawae*. *A. sativum and C. macrostachyus* had significantly higher inhibitory effect on C. kahawae with both aqueous and ethanol.

2.4. Coffee leaf rust (CLR)

Coffee leaf rust (CLR) which is caused by *H.vastatrix* was first detected in East Africa (Kenya) but the first record on cultivated coffee tree was reported from Sirilanka in 1869.The disease spread to India and South West Asia and reported from Brazil in 1970 and spread to southern and northern part of the countries covering major coffee growing areas in Latin America. In spite of devastating coffee plantation and its replacement by tea or rubber in Sirilanka termination of coffee export to United Kingdom compelled habituated consumers to adapted tea drinking. More ever it was responsible for sharp decline of coffee yields from 1500 to 300kg/ha. Generally rust incurs an estimated yield loss between 10-40% in different countries and cost of control with fungicide is very high. In Brazil annual loss was estimated to about 30% and entailed expense for chemical control adds up to equivalent US $ 100-120/ha (Muller *et al*., 2004).

Coffee leaf rust was first reported in 1934 in Ethiopia but it has never reached to epidemic level to cause eradication of Arabica coffee. This may be as a consequence of long term coexistence of rust and coffee which created a balanced pathosystem. Currently, CLR is widely distributed all over coffee growing regions of the country with varying intensities. Highest diseased trees with mean percent 42.5 in Keffa, 41.9 in Illubabor, and 39.6 in Hararghe. The average national infected trees were estimated to 12.9%, in 1980 and increased by three fold 36.3% after ten years in 1990 (Mesret,1996). Eshetu *et al*., (2000) reported as high as 27% CLR severity in Hararghe and this might be attributed to the distribution of susceptible host, occurrence of virulent races and the type of coffee production systems. Although the disease has been present for such long period and increasing from time to time, it was considered as minor significance and neither inflicted yield loss nor have management strategies been practiced to combat the disease in the country.

In Arabica coffee, vertical (complete), horizontal (race non-specific) and incomplete (partial) resistance to the leaf rust disease was reported. Complete resistance inhibit the infection process and prevent production of inoculums while partial resistance which may be also called incomplete resistance does not inhibit infection process completely but allow the production of inoculums through increased latency period and reduced lesion density (Muller *et al*., 2004).

So far over 40 different races have been identified all over the world out of this, in Ethiopia, the existence of six physiologic races in different coffee growing regions was reported. Race III the most dominant (52.7%) and mostly prevalent in south west forest coffee regions followed by race II which is distributed in all areas where rust existed and in garden and plantation areas. Recent reports also confirmed the existence of races III and X in the forest coffee at Bonga and race II, at Birhan-Kontir (Meseret 1996, Hindorf and Arega, 2006).

In Ethiopia, large genetic diversity of *C.arabica*, high level of horizontal (non specific) resistance to CLR and availability of at least some incomplete resistance likely protects coffee against rust under prevailing conditions. Meseret (1996) identified coffee plants with partially (incomplete) resistance to CLR from lowland forest coffee of south-western

Ethiopia. Catimor J21 and Catimor J19 were released coffee cultivars by JARC for their resistance to CLR. Chala (2008) reported Catiomor lines manifest rust incidence greater than 60% in September with leaves completely covered by abundantly sporulating lesion indicating their susceptibility to in the field at Tepi. Catimor J21 showed highest rust severity of 47.6% at Bebeka.

2.5 Coffee Wilt Disease (CWD)

2.5.1. History of Coffee Wilt Disease (CWD) in Ethiopia

Historically, CWD on *C. 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*. Later, based on comparative studies of the isolates collected from dying arabica coffee trees from different origins and different *Coffea* spp., the causal 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). Even in some localities like Bebeka and Teppi, CWD outbreaks were noticed in large-scale plantation coffee (Girma 1997, Eshetu *et al.* 2000). During recent years, the prevalence and importance of CWD have been markedly increasing throughout coffee producing areas of the African countries (Girma *et al*. 2001; CABI, 2003; Girma, 2004; Oduor *et al*. 2005).

2.5.2. Disease Symptoms

CWD (also known as tracheomycosis) kills the whole coffee tree within a short period. Infected arabica coffee plants usually occur singly or at random in groups in the affected fields. The earliest symptom of infection on both mature and young coffee trees is

epinasty of leaves on some branches in the lower tree canopy. The leaves become brown or dark brown within two or more weeks and finally drop off the branches. These external symptoms most frequently start on one side of an infected coffee tree (unilateral or partial wilting) but eventually advance so that the whole plant is affected. Later in the season, completely wilted trees become desiccated and severely defoliated. These trees cannot be easily pushed over and uprooted as opposed to coffee trees that have died from root rot disease (*Armillaria mellea*). Internally, brown or blue–black discolored bands are seen on the exposed wood of the stem. Dark fruiting bodies (perithecia) of the pathogen can be observed on the bark of stems especially around the collar region and occasionally on branches of dead coffee trees (Girma *et al*. 2001). Similar symptom description was given by (Van der Graaff and Pieters 1978, Flood 1997, Girma 1997, and Girma *et al*. 2008b).

The characteristic partial wilting symptom accompanied by discolored internal tissues effectively facilitates diagnosis and recognition of infected coffee trees in the field. This early detection allows rouging out of the infected trees early in the season before fungal sporulation at the advanced stage of pathogenesis (Girma and Hindorf, 2001).

2.5.3. Biology and Taxonomy of *Fusarium xylarioides* (*Gibberella xylarioides*)

Though little is known about the fungus that causes tracheomycosis, it lives in the soil, on infected debris, in an alternative hosts or as resistant propagules of species and enters the coffee tree through wounds in the base of the tree or in the roots (Flood 2003, Girma and Mengsitu 2000, Rutherford, 2006).

Two asexual spores (macroconidia, microconidia) and the third sexual spore (ascospores) allow the pathogen for the production of highly variable population, in addition to the parasexual cycle (Flood 2003, Girma 2004, Rutherford 2006). *Fusarium xylarioides* survives for two to eleven years in the soil as “saprophyte” because it produces resting spores or chlamydospores. Moreover, the sexual spores (ascospores) produced in the perithecia may be able to act as survival spores (Flood, 2003; Girma *et al*., 2009b).

The taxonomy of *Fusarium* is based on the morphological characters including the presence or absence, the shape and the dimensions of micro conidia, macro conidia basal

cells and the growth and color development on different media are used as markers in practice (Flood and Brayford, 1997; Girma, 2004). All *Gibberella* species are sexual states or teleomorphs of *Fusarium* species, which are destructive plant pathogens (Desjardins, 2003). The anamorphic stage F. *xylarioides* was first described by Steyaert from stem samples of diseased coffee trees obtained from *coffea excelsa (*Lewis Ivey *et al*., 2003). The teleomorph form was observed by Saccas in 1949 on dead trees of *C. noearnolandiana* and described and renamed as the *Gibberella xylarioides* (Nelson *et al.* 1983). The fungus was indicated as one of heterothallic ascomycetes having male and female strains, which can be identified based on the colony appearance and conidial morphology (Booth 1971, Nelson *et al.* 1983).

Girma and Mengistu (2000) described the pigementation and frequent shape of condia. The fungus consistently produces characteristic pigmentation more dominant in potato sucrose agar (PSA) than in potato dextrose agar (PDA) at 22±2^{o}c temperature under 12 hours light and dark cycling conditions than in constant dark or light. The most frequent pigments of Arabica isolates were grayish or pale white, light purplish or light violet that become dark bluish or dark violet in older colonies. Robusta and Excelsa strains produce a distinctly orange diffusate color. Apparently *Gibberella xylarioides* colony grows at as slower rate than colonies of *Fusarium oxysporium*, *F. solani*, *F. stillboides* and *F. lateritum (*Girma, 2004*).*

The majority of macroconidia of *Gibberella xylarioides* are distinctly curved while some were cylindrical, falcate, or fused with visibly pointed apical and foot shaped basal cells. Macro conidia may occur as 1-3 septate cells 18.7 x3.0 for one stated to 34x 3 µm for three septated macro conidia. Micro conidia are morphologically more variable than macro conidia with cylindrical, curved, allentoid, u and comma shaped and measures 11.2 x2.6 µm on average.The perithecia are apparently dark in colour globose in shape and containing enormous of ascospores. Asci are cylindrical and each ascus often contains 8 ascospores. The ascospores are fusoid and 1-2 septate with a slight constriction at the septum and measures 5.2 x 12.1 µm on average. Mature ascospores germinate by forming germ tubes one end (1-celled) or both ends (2-celled) and about 90-100% germination was estimated on water agar (Girma, 2004; Girma *et al*. 2007).

2.5.4. Molecular and host pathogen interactions studies

In RAPD-PCR analysis of 22 *G. xylariodes* isolates of the recent and historical collections from Arabica , Robusta and Excelsa coffees, the Ethiopian Arabica isolates clustered in to a single homogenous population although distinctly polymorphic to the recent and historical strains from Robusta and Excelsa coffees. The historical Arabica strain was slightly different from all the recent isolates in Ethiopia illustrating little genetic change in the population structure over the last 3 decades (Adunga *et al*. 2005). The result of three independent sets of cross inoculation experiment (Girma *et al.* 2009b) to determine regional diversity of the current and historical strains of the coffee wilt pathogen collected from Coffee spp. discovered that *C. arabica* isolate from Ethiopia shown to be aggressively pathogenic to seedlings of the Kenya (K7 and SL28), Ethiopia (7454 and 74165) and Costa Rrica (Yemen, Java and E-238), but the isolate did not induce disease in *C.canephora*. On the other hand *C. canephora* isolates obtained from Uganda and Tanzania attacked four *C. canephora* lines from Democratic Republic of Congo, Ivory Coast and seedlings of *C. liberica* lines.

Neither of Robusta isolates induced symptoms in *C. arabica*. The results of host pathogen interaction in coffea- *Gibberella xylarioides* pathosystem proof host specializaition occurs in *Gibberella xylarioides* population.

Following on from the detailed host pathogen interactions supported by random amplified polymorphic DNA analysis (Girma, 2004; Adunga *et al*. 2005) introduced the existence of host specialization in to at least two pathogenic forms with in *Gibberella xylarioides* populations *Gibberella xylarioides* f.sp. *abbyssiniae (anamorph: Fusarium xylarioides* f.sp.*abyssiniae)* for the fungus strains attacking only *C.arabica* and limited to Ethiopia and *Gibberella xylarioides* f.sp *canephorae (anamorph: Fusarium xylarioides* f.sp *canephorae)* pathogenic to *C. anaphora* and *C.excelsa*.

2.6. Coffee Production Systems and Coffee Wilt Distribution in Ethiopia

Coffee production in Ethiopia is broadly grouped into four systems on the basis of biological diversity of the species and level of management, namely, forest, semi-forest, garden and plantation coffee (Meyer, 1965; Paulos and Demel, 2000). Forest coffee regenerates spontaneously from self-sown seedlings as in the understory of intact multilayered tropical rainforests. The system is characterized by a very rich genetic diversity of both coffee and other flora and fauna. This coffee growing system is situated in the West and the South-West of the country.

Coffee wilt disease was found in forest coffee areas that has being posing considerable loss. Its incidence was reported 0-16%, 9-10%, 0-6%, and 0-30% in forest areas of Harena, Bonga, Sheko, and Yayu respectively. The mean incidence varied between 2.4% at Sheko and 16.9% at Yayu. The disease was found expanding and damaging the coffee trees particularly in Yayu and Harena. This was the first documented report that showed presence of CWD on forest coffee trees (Arega, 2006).

Semi-forest or semi-domesticated coffee is simply derived from the wild forest coffee through human intervention and domestication, i.e. by thinning the dense ‘overstorey’ of the forest trees and slashing the understorey bushes and shrubs. The open areas are filled mostly by transplanting naturally propagated coffee seedlings from under the mother trees resulting in irregularly spaced trees with a high population density.

Slashing is practiced once a year just before or during coffee picking season. Semi-forest coffee also contains diverse populations of coffee and contributes 35% to the total coffee production of the country, although its productivity is low (Paulos and Demil, 2000).

The mean incidence in semi-forest coffee ranged from 3.6% at Mettu to 15.5% at Gera situated in South-West coffee-producing areas and the severity varied between 18.6% and 25.4% in some coffee fields at Yirgacheffe (Girma, 2004). A similar situation was observed in Bale, Jimma, Iluababor, and West Welega zones (CABI, 2003).

The garden coffee system is predominant in the southern and southeastern regions, with plots of varying size (usually less than 0.5 ha) around farmers' dwellings and predominantly intercropped with a variety of fruit, root and cereal crops.

The coffee population is less diverse consisting of many landraces. This system involves more intensive management practices such as slashing and hoeing (three to five times per year), and to some extent, the coffee trees are pruned, mulched and fertilized with organic materials (Workafes and Kassu, 2000).

CWD is prevalent in the southern region, specifically in the three major quality-coffee-producing districts, namely, Wonago, Kochore and Yirgacheffe of Sidama and Gedeo zones, with highest incidence in Yirgacheffe followed by Kochore and Wonago. The severity of wilting in the sample fields in Yirgacheffe varied between 27.2% and 43.5% in the garden coffee as compared to that of the semi-forest coffee (Girma, 2004).

Plantation coffee is a relatively new farming system for coffee cultivation in Ethiopia. The system involves monoculture of plants derived from CBD-resistant and high-yielding cultivars in nurseries and transplanting them into well-prepared land. Improved agronomic practices such as row planting, correct spacing, intercropping, mulching, pruning, shade regulation and to some extent advanced postharvest technologies are being applied. Higher yield and good-quality coffee are obtained from plantation coffee.

The disease incidence is more severe in plantation coffee such as at research centers, on larger farmer holdings (1 to 5 ha) and in large estate commercial farms. CWD is commonly encountered in the research plots at Gera and Jimma amounting 42.5% and 48.2%, respectively. It is serious in the farmers' coffee plantations at the Gera, Chira and Gechi districts, with respective mean incidence ranging from 21.7% to 25.5%, from 32.3% to 77% and from 35% to 60%, respectively (Girma, 2004).

The overall mean coffee tree loss in the farmers' plantation was more than 30%, furthermore the highest was at Bebeka 65.2% (Girma, 2004).Van der Graaff (1979) remarked that some spectacular failures of the modern plantations system could be due to *G. xylarioides*, and when comparisons are made across production systems, the disease is

more destructive in garden and plantation coffees than in forest and semi-forest coffee systems. The latter two systems are composed of heterogeneous coffee populations possessing varying levels of resistance and less human interference.

However, in the former systems, characterized by relatively homogenous coffee trees and high levels of intervention, the disease spreads from tree to tree, from row to row and from one block to the other developing throughout the field , a remarkable increase in CWD severity of 11.5% was recorded over a 6-month period in nine districts (Weredas) of Gedeo and Sidama zones of Ethiopia (CABI, 2003; Girma, 2004).

CWD occurs in all of the above coffee production systems to varying extent of damage among and within coffee fields and districts (Weredas) depending on different interacting factors, mainly susceptibility of coffee trees, intensity of cultural practices and environmental conditions (Merdassa, 1986; Girma *et al*. 2001; CABI, 2003; Girma, 2004).

2.7. Management of Coffee Wilt Disease (CWD)

2.7.1. Quarantine measures

Quarantine is an important control method for CWD and is aimed at preventing the movement of infected plant material, infected soil and infected implements to 'clean' areas where it can be a source of new disease outbreaks. Quarantine can be used at farm, national and international levels (Hakiza and Mwebesa, 1997; Phiri *et al*. 2009). Quarantine also involves monitoring and restricting movement of plant material between affected and non-affected countries. Coffee growing countries like Burundi, Rwanda and Kenya, which have neighbors with CWD outbreak, should be diligent in preventing cross-border movement of coffee material (germplasm, coffee in parchment or green coffee) to prevent spread of CWD from the infected neighboring countries. This has already occurred between DRC and Uganda (CABI, 2003; Phiri *et al*. 2009). Enforcement of intercountry quarantine is probably the only chance for Kenya's *C. arabica*, which faces threat from the arabica strain of *G. xylarioide* that is affecting *C. arabica* in Ethiopia. Studies by Girma *et al*. (2009b) showed that most of the existing Kenyan coffee varieties, except for Ruiru 11, which was not included in the study, were susceptible to the Arabica

strain. Quarantine measures need to be backed up with dissemination of information about the disease to farmers, extension workers, scientists and the general public. Dissemination of information on the symptoms of the disease is essential to allow monitoring and early detection of the disease (Phiri *et al*. 2009**)**.

2.7.2. Cultural Practices

Uprooting and burning is probably one of the oldest CWD management methods. It dates back to the first CWD outbreak in the 1950s (Muller, 1997; Saccas, 1956) and contributed in limiting the spread of *G. xylarioides* inoculum from the plant material and the contaminated soil. *G. xylarioides* is considered to be an endemic soil-inhabiting fungus (Rutherford *et al*. 2009; Wrigley, 1988). The method works by removing the source of pathogen inoculums and involves frequently inspecting the coffee farm to identify infected coffee bushes. Once identified, the infected coffee bushes are uprooted by digging out as much of the root system as possible then burning them on the spot at the earliest opportunity in the hole where the coffee bush was uprooted. To reduce further risk of inoculums in adjacent coffee trees, those coffee trees surrounding infected coffee trees also need to be uprooted and burnt. Uprooting the coffee tree that has already died from CWD is too late; the pathogen will have already spread to surrounding and distant coffee trees in the farm and even to adjacent farms. Conidia and ascospores of *G. xylarioides* spread through wind, rain and human activities (harvesting, pruning). When CWD infection levels are high, such as 70% of coffee trees being infected in a coffee garden, it is advisable to uproot and burn all coffee trees in the farm and replant with resistant or tolerant coffee germplasm (CABI, 2003; phieri *et al*. 2009).There are a number of examples of use of uprooting and burning. When CWD first appeared in West Africa from 1927 to the 1950s, it was controlled in Cameroon by mobilizing soldiers to uproot and burn all infected coffee in the country (Wrigley, 1988).

G. xylarioides penetrates through wounds, so any agent causing wounds will aid the spread of the fungus. Kranz and Mogk (1973) noted that most dying and dead trees had been wounded during weeding. This is worsened by the fact that most of the coffee are grown using very close spacing in Ethiopia, and some of the coffee are actually semiforest type, i.e. it was not planted by farmers, hence may grow at even closer spacing than the

recommended one. Coffee tree wounding is also prevalent in most countries in Africa, and it is mainly due to weed management. Hand weeding around coffee tree as opposed to the normal practice of slashing and the use of cover crops in coffee fields significantly reduced the incidence of CWD (Phieri et al., 2009, Girma *et al*. 2009a).

There are other coffee farm operations that have to be carried out which cause wounds on the coffee bush. Particular operations that pose a huge challenge in the prevention of CWD spread is the rejuvenation of coffee or changing of a coffee cycle that involves stumping. Changing a coffee cycle is a routine practice that has to be carried out when coffee bushes have aged or have become unproductive. The operation requires cutting of coffee stems at about 45 cm above the soil level, at an angle, with a pruning saw so that new coffee shoots that sprout below the cut point are selected and one or two are allowed forming the next coffee cropping cycle. If a farmer can afford, it is advisable for the stumps to be painted with a fungicide paste. An ordinary copper-based fungicide can be used for this purpose. The copper-based fungicide should be mixed with water at the rate of 300 g copper-based fungicide to 1 l of water. The paste can be applied with a paint brush on the cut surface of the stump. Pruning to cut off dead or interlocking branches or branches that are pointing inwards is also a potential way of spreading CWD and should be managed properly. Secateurs that are used in the pruning process should be sterilized (CABI, 2003; Phieri *et al*. 2009; Girma *et al*. 2009a).

2.7.3. Biological control

Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms accomplishing naturally or through manipulation of the environment, host or antagonists, or by mass introduction of one or more antagonist (Baker and Cook, 1974). Biological control is the strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms (Tesfaye and Kapoor, 2004). Antagonists that produce antibiotics kill pathogens and eradicate or control them from substrate. Some microorganisms occupy the niches and exclude pathogens from becoming established, thereby protecting plants from infection. Biological control has attracted great interest because of increasing regulation and restriction of fungicides or unnecessary control

attempts by other means. It is especially attractive for soil borne diseases because it needs critical evaluation of economics of the country and the pathogens that are difficult to reach with specific fungicides (Montealegre *et al.* 2003). The result of a recent in vitro study conducted by (Muleta *et al*. 2007and Negash, 2011) on antagonistic effects of some rhizobacteria and Tricoderma isolates against the *F. xylarioides* were promising. Of 23 bacterial isolates obtained from rhizospheres of arabica coffee trees in south-west Ethiopia, 21 significantly inhibited the mycelial spread of *F.xylarioides. Bacillus subtilis*, designated as isolate 'AUBB20', was the most antagonistic to this pathogen. *T. viride* and *T.harzianum* has shown good potential in inhibiting the mycelial growth of *F. xylarioides*.

2.7.4. Resistant Coffee varieties

CWD destroyed coffee trees during the 1927s to the 1950s in African countries, particularly in Cameroon and Central African Republic and Ivory Coast. In contrast, several varieties of *C. Canephora* imported from the Democratic Republic of Congo (DRC) between 1914 and 1933 exhibited some level of field resistance, which was later confirmed through artificial inoculation (Muller, 1997). Muller, (1997) also reported apparent differences for the same materials planted in different areas of the region, i.e. certain *C. liberica* and *C. canephora* varieties showing resistance in Ivory Coast were completely susceptible in CAR, suggesting the resistance was either being influenced by environmental conditions or there were different physiological races of the pathogen in different localities of this region.

Van der Graaff and Pieters (1978) reported that coffee lines of *C. arabica* in Ethiopia showed differences in resistance to the CWD pathogen, thus providing potential for controlling CWD using resistant varieties in arabica coffee. They suggested that resistance in *C. arabica* was quantitative in nature and horizontal, and there was no evidence of single-gene (vertical) resistance that could be readily overcome by pathogen adaptation.

As CWD threatened the coffee industry throughout Africa, affected countries decided in 1956 to implement systematic elimination of all affected plants over large areas and to

search for resistance both in wild and cultivated varieties. Following this initiative, *C. canephora*-resistant varieties identified in DRC were used for replanting within DRC and Ivory Coast (Saccas, 1956). In 1986, new large-scale outbreaks of CWD were reported on *C. canephora* in the north-east of DRC (Flood and Brayford, 1997), from where it spread rapidly into Uganda (1993) and north-west Tanzania (1996). Because the disease appeared in these countries for the first time, there were no resistant varieties available for replanting in infected areas and all available commercial varieties were susceptible to CWD.

Thus, following the successful use of resistance in Ivory Coast and the CAR, in Uganda, a breeding programme was initiated at the Coffee Research Centre (COREC) (now CORI) which aimed at developing resistant germplasm for managing the disease. Similar breeding programmes were initiated by TaCRI in Tanzania and the University of Kinshasa in DRC, Intra and interspecific differences among and between coffee species respectively provide potential genetic variability, which is exploited for resistance against CWD. Intraspecific variability is the best and easiest to exploit since resistant individuals are easily released as new varieties without undergoing hybridization, provided they posses other agronomic traits such as being high yielding; having resistance to other major diseases, mainly leaf rust and red blister disease and coffee berry disease and having good market qualities (Musoli *et al*.2009).

A breeding programme in Uganda resulted in screening of thousands of Robusta plants for resistance to CWD. The initial screening produced over 1,500 lines potentially resistant to the disease. Further screening and agronomic trials have reduced this to seven clones which have been officially released in Uganda (Phirii *et al*. 2010). Similar achievements were reported from Tanzania (Kilambo *et al*. 2010). Out of 875 lines 201 were found to resist CWD. Six clones were selected for multi-locations.

Girma *et al*. (2001) Girma and Hindorf, (2001) and Girma, (2004) reported varietal differences in Arabica coffee. Gambella origin accessions revealed significantly more wilt incidence as compared to French collections and Catimor lines. In the national coffee collection plots, SN5, F-35, and F-51/53 and 248/71 appeared to be highly susceptible with a 100% loss as compared to F-35 and F-51 conferring resistant reactions with

significantly low death rates of 9.3 and 27.9% respectively. Chala *et al*. (2011) reported some released Sidama/Yirgachefe varieties like 971 (Fayate) and 974 (Odicha) shows high to moderate resistance with low death rates of 2.9 and 7.3% respectively. Generally it is difficult to comment with certainty on host resistance of such soil borne pathogen under field conditions, as there are a number of misleading factors; field resistance gives clue to select tolerant cultivars or lines that can be proved by seedling test under controlled environment. The experience from Uganda showed for clonal Robusta materials, that were presumed to be resistant during early years of outbreaks, were later found to be susceptible both in the field and seedling tests (Girma, 2004; CORI, 2001).

2.7.4.1. Reaction of coffee cultivars in the field and artificial inoculation

A number of researchers have reported existence of marked differences in resistance levels in arabica coffee populations to CWD under field conditions at various locations (Van der Graaff and Pieters 1978, Merdassa 1986; Girma *et al*. 2001; Girma 2004). 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%. In a field at Bebeka, 23 cultivars (including four introduced Catimor lines) were planted in a completely randomized block design with three replications and 90 trees per plot, cultivar 785, 1185, 1785 and 4485 were uniformly attacked in all plots and showed significantly high mean death rates of 80.0%, 72.9%, 83.4%, and 97.4%, respectively, indicating their susceptibility to coffee wilt disease. In contrast, the Catimor lines (1579, 1779, 1979 and 2179) and some French collections (F-15, F-27 and F-59) had the lowest infection levels of less than 10% (Girma, 2004). The introduced coffee lines such as Caturra Rojo, Caturra Amerello and Catuai showed significantly ($P < 0.05$) higher mean incidences of 83.0%, 80.5% and 80.0%, respectively, and were more susceptible to CWD than the indigenous cultivars 7454, 74110, 74112, 74140 and 74165 at Teppi (Girma, 2004).

2.7.4.2. Inoculation Methods

Use of a quick and effective procedure is crucial to the successful identification of reliable resistance. Where breeding for resistance against CWD is being initiated for the first time, it is necessary to have the basic skills and knowledge, which can lead to successful screening of the germplasm and identifying genuinely resistant genotypes. This will include having easy but effective protocols for testing and quantifying the resistance. The screening protocols involve assessing genotypes for resistance under natural and artificial infections. There are almost no special manipulations involved when assessing coffee plants for resistance in naturally infected gardens, and limited variation in methodologies is anticipated, except perhaps in the quantification of resistance. There are more methodological variations when assessing genotypes for CWD resistance in artificial conditions, perhaps due to variation of costs required by different methods and the facilities available for the studies (Musoli *et al*. 2009, Girma *et al*. 2009b).

Many different inoculations techniques are and have been employed in various laboratories in different countries over many years. Stem-nicking method of young coffee seedlings with inoculum suspension (2–2.5 × 10^6 concentration) of *G. xylarioides* isolate at cotyledon stage (2 to 2.5 months old) using a scalpel has been adopted as the preferred standard practice on *C. arabica* in Ethiopia (Pieters and Van der Graaff 1980 ,Girma and Mengistu, 2000; Adunga *et al*. 2005; Girma *et al*. 2007), whereas syringe injection of inoculum (1 × 10^6 concentration) into the stem of growing seedlings (at 9 to 10 months old) has been routinely used at Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD); and the root dip inoculation procedure is employed (1.3 × 10^6 concentration) in screening *C. canephora* seedlings (6-8 months old) in Uganda, DR Congo and Tanzania (Kilambo *et al*. 2007, Musoli *et al*. 2009, Girma *et al*. 2009b).

Plants inoculated by root dipping developed the disease symptoms earlier than plants inoculated by other methods and had a higher incidence of diseased plants, and there was clear contrast between susceptible and resistant genotypes. This method was therefore adopted for large-scale germplasm screening by scientists in Uganda and Tanzania. However, root dipping required a lot more inputs (polythene pots, soils and manpower)

than the other methods and therefore is more costly. It was also suspected that some of the plants infected by this method could have developed the disease because of extra stress resulting from root damage incurred when stripping off soils from roots in preparation for dipping. This method can also clearly enable differentiation of resistant and susceptible genotypes. Stem nicking was also adopted for germplasm screening in Ethiopia, as it is considered to be less expensive But, The stem injection method was technically inefficient in screening large number of accessions, as it is difficult to pierce the seedling stems and place the required amount of inoculum at the later growth stages (Girma, 2004; Girma *et al*. 2009; Musoli *et al*. 2009).

2.7.4.3. Quantifying CWD Resistance in Artificial Inoculation

An effective and reliable method of quantifying resistance was necessary for comparison of results and for the selection of genuinely resistant genotypes, irrespective of whether the evaluation was carried out on mature plants in the field or young plants in the screen house. The method adopted will depend on the purpose of the study. Where the study aims at determining relative resistance between progenies or clones, resistance can be assessed as numbers of infected plants compared to those uninfected. The numbers can be expressed as percentage infection (Girma, 2004). In Uganda plants studied in artificial inoculations were commonly assessed on a scale of 1 to 5, where 1 = no disease, 2 = curling leaves and stunted growth, 3 = leaf wilting and yellowing, 4 = leaf necrosis, leaf wilting, and abscission and 5 = plants are dead. Mature plants studied in fields were also assessed on a scale of 1 to 5, but the quantification of the disease levels in the field was slightly different. In field assessment (mature coffee trees) 1 = no disease, 2 = 1%–25% defoliation, 3 = 26%–50% defoliation, 4 = 51%–75% defoliation and 5 = 76%– 100% defoliation. Plants scored as level 5 are normally considered dead. Since all plants that develop CWD symptoms eventually die, only plants without the symptoms after a long period of infection (6 months for plants in artificial inoculations and not less than 5 years for plants evaluated in heavily infested fields) were considered resistant. Where necessary, these plants can be re-inoculated and assessed again for another 6 months to ascertain their resistance. The plants that remained healthy after the re-inoculation were considered to have complete resistance, and such plants were planted in mother gardens for cloning and further assessments (Musoli *et al*. 2009).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in the laboratory and greenhouse at Jimma Agricultural Research Center during 2011-2013. The center is found in Oromiya Regional State in Jimma zone, Ethiopia, located around 07°46'N latitude and 36°47'E longitude coordinate and at an elevation of 1753 m.a.s.l. It is 365 kilometer away from Addis Ababa and 12 kilometers from Jimma town in the West direction. It represents the medium agro ecological zones which receive annual rainfall of 1572mm, with mean minimum and maximum temperatures of 11.6^0c and 26.3 ^{0}c, respectively.

3.2. Experimental Materials

The experimental materials consisted 100 coffee accessions with two released lowland varities (Gesha and Catimor J-19) and SN-5 as a check. These materials were collected from different coffee producing regions of the country Southern Nations, Nationalities, and Peoples Regional state (SNNPRS), Oromiya and Amhara between 1966 and 2006. The initial objective of the collection mission was to collect and conserve as many coffee germplasm as possible and to evaluate for different desirable agronomic traits and resistance to major diseases. The accessions were established at Jimma Agricultural Research Center which has the mandate of serving as one of gene banks for coffee land races. The coffee accessions were field planted in conservation block between 1995-2007, in single rows of 10 - 12 trees per plot. Coffee accessions in the conservation block were asssed for their survival rate under field condition and tree death record were taken every month until the termination of the experiment. As a result 100 coffee accessions which have survival rate of more than 80% and which had enough amount of seeds for conducting seedling test was selected as experimental material (Table 2).

3.3. Raising Coffee Seedlings

Ripe cherries were picked from uniform mother tress of each accession, and then dried under shade after carefully removing the skin and the pulp by hand. Sowing was accomplished in two baches 52 accessions in first batch and the remaining 48 accessions as second batch. The seed lots of each germplasm first soaked in distilled sterile water for about 48 hours after removing the parchment. The soaked seeds (40 seeds/pot) of each accessions were sown into heat sterilized and moistened sandy soil in disinfected plastic pots (10 % sodium hypochlorite) with 2295cm^3capacity. Sterile water was regularly applied every two days, to maintain adequate moisture for seed germination, emergence and growth of the plants throughout the experimental period (Girma, 2004).

Table 2: Description of selected coffee accessions for testing against coffee wilt disease *Gibberella xylarioides*) resistance

No	Acc. No.	Region	Zone	District	Site	Altitud. mA. s. l
1	F-2	SNNPRS	Kaffa	Gimbo	Gojeb valley	1420
2	F-13	Oromiya	Jimma	Goma	Dembi	1660
3	F-24	SNNPRS	Sheka	Yeki	Tepi	1200
4	F-25	SNNPRS	Sheka	Yeki	Tepi	1200
5	F-31	SNNPRS	Sheka	Yeki	Tepi air port	1300
6	F-37	SNNPRS	Kaffa	Decha	Shasha	2000
7	F-39	SNNPRS	Kaffa	Decha	-	2000
8	F-51	SNNPRS	Kaffa	Decha	Catolic mission	2050
9	1/70	SNPPRS	Kaffa	Chena	Wushi	1700
10	3/70	Oromiya	Jimma	Seka chekorsa	Melko	1750
11	4/70	Oromiya	Jimma	Seka Chekorsa	Melko	1750
12	5/70	Oromiya	Jimma	Seka Chekorsa	Melko	1750
13	6/70	Oromiya	Jimma	Gera	Gera	1900
14	8/70	Oromiya	Jimma	Gera	Gera	1900
15	16/70	SNNPRS	Sidama	Aletawondo	Wondogenet	1700
16	25/70	SNNPRS	Wolayita	Boloso Sore	Dubo	1780
17	28/70	SNNPRS	Gedeo	Yirgachefe	Yirgachefe	1880
18	30/70	SNNPRS	Gedeo	Yirgachefe	Yirgachefe	1880
19	32/70	SNNPRS	Gedeo	Wonago	Dilla	1550
20	43/70	Oromiya	Jimma	Goma	Choche	1520
21	54/70	Oromiya	Jimma	Gimbo	Gogoma	1750
22	56/70	SNNPRS	Kaffa	Gimbo	Wush wush	1940
23	59/70	SNNPRS	Kaffa	Gimbo	Wush wush	1940
24	74/70	Oromiya	Illubabor	Yayu	Saki	1380
25	1/71	Amhara	W.Gojam	Bahirdar zuria	Zeghei	1800
26	2/71	Amhara	W.Gojam	Bahirdar zuria	Zeghei	1800
27	120/71	SNNPRS	Gedeo	Wonago	Chichu	1550
28	195/71	SNNPRS	B.Maji	Dizi	Maji kersi	-
29	197/71	SNNPRS	Gedeo	Wonago	Chichu	1550
30	199/71	SNNPRS	B.Maji	Dizi	Maji	-
31	200/71	SNNPRS	B.Maji	Dizi	Bero Kaffa	1575
32	201/71	SNNPRS	B.Maji	Dizi	Bero Kaffa	1575
33	203/71	SNNPRS	B.Maji	Dizi	Garo	1590
34	213/71	SNNPRS	B.Maji	Dizi	Geba	1590
35	226/71	SNNPRS	B.Maji	Dizi	Kassi	1500
36	236/71A	SNNPRS	B.Maji	Dizi	Bero	1570
37	243/71A	SNNPRS	B.Maji	Meanit	BardaTown	1620
38	244/71	SNNPRS	B.Maji	Meanit	Barda	1650
39	245/71	SNNPRS	B.Maji	Meanit	Barda	1660
40	246/71	SNNPRS	B.Maji	Meanit	Barda	1640
41	247/71	SNNPRS	B.Maji	Meanit	Barda	1640
42	250/71	SNNPRS	B.Maji	Meanit	Barda	1640
43	258/71	SNNPRS	B.Maji	Meanit	Barda	1660
44	259/71	SNNPRS	B.Maji	Bench	Geisha	1930
45	276/71	Oromiya	Jimma	L.Kossa	Dambi	1410
46	279/71	Oromiya	Jimma	Sekoru	Ushane	-
47	4/72	Oromiya	Jimma	-	-	-
48	5/72	Oromiya	Jimma		Limu	-
49	**13/72**	Oromiya	Iluababor	Bedele	-	-
50	12/74	SNNPRS	Sidama	Awassa	Awassa Zuria	-
51	13/74	SNNPRS	Sidama	Awassa	Awassa Zuria	-
52	3/77	Oromiya	Jimma	Gomma	Choche	**1520**

Table 2**: Cotnuied**

No	Acc. No.	Region	Zone	Woreda	Site	Altitude
53	**5/77**	Oromiya	Jimma	Gomma	Choche	**1520**
54	10/77	Oromiya	Jimma	Gomma	Choche	1520
55	11/77	Oromiya	Jimma	Gomma	Choche	1520
56	19/77	SNNPRS	Gedeo	Wonago	Sokicha	1920
57	25/77	SNNPRS	Gedeo	Wonago	Sokicha	2050
58	26/77	SNNPRS	Gedeo	Wonago	Sokicha	2050
59	27/77	SNNPRS	Gedeo	Wonago	Sokicha	2050
60	46/77	SNNPRS	Keffa	Gimbo	Wush Wush	-
61	4/78	Oromiya	W.Wellega	Haru	MelkaDemeke	1860
62	5/78	Oromiya	W.Wellega	Haru	MelkaDemeke	1860
63	6/78	Oromiya	W.Wellega	Gimbi	Homa Giorgis	1680
64	8/78	Oromiya	W.Wellega	Gimbi	Homa Giorgis	1680
65	9/78	Oromiya	W.Wellega	Gimbi	Worabu Keta	1650
66	11/79	SNNPRS	B.maji	Bench	Bebeka	-
67	12/79	SNNPRS	B.maji	Bench	Bebeka	-
68	13/79	SNNPRS	B.maji	Bench	Bebeka	-
69	7932	Unnown	-	-	-	-
70	11/81	Oromiya	Iluababor	-	-	-
71	44/83	Oromiya	Bale	Gololcha	Gololcha	2220
72	48/83	Oromiya	Bale	Hrena	Meana	1450
73	4/84	Oromiya	W.Wellega	Gimbi	Gambela	1710
74	5/84	Oromiya	W.Wellega	Haru	Gambela	1710
75	18/84	Oromiya	W.Wellega	Gimbi	Siba Yesus	1710
76	19/84	Oromiya	W.Wellega	Haru	Waljaleta	1660
77	20/84	Oromiya	W.welega	Haru	Waljaleta/ kebele	1661
78	22/84	Oromiya	W. Wellega	Gimbi	Homa Giorgis	1710
79	24/84	Oromiya	W. Wellega	Gimbi	Siba yesus	1680
80	26/84	Oromiya	W. Wellega	Haru	Sedale	1800
81	27/84	Oromiya	W. Wellega	Nole Kaba	Karaleku	1990
82	29/84	Oromiya	W. Wellega	Nole Kaba	Aye Bollo	1850
83	30/84	Oromiya	W. Wellega	Nole Kaba	Bechero Kebele	1960
84	33/84	Oromiya	W. Wellega	Haru	Sedale	1790
85	36/84	Oromiya	W. Wellega	Nole Kaba	Abaglemo	1870
86	39/84	Oromiya	W. Wellega	Yubdo	Maneti Bollo	1840
87	49/94	Oromiya	W.welega	Ayra	Katto Aba korma	-
88	75/84	Oromiya	W. Wellega	Mana sibu	Kiltu Kara	1520
89	79/84	Oromiya	W. Wellega	Boji	Billa 01 Kebele	1820
90	85/84	Oromiya	W. Wellega	Nejo	Kilitu Farra	1680
91	86/84	Oromiya	W. Wellega	Nejo	Kilitu Farra	1680
92	87/84	Oromiya	W. Wellega	Jarso	Jarjo Mariam	1700
93	88/84	Oromiya	W. Wellega	Jarso	Neda Budi	1640
94	89/84	Oromiya	W. Wellega	Jarso	Hindebu Georgis	1560
95	92/84	Oromiya	W. Wellega	Lalo hasbi	Dongoro Keta	1720
96	98/84	Oromiya	W. Wellega	Lalo hasbi	Lalo Wanjo	1600
97	100/84	Oromiya	W. Wellega	Lalo hasbi	Degaga Burko	1560
98	B1/2006	Oromiya	Bale	Manan-getu	Majette	-
99	B2/2006	Oromiya	Bale	Man-angetu	Majette	1490
100	B3/2006	Oromiya	Bale	Man-angetu	Majette	1490
101	Catimor J-19+	International coll.	Introduced from Portugal			
102	Geisha +	International coll.	Introduced from India			
103	SN-5	SNNPRS	Kaffa	Gimbo	Near Bonga	1730

Source: Extracted from data base of coffee breeding and genetics reserch division, JARC
- =Data not available, + released coffee varieties

W.Gojam= West Gojam, B. Maji= Bench Maji, W.Wollega= West Wollega

3.4. Isolation of coffee wilt pathogen (*Gibberella xylarioides)*

Isolation from partially wilting stem specimen was accomplished methods described by Girma (2004), Sihen *et al.* (2012) by using potato sucrose agar and synthetic low nutrient agar. Stems of CWD infected coffee tree samples with bluish black internal discoloration were collected from Gera Reserch Center and brought to Plant Pathology laboratory at JARC. The bark from the specimen was carefully removed and small section (0.5 cm x0.5 cm) was excised from intervening regions of discolored and white healthy wood using a sterile scalpel. About four to six small sections were transferred into plastic Petri dishes having 5 ml of 10% sodium hypochlorite uniformly agitated for 1 min and immediately rinsed in 3 changes of steriled water. After surface disinfecting and blotting, 4 sections were aseptically plated using sterile forceps onto petridishes (9 mm) containing potato sucrose agar .The cultures were incubated under 12 hour fluorescent light and dark cycles at 22 ± 2^0c.The fungus colony grown out of the plated sections were purified and grown on SNA. The fungus isolates identified as *Gibberella xylarioides* was purified and preserved on sterile sandy soil until coffee seedlings grown and ready for inoculation.

3.5. Media used for isolation of *Gibberella xylarioides*

The identification of *Fusarium* is based on the morphological characters including the presence or absence, the shape and the dimensions of microconidia, macroconidia. Growth and color development on different media are used as markers in practice. Potato sucrose agar which encourage development of pigmentation was prepared containing potato sucrose agar (500 ml of the fresh potato extract, 20 g sucrose, 20 g of agar with 500 ml of water) adjusted to pH 6.5-7.0 and amended with 20 ml (per liter medium) of 5 % streptomycin sulphate solution. For good sporulation and short term storage of *Gibberella xylarioides* synthetic low nutrient agar (SNA) was prepared per one liter distilled water 1.0g KH_2PO_4, 1.0gKNO_3 , 0.5g $MgSO_4$, 0.5 g KCl, 0.2g Dextrose, 0.2g Sucrose, 0.6ml NaO, and 2.3 g Agar (Booth, 1971; Nirenberg, 1976).

3.6. Inoculum Preparation and Inoculation of Coffee Seedlings

The preserved stock was 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 two weeks at the same time fresh branch was collected from healthy trees and cut into small pieces (15cm long) and the bark were slightly scratched off to expose the wood. The branches were placed in a large Petri dishes having small role of well moistened cotton wool underneath and then sterilized in autoclave each twig were inoculated with 2-3 ml of conidia suspension for 10 days under standard conditions (Girma, 2004).

The conidia used for inoculation were obtained by thoroughly rinsing of the branches with good colony growth with sterile water in a sterile beaker and stirred up with magnetic stirrer and filtered through double layer cloth, suspension were adjusted to the desired concentration $2.3X10^6$ conidia per ml. The coffee seedlings were inoculated with a viable conidial suspension of the isolate by stem nicking procedures (Pieters and van der Graaff, 1983; Girma *et al*. 2009). A sterile scalpel was immersed in to the suspension, then the stem of each seedling were nicked at about 2 cm from the soil level and a drop of nearly 1 milliliter was placed in the notch. The treated plants were kept in air conditioned growth room with high relative humidity (>95%) and optimum temperature ($23\text{-}25^0$C) for infection. After 10 days, the inoculated seedlings were transferred to a green house (with temperature of $15\text{-}30^0$C and relative humidity of 60-80%).The experiments were laid out in completely randomized design with three replication in two batches. Batch I and II consists 52 and 48 coffee accessions respectively. The numbers of wilting seedlings shown external symptom were recorded fortnightly for 6 months. The dates for incubation period on which symptoms first appearance and types of induced wilting was periodically noted.

3.7. Assessment of Coffee Berry Disease, Coffee Leaf Rust and Coffee Wilt Disease

Disease assessments of coffee berry disease and coffee leaf rust was conducted in August 2012 and in November 2012 conservation block in the field at JARC, following Van der Graaff's method (1981). CBD and CLR were assessed to evaluate selected coffee accessions for their tolerance under natural inoculum, CWD data were used to estimate

correlation between tree and seedling deaths. Assessment of levels of natural infection was estimated using visual assessment technique.

Field Score

Vissual assessment of CBD: to estmate the level of CBD under field conditions the percentage of diseased berries on individual trees were assessed

Vissual assessment of CLR: to estmate the level of CLR under field conditions the percentage of diseased leaves on individual trees were assessed.

CWD assessment: Healthy and diseased trees showing characterstic symptoms (internal and external) were recorded for ten months and the incidence of CWD was computed as the number of diseased trees divided by the total number of observed coffee trees x 100.

3.8. Statistical Analysis

Statistical analysis system (SAS), software version 9.2 was employed for the analysis of variance (ANOVA), mean separations and correlation analysis. Treatment mean separation was made using Tukey's test.

Area under disease progress curve (AUDPC) was computed from severity data of greenhouse experiment using the formula suggested by Campbell and Madden (1990) and used by Tshilenge *et al.* (2011) for coffee wilt on Robusta coffee.

AUDPC was calculated using the formula:

$$AUDPC = \left[\sum (x_1 + x_2)/2\right]\left[t_2 - t_1\right]$$

Where x1 and x2 represent the severity at time 1 and time 2, t2-t1: time interval between two observations. The percentage of wilt or dead seedlings was computed from the cumulative number of wilted seedlings over the total number of inoculated seedlings and the death rates were analyzed after angular transformation. Stem and root tissue from symptomatic and symptomless seedlings were plated to reisolate the pathogen in order to verify Koch's postulates. The daily minimum and maximum temperature in (^{0}C) in the green house were recorded throughout the experimental period.

Correlation and cluster analyses: The coefficients of correlations of seedling test vs. tree death, and other traits (disease severity vs incubation period and AUDPC) were tested

for their significance by comparing the value of correlation coefficient with tabulated r-value at g-2 degree of freedom. The calculated‘t’ value was compared with the tabulated’ value at g-2 degree of freedom at 5% level of significance, where, g = number of coffee accessions.

Cluster analysis includes a broad suite of techniques designed to find groups of similar items within a data set. Partitioning methods dividing the data set into a number of groups are designated by the user. Hierarchical cluster methods produce a hierarchy of clusters from small clusters of very similar items to large clusters that include more dissimilar items. Hierarchical methods usually produce a graphical output known as a dendrogram or tree that shows this hierarchical clustering structure.

In this study, CWD parameters (disease severity, incubation period and AUDPC) were used for clustering the accessions in to homogeneous groups. Data were subjected separately to the analysis so as to determine the variability among the accessions. Hierarchical clustering was employed using the similarity coefficients among the 55 and 51 coffee accessions of batch I and batch II respectively. Clustering was performed using the proc cluster procedure of SAS version 9.2 (SAS institute, 2008) by employing the method of average linkage clustering strategy of the observation. The numbers of clusters were determined by following the approach suggested by Copper and Miligan (1988) by looking in to two stastics namely Pseudo F and Pseudo t^2 clustering criteria.

4. RESULTS AND DISCUSSION

4.1. Description and Progression of Coffee Wilt Symptoms on Coffee seedlings

The different symptoms observed in this trial focused on changes in appearance and leaf drying of seedlings. These symptoms varied, both in their nature and their chronological sequence from the time of their appearance. Two types of external symptoms were recognized in the green house. The first symptom were loss of turgidity in green leaves (Figure 1) and wilt completely turning brown or dark brown in colour, without defoliation of leaves (Figure 2A). In the second type, epinasty, dullness and loss of turgidity in green leaves. These epinastic leaves latter desiccate and wilt completely turning brown or dark brown in color. This type of symptom did not involve defoliation of leaves (Figure 2B). In both cases partial wilt of the leaf on one side of the stem, continuation of similar symptom on the adjacent cotyledonary leaf (Figure 1). The loss of turgor can be considered as early stage wilting symptom. The seedlings of accession such as 1/70, Geisha, 18/84, 8/70 from batch I and 20/84, 46/77, 24/84, 12/79, 9/78, 87/84 uniformly manifested chlorotic defoliation.

Coffee accession 1/70 and 20/84 shows early symptom expression after 52.6 and ,51.7 days of incubation followed by coffee accessions 195/71, 197/71, 203/71 which shows symptom expression three months after inoculation. Symptom expression was delayed on accession 226/71 and 26/77 which expressed six and five month after inoculation, respectively. Coffee wilt seedling symptoms expressed are similar to those previously described by Girma (2004). The reisolation results revealed the presence of *G. xylarioides* on coffee accession of 276/71, 1/70, and 20/84 however of coffee wilt pathogen was not retrieved from resistant and moderately resistant accession of 279/71, 3/70 and 12/74.

Figure 1. Partial wilting symptoms of coffee wilt disease (CWD) after inoculating with *G. xylarioides*

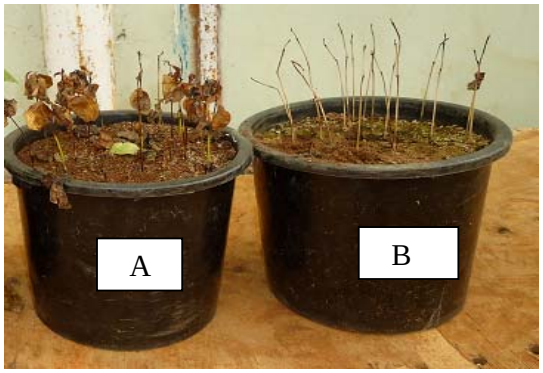

Figure 2 . Coffee wilt disease (CWD) symptoms without defoliation (A), defoliation of leaves (B) after inoculating with G. *xylarioides*

Figure 3 . Comparison among coffee accessions standard resistant Check Catimor J-19 (III), standard susceptible check SN-5 (II) and coffee accession 279/71(I) resistant from national coffee collection inoculated with *Gibberella xylarioides*

4.2. Severity of Coffee Wilt Disease on Coffee accessions

There existed highly significant (P<0.001) difference among coffee accessions both in percent dead (wilt) seedlings and incubation period (Table 3, 4 and Annex1-6). Disease severity and incubation period varied between 0.0-86.3, 0-182.7 and 7.1-86.4, 51.7-155 for batch I and II, respectively.

Accession 279/71 showed no seedling death until the termination of the experiment (Figure 3). Accessions 226/71, 259/71, 244/71, 120/71, 3/70, 16/70, 245/71 and 30/70 resulted significantly (p=0.05) low percentage (<30%) of dead seedlings (moderately resisistant i.e. 6.2, 10.5, 16, 19.1, 22.8, 29, 30.7, 34.5% and with incubation period of 182.7, 92 158.7, 134,141.7 108.7, 123.3 and 97 days, respectively. From Bach II

accession 27/77, 12/74, 26/77, B3/06, B2/06, 44/83, 48/83, B1/06, 11/77 and 13/74 resulted low percentage of seedling death (moderately resisistant)
i.e. 7.1, 8.5, 9.2, 11.2, 17.8, 20.8, 21.1, 25.8, 30.9, 37.2 with incubation period of 150.7, 129.7, 155, 137.7, 93, 148.3, 82.3, 85, 96.3, 96.7 days, respectively as compared to standard resistant check catimor J-19 which showed 41.2 % seedling death and incubation period of 67 days (Table 3 and 4).

Coffee accession 236/71A, 200/71, 276/71, F-2 from batch I and accession 39/84, 49/84, 5/77, 86/84, 12/79 from batch II showed moderately susceptible reaction with percent seedling death of 42.9, 55.6, 55.6, 58.9, 52.3, 54.9, 56.6, 60.6, 60.7 % with incubation period of 75, 98 , 87 ,89, 93.5,90,84.5,81.5,78,81.3,94,76.3, and 97.6 days, respectively.
Coffee accessions from batch I 1/70,18/84, 5/84, 5/72, F-31, 54/70, 250/71, 4/84 13/79,199/71 exhibited high susceptible reaction with CWD severity value of 86.3 ,86.3, 85.2,85.1, 85, 83.4, 81.5,81.4,81.4 ,81.3 with incubation period of 52.7, 56, 57.5,76, 66, 60.7 , 57, 67, 67, 63.5 and 98.3 days respectively. Coffee accessions 9/78, 26/84, 20/84, 24/84, 22/84 from batch II shows highly susceptible reaction with seedling death of 86.4, 83, 82.5 ,81.5, with incubation period of 74, 63.7, 51.7, 59, and 92 days, respectively. As already reported by Girma and Chala (2007) and Adugna *et al*., (2009), Geisha which is a released lowland cultivar exhibited susceptible reaction with seedling death of 84.4 % incubation period of 60.7 days.

The current screening result was in agreement with findings of many investigators who reported significant variations among coffee accessions for CWD resistance, collected from major coffee growing regions of the country. Arega, (2006) reported 50% of Harena coffee collections showed less than 15% seedling deaths as opposed to coffee accessions collected in Bonga, (Kaffa) Birhan-kontir (Bench Maji) and Yayo (Iluababor) forest areas which showed more than 85% average infections. Girma, *et al* (2008a) also reported significant difference among 70 coffee accessions in seedling death and incubation periods. Result of greenhouse experiment of Sihen *et a*l. (2012) proved diversity of coffee population (with in and among forest) against CWD resistance.

The presence of of CWD resitance in Ethiopia *C.arabica* germplasm gives way to do more large scale screening works. Development of CWD resistant program has to go in line with local land race development program which aims development of varities for each specific agroecologies (Bayeta and Labouisse, 2006).

4.3. Area under Disease Progress Curve (AUDPC) on Coffee accessions

The area under disease progress curve (AUDPC) was highly significant ($p<0.001$). The AUDPC value varied between 3.43-188.2 and 2.3-182.3 for batch I and BII of coffee accessions, respectively (Table 3 and 4). The lowest AUDPC value 3.4, 5.2, 6.9, 12, 21.2, 41.2, 47.6, 49.1 on coffee accessions 226/71, 259/71, 244/71, 120/71, 3/70, 16/70, 30/70, 245/71 from batch I; AUDPC value 2.3, 3.3, 10.2, 10.7, 12, 13.4, 15.7, 22.3, 62.1 were obtained from coffee accession 27/77, 12/74, B3/06, 26/77, B2/06, 48/83, 44/83, B1/06, 11/77, 13/74 were obtained from batch II. Coffee accession 59/70 , 4/84, F-31, 199/71 , 4/70 , 250/71 , F-39, 18/84, 1379, F-13 from batch I accession 4/78, 24/84, 11/79, 19/77, 20/84, 5/78, 9/78, 87/84, from batch II shows high percent day AUDPC 188.2, 186.3, 185.3, 185.2, 181.9, 181.8, 180.9, 180.1, 179.1, 177.9, And 181.9, 181.1, 175.3, 174.6, 172.3, 168.7, 165.5, 165.1,164.6, respectively. High percent AUDPC value of 182.3 was recorded on released low land coffee variety known as Geisha. The moderately resistant accessions of set I and II showed less disease severity with lesser AUDPC values, similarly high severity value of susceptible accessions was also associated with higher AUDPC value.

Chala (2008) reported the higher initial rust incidence resulted higher AUDPC values. Tshlenge-Djim *et al*. (2011) also reported some strain of G. *xylarioides* MUCL 35223, B101101Zobolia and B101101 (2) J expressed low value of AUDPC which indicates low level of aggressiveness of the strain.

Table 3. Resistance level of coffee collections to coffee wilt disease in the greenhouse at Jimma Agricultural Research Center, Ethiopia (Batch I)

Accession No	Severity (%)		Incubation period	AUDPC	Tree death
	Actual Value	Transformed value	(days)		
1/70	98.7	86.3 a	52.7 gf	160.8 ac	0
3/70	15.1	22.8 h-j	141.7 ac	21.2 gh	0
4/70	92.9	75.2 ab	70.3 dg	181.9 a	0
5/70	91.2	73.1 ab	72.7 cf	154.2 ac	0
6/70	96.4	79.1 ab	70 dg	166.4 ac	0
8/70	91	73.3 ab	63.3 gf	160.1 ac	0
16/70	23.6	29 gh	108.7 bf	41.2 fh	0
25/70	77.5	62.7 ae	110.7 bf	102.8 cf	0
28/70	82.8	65.8 ad	75.3 cf	135 ad	0
30/70	33.8	34.5 ei	97 bf	47.6 eh	0
32/70	83.6	66.5 ad	122 af	143.2 ac	0
43/70	92.2	76.7 ab	76.7 cf	168.8 ab	0
54/70	96.2	83.4 ab	57 ef	170.8 ab	0
56/70	83.3	67.5 ad	87.3 cf	153.8 ac	0
59/70	95.3	79.8 ab	69.5 ef	188.2 a	0
74/70	94.4	78.8 ab	64 df	166.1 ac	0
F-2	73.1	58.9 af	78.5 cf	127.3 ad	0
F-13	94.6	79.5 ab	71 ef	177.9 a	8.3
F-24	90.8	75.9 ab	75 cf	168.2 ab	0
F-25	89.9	76 ab	72.5 cf	169.4 ab	8.3
F-31	97.8	85 a	66 df	185.3 a	0
F-37	76.6	61.8 ae	102 bf	139.4 ac	10
F-39	94	78.7 ab	78 cf	180.9 a	0
F-51	85	68.2 ad	83.5 cf	134 ad	0
1/71	83.4	66.4 ad	73.5 cf	146.7 ac	0
2/71	88.9	74.6 ab	71.5 cf	169.6 ab	0
120/71	11	19.1 hj	134 ad	12 h	0
195/71	94.4	76.5 ab	94.3 bf	170.7 ab	0
197/71	93.1	75.1ab	91 bf	178 a	0
199/71	96.5	81.3 ab	98.3 bf	185.2 a	0
200/71	67.2	55.6 bg	98 bf	93.7 cf	0
201/71	94.3	78.8 ab	90 bf	174.3ab	0
203/71	78.9	62.7ae	88.7 bf	138.9ac	0
213/71	82.6	65.4 ad	97 bf	160.4ac	0

Table 3. Continued

Accession No	Severity (%)		Incubation Period	AUDPC	Tree death
	Actual Value	Transformed Value	(days)		
226/71	3.4	6.2 ij	182.7 a	3.4 h	0
243/71A	95.4	79.8 ab	70 df	162.6 ac	0
244/71	8.1	16 hj	158.7 ab	6.9 h	0
245/71	28	30.7 fh	123.3 ae	49.1 eh	0
246/71	85.8	71.8 ac	60 ef	152.2 ac	0
247/71	86.3	69.3 ad	45 ef	162.4 ac	0
250/71	96.6	81.5 ab	67 df	181. 8a	0
258/71	93.1	75.1 ab	71.7 cf	175.7 ab	0
276/71	68	55.6 bg	87 cf	115.7 ae	0
279/71	0	0 j	0 g	0 h	0
5/72	97.8	85.1 a	76 ef	167.3 ac	0
13/79	96.6	81.4 ab	63.5 ef	179.1 a	0
79/32	93.5	75.5 ab	93.5 bf	131.5 ad	10
4/84	96.6	81.4 ab	67 df	186.3 a	0
5/84	97.9	85.2 a	57.5 ef	158.6 ac	0
18/84	98.8	86.3 a	56 ef	180.1 a	0
236/71A	46.4	42.9 ch	75 cf	64.3 dh	0
259/71	5.2	10.5 ij	92 bf	5.2 h	0
Geisha**	97.2	84.4 ab	60.7 ef	182.3 a	-
Catimor J-19 *	43.5	41.2 dh	67.6 ef	62.7 dh	-
SN-5 **	87.3	69.2 ad	84 cf	160.5 ac	
Mean		64.05	84.2	133.9	0.7
CV(%)		13.5	24.7	16.4	

*Resistant check, ** Susceptible check.
Incubation periods indicate the number of days between inoculation and the first date of symptom appearance.
0 (zero) values indicate no incubation period, i.e.; there was no infection symptom until termination of the experiment.
Means followed with the same letter are not different according to Tukey test.
-= no data
AUDPC= Area under Disease Progress Curve.

Table 4 . Resistance levels of coffee collections to coffee wilt disease in the green house at Jimma Agricultural Research Center, Ethiopia (Batch II)

Accession no	Severity		Incubation period	AUDPC	Tree Death
	Actual value	Transfrmed			
19/84	85.6	68.1 ac	64 fg	153.8 ag	0
20/84	97.5	82.5 a	51.7 g	172.3 ad	0
22/84	92.7	80.7 a	92 eg	156.6 ag	0
24/84	96.6	81.5 a	59 fg	181.1 ab	0
26/84	95.7	83 a	63.7 fg	156.8 ag	0
27/84	90.7	75.5 ab	70.3 fg	140 bg	0
29/84	92.1	76.6 ab	89.3 df	156.5 ag	0
30/84	84.3	71.8 ac	106 af	158.8 af	0
33/84	77	63.2 ad	106 af	141.4 ag	0
36/84	86.1	69.5 ac	68.3 fg	125.6 eg	0
39/84	62.4	52.3 af	78 eg	114.9 g	0
49/84	65.8	54.9 af	81.3 eg	117.1 fg	0
75/84	82	69.1 ac	77.7 eg	154.2 ag	0
79/84	93	75.5 ab	82 eg	159.4 ad	0
85/84	89.1	74.5 ab	88 ef	135.8 cg	0
86/84	75.6	60.6 ad	76.3 ef	130.6 dg	0
87/84	93.1	77.5 a	63 fg	164.7 ad	0
88/84	90.2	75 ab	67.7 dg	160.1 ad	0
89/84	86.9	72.4 ac	70 fg	154.7 ag	0
92/84	85.4	67.6 ac	86.7 eg	146.1 ag	0
98/84	88	77.7 a	67.3 fg	151 ag	0
100/84	86.3	68.7 ac	90 dg	146.5 ag	0
11/81	91.3	76.3 ab	85.7 dg	162.4 ad	0
3/77	81.4	64.8 ad	81 eg	134.3 cg	0
5/77	69.7	56.6 af	94 eg	134.7 cg	0
13/72	81.2	64.4 ad	75 fg	144.3 ag	0
10/77	82.7	66.7 ad	108 af	150 ag	0
11/77	27.2	30.9 dg	96.3 cg	34.4 hi	0
19/77	90	76.1 ab	72.3 fg	174.6 ac	10
25/77	87.2	72.8 ac	105.7 ag	152.2 ag	0
26/77	7.1	9.2 h	155 a	10.7 i	0
27/77	2.3	7.1 h	150.7 a	2.3 i	0
8/78	90.6	73.5 ab	58.3 fg	146.9 ag	0
46/77	85.7	72.6 ac	102 ag	147.3 ag	0

Table 4. Continued

Accession No	Severity		Incubation period	AUDPC	Tree death
	Actual value	Transformed value			
4/78	92.9	75.1 a	69.3fg	181.9 ab	0
5/78	88.8	70.6 ac	67.3 fg	168.8 ad	0
6/78	88.2	70.1 ac	61.7 fg	164.6 ad	0
9/78	98.8	86.4 a	74 fg	165.1 ad	0
44/83	14.5	20.8 fh	148.3 ac	15.7 d	0
48/83	13.4	21.1 fh	82.3 eg	13.4 d	0
12/74	3.4	8.5 h	129.7 ad	3.3 d	0
13/74	36.6	37.2 ch	96.7 bg	62.1 bd	0
B1/06	20.8	25.8 eh	85 dg	22.3 d	0
B2/06	9.6	17.8 gh	93 dg	12 d	0
B3/06	5.6	11.2 h	137.7 ad	10.2 d	0
4/72	81.7	64.7 ad	79.3 eg	139.8 bg	11.1
11/79	93.3	75.3 ab	73.3 gf	175.3 ac	0
12/79	74.4	60.7 ae	97.7 bg	145.5 ag	0
Geisha **	97.2	84.4 a	60.7 fg	182.3 a	-
Catimor J-19 *	43.5	41.2 bh	67.6 fg	62.7 h	-
SN-5 **	87.3	69.2 ac	84 dg	160.5 ad	-
Mean		60.6	85.8 dg	124.6	0.4
CV(%)		17.7	26.9	20.9	

*Resistant check, ** Susceptible check.
Incubation periods indicate the number of days between inoculation and the first date of symptom appearance
Means followed with the same letter are not different according to Tukey test.
- =no data
AUDPC= Area under Disease Progress Curve.

4.4. Comparisons of Resistance in the Field and Artificial Inoculations

In this study 100 coffee collections which were planted at Jimma Agricultural Research Center in single tree row 10trees/plot and their seedling death in greenhouse were evaluated. About 97 % of the accessions showed no tree mortality except F-13, F-25, 4/84 19/77, and 4/72 which exhibited tree death of 8.3, 8.3,10, 10, and 11.1%, respectively (Table 3 and 4). On the other hand accession 279/71 showed no seedling death, whereas most of the accessions which were resistant in the field found susceptible under artificial inoculations (Table 3 and 4). Correlation between mortality of trees in the field and

artificial inoculation of seedlings were not significant (Table 5). Girma (1998) in field of 1981 national collection at Gera reported some cultivars such as SN-5, F-51/53 and 248/71 showed 100 % tree loss, similarly high proportion of tree death was recorded on the Gambella and Harar accessions. High tree mortality was associated to coffee cultivars, tree age, intensity of cultural practice and environmental conditions at specific location.

Where as an attempt to quantify the relative resistance among *C.arabica* accessions using incubation period (time taken by plants from appearance of first symptoms) and AUDPC was found significant p=0.05 (Table 5). The result obtained contradicts the findings of Musoli et *al*. (2009) with traits of seedling death, field mortality and seedling death, incubation periods. However, the result obtained with regard to seedling death and AUDPC was in agreement with the findings of Musoli *et al*. (2009). Correlation between seedling death and field mortality become significant when coffee accessions are planted in wilt devastated farms or sick plot and with minimum of five years data. The current study was superimposed for one year on already established coffee trees which is targeted for *ex-situ* conservation. Negative relationship between seedling death and incubation period gives evidence tolerant accessions which have low seedling death expressed prolonged of symptoms expression. Girma *et al*. (2008) also reported lowest wilt severity along with longest incubation periods implies high resistance levels to coffee wilt disease.

Table 5. Correlation between coffee wilt disease (CWD) severity on seedling in the greenhouse and tree death in the field on Arabica coffee collection at Jimma Agricultural Research Center, Ethiopia

	Incubation period	AUDPC	Field mortality
Disease severity	-0.5 (0.2353)*	0.97 (0.2353)*	
Seedling test			0.21 (0.2353)

Figures in parentheses are probability values. AUDPC = Area under Disease Progress Curve.
* = significant at ($p < 0.05$)

4.5. Cluster Analysis

4.5.1. Cluster composition

The clustering patterns of 55 and 51 coffee accessions (Table 6 and 7) were generated from means of three CWD parameters (disease severity, incubation period and AUDPC). These accessions were grouped in to three clusters in both sets of experiment. In the first batch, the first cluster comprises of 44 accessions (80%) and the second cluster consists of ten

accessions (18.2%) and third cluster contain one accession (1.8%). In batch II accessions grouped in three clusters, the first cluster comprises 40 accessions (78.1%) and the second cluster consists of five accessions (9.8%) and the third cluster contains six Accessions (11.8%). In both sets of experiment the first clusters are characterized by susceptible reaction to CWD, cluster three in batch one express resistant reaction, where as cluster two in batch one and cluster two and three in batch two express moderately resistant reaction to CWD.

Cluster analysis in coffee was conducted by few researchers, for instance, Mesfin (2008) reported that cluster analysis grouped 104 coffee accessions collected from 16 weredas of east and west Hararrege in to six clusters. Similarly, Mesfin reported that 41 coffee genotypes collected from south Ethiopia have been grouped in to nine clusters using quantitative and morphological characters.

Chala (2008) also reported that cluster analysis grouped 56 coffee accessions in to four clusters to differentiate resistant and susceptible genotypes to CLR. Olika *et al*. (2011) also grouped 49 coffee accessions collected from Limu Kossa wereda in to 4 clusters based on 22 quantitative morphological characters.

Table 6 . The distribution of germplasm accessions in to three clusters, for 55 coffee accessions tested at Jimma Agricultural Research Center (2011/12), Ethiopia.

Cluster no	Number of accessions	Percent	Coffee Accessions
I	44	80	1/70, 5/84, 54/70, 247/71, 5/70, 1/71, 8/70, 246/71, 4/70, F-13, 258/71, F-39, 59/70, 4/84, F-31, 250/71, 13/79, 1884, Geisha,* 6/70, 243/71, 74/70, 43/70, F-24, F-25, 2/71, 5/72, 56/70, SN-5*, 213/71, 195/71, 197/71, 201/71, 199/71, 25/70, 200/71, 28/70, F-15, F-2, F-37, 203/71, 7932, 276/71, 32/70
II	10	18.2	3/70, 120/71, 244/71, 226/71, 16/70, 30/70, 245/71, 236/71, Catimor J-19**, 259/71
II	1	1.8	279/71

**Susceptible check,* ** Resistant check

Table 7. The distribution of germplasm accessions in to three clusters , for 51 coffee accessions tested at Jimma Agricultural Research Center (2011/12), Ethiopia.

Cluster no	Number of accessions	%	Coffee Accessions
I	40	78.1	19/84, 88/84. 89/84, 98/84, 26/84, 8/78, 22/84, 29/84, 79/84, 11/81, SN-5*, 75/84, 27/84, 86/84, 3/77, 4/72, 13/72, 85/84, 92/84, 100/84, 36/84, 30/84, 10/77, 25/77, 46/77, 33/84, 12/79, 5/77, 20/84, 24/84, Geisha*, 87/84, 5/78, 6/78, 19/77, 11/79, 4/78, 9/78, 39/84, 49/84
II	5	9.8	11/77, 48/83, B106, B2/06, 13/74, Catimor J-19**
III	6	11.8	26/77, 27/77, 44/83, 12/74, B3/06

**Susceptible check,* ** Resistant check

4.6. Evaluation of Coffee Collections for CBD and CLR

Coffee berry disease (*Colletotrichum kahawae*) and coffee leaf rust, (*Hemilea vatatrix*) were observed in conservation block field of Jimma Agricultural Reserch Center (Table 8, 9). The disease assessment result indicated that coffee berry disease varied between 0-95.6 and 0-83.1 in batch I and batch II, respectively. In both set of experiment, coffee berry disease was not observed on eighteen coffee accession (F-2, F-13, F-24, F-31, F-37, F-39, F-51, 195/71, 13/79, 79/32, 33/84, 48/83, 12/74, 13/74, B1/06, B2/06, B3/06, 11/79). On the other hand high CBD severity value 95.6, 94.7, 69.3, 66.7 on coffee accessions 279/71, 25/70, 120/71, 3/70 from batch I and 83.1, 80.8, 78, 75, 71.4, 71.1, 69.5 % was observed on coffee accession 13/72, 3/77, 85/84, 44/83, 89/84, 9/78 , 92/84 from batch II. The mean rust severity varied from 0-34.4 in batch I and 0-13% in batch II. The corresponding incidence ranged from 0-47.3, 0-29.9% in batch I and batch II respectively. Coffee leaf rust was not observed on coffee accession F-2 and F-25 from set I and 12/79 from batch II. Two accessions (195/71, 2/71) from set I showed greater than 20% rust severity. Conversely coffee accessions in batch II showed less than 20% severity. Hyperparasites, *Verticillium hemileiae* was observed on majority of coffee accessions except 5/70, 8/70, 74/70, F-2, F-13, F-24, F-25, F-31, F-39, F-51, 1/71, 245/71 and 26/84, 75/84, 10/77, 26/77,12/74, 13/74, B1/06 and 48/83 for batch I and II respectively.

Arega (2006) reported indigenous forest coffee selections that were selected from four different coffee areas revealed significant variations in percent CBD infections. JARC, Coffee Project progress report 2009/2010 also reports, variation in CBD infection 0.0-47.5%, 0.0-22% and 0.23-56.7% in eighty seven promising Arabica coffee collections planted at Jimma in Set I, II, and set IV respectively. The low level of coffee leaf rust was associated with effect of altitude on rust development and the presence of hyperparasites, *Verticillum hemileiae*. Inverse relationship between rust incidence and altitude was reported by Chala (2008).

Table 8. Reaction of coffee collections to coffee berry disease (CBD) and coffee leaf rust (CLR) under field conditions at Jimma Agricultural Resarch Center, 2012 (Batch I)

Coffee accessions	Severity (%)		CLR (%)		
	CBD	CLR	Incidence		Hyperparasite
			Actual value	Transformed Value	
1/70	23	4.71	32.3	34.6	P
3/70	66.7	3.7	33.1	35.1	P
4/70	31.25	0.66	10	18.4	P
5/70	51.25	1.02	4.9	12.8	A
6/70	0.03	0.83	1.9	7.9	A
8/70	0.9	2.32	5.7	13.8	A
16/70	15.14	7.44	25.3	30.2	P
25/70	94.7	8.3	20.6	27	P
28/70	18.1	14	30	33.2	P
30/70	14.8	2.8	9.9	18.3	P
32/70	10.28	6.9	8.8	17.3	P
43/70	18.5	0.9	2.6	9.3	P
54/70	23.7	1.73	3.4	10.6	P
56/70	2.23	4.3	20.6	27	P
59/70	19.4	0.6	10.5	18.9	P
74/70	30.6	5.02	21.7	27.8	A
F-2	0	0	0	0	A
F-13	0	0.01	0.7	4.8	A
F-24	0	0.03	0	0	A
F-25	0.29	0	0	0	A
F-31	0	0.21	0	0	A
F-37	0.1	0.13	0.9	5.4	A
F-39	0	0.42	1.7	7.5	A
F-51	0	0.01	0	0	A
1/71	36.3	16.6	29.4	32.8	A
2/71	39	24.5	47.3	43.5	P
120/71	69.3	2.8	5.4	13.4	P
195/71	0	34.4	54.4	47.5	P
197/71	32.5	17.5	28.2	32.1	P
199/71	4.1	18.2	39.4	38.9	P
200/71	0.29	18	37.5	37.8	P
201/71	2.6	11.8	29.4	32.8	P
203/71	0.8	14.5	25.5	30.3	P
213/71	7.6	9.2	27.1	31.4	P
226/71	1.33	13.25	21.8	27.8	P
243/71A	0.1	7.3	34.1	35.7	P
244/71	0.23	3.5	20.9	27.2	P
245/71	0.6	4.7	14.7	22.5	A

Table 8. continued.

Coffee accessions	Severity (%)		CLR (%)		
	CBD	CLR	Incidence		Hyperparasite
			Actual Value	Transformed value	
246/71	0.3	3.1	13.1	21.2	P
247/71	10.2	4.3	7.2	15.6	P
250/71	0.01	17.5	28.2	32.1	P
258/71	0.14	5.31	3.4	10.6	P
276/71	50	15	25.2	30.1	P
279/71	95.6	5.8	30.5	33.5	P
5/72	4.8	7.6	20.5	26.9	P
13/79	0	0.1	6.7	15	P
79/32	0	9.7	25.1	30.1	P
4/84	0.9	4.75	12.5	20.7	P
5/84	47.6	1.2	4.1	11.7	P
18/84	14.4	4.8	23.2	28.8	P
236/71A	0.01	12	12	20.3	P
259/71	5.22	14	14	22	P
Mean	16.2	7.1	17	20.7	
LSD(.05)				14	
CV(%)				41.6	

+P= present
A= Absent

Table 9. Reaction level of Coffee collections to coffee berry disease (CBD) and coffee leaf rust (CLR) under field condition at JimmaAgricultural Research Center , 2012 (Batch II)

Coffee Accessions	Severity (%)		CLR		
	CBD	CLR	Incidence		
			Actual value	Transformed value	Hyper parasite
19/84	1.4	3.8	22.3	28.2	P
20/84	14	10	17.2	24.5	A
22/84	16.1	8.3	22.3	28.2	P
24/84	15.9	5.6	16.3	23.8	P
26/84	0.14	3.12	8.9	17.4	A
27/84	18.5	7.1	31.3	34	P
29/84	32.8	8.1	22.2	28.1	P
30/84	0.7	7.8	27	31.3	P
33/84	0	2.1	4.8	12.7	P
36/84	10.1	4	8.1	16.5	P
39/84	28.8	2.5	15.2	22.9	P
49/84	27	0.1	0	0	A
75/84	12.9	0.5	0	0	A
79/84	14	2.1	21.9	27.9	P
85/84	78	5.6	21.9	27.9	P
86/84	12.8	3.8	19.6	26.3	P
87/84	22.4	6.9	31.7	34.3	P
88/84	3.3	4.8	23.7	29.1	P
89/84	71.4	3.3	9.8	18.2	P
92/84	69.5	10.6	22.9	28.6	P
98/84	0.1	6.7	11.4	19.7	P
100/84	29.4	12.8	18.5	25.5	P
11/81	1.7	8.6	16.7	24.1	P
3/77	80.8	1.44	5.1	13.1	P
5/77	5.9	5.03	5.7	13.8	P
13/72	83.1	8.333	19.6	26.3	P
10/77	7.2	0.8	0	0	A
11/77	20.2	6	11.5	19.8	P
19/77	24.4	13.1	19.6	26.3	P
25/77	1.4	0.9	1.6	7.3	A
26/77	10.3	4.3	2.2	8.5	A
27/77	0.08	1.8	3	10	P
8/78	13.3	0.43	0	0	P
46/77	8.5	5.12	16.6	24	P
4/78	12.4	3.1	8.3	16.7	P
5/78	0.9	2.5	10.8	19.2	P
6/78	2.01	0.27	13.7	21.7	P

Table 9. continued.

Coffee accessions	Severity (%)		Incidence		Hyper parasite
	CBD	CLR			
			Actual value	Transformed Value	
9/78	71.1	6.3	9.4	17.9	P
44/83	75	5	18.6	25.5	P
48/83	0	1.3	6.5	14.8	A
12/74	0	0.1	0	0	A
13/74	0	0.2	0	0	A
B1/06	0	4.429	17.1	24.4	A
B2/06	0	4	17	24.4	P
B3/06	0	1.571	6.3	14.5	P
4/72	8.4	10	13.9	21.9	P
11/79	0	2	2.1	8.3	P
12/79	0.2	0	1.4	6.8	A
Mean	18.9	4.5	12.6	16.3	
LSD(.05)				9.1	
CV(%)				34.4	

P= present
A= Absent

4.7. Performance of moderately CWD Resistant Accessions to Coffee Berry Disease in the field

Coffee accession 279/71 which shows no seedling death in the greenhouse expressed highest CBD with severity value of 95.6 %, and coffee accession 120/71, 3/70, and 44/83 showed severity value 66.7-95.6 % to CBD. On the other hand accession 226/71, 259/71, 244/71, 27/77, 12/74, B3/06, B2/06, 48/83 and B1/2006 expressed less than five percent CBD (Figure 4 and 5). Coffee berry disease (CBD) infection less than five percent under field condition is acceptable level of resistance (Van der Graaff, 1981). Girma et al., (2009c), also reportred the presence of multiple disease resistance in some arabica cultivars.

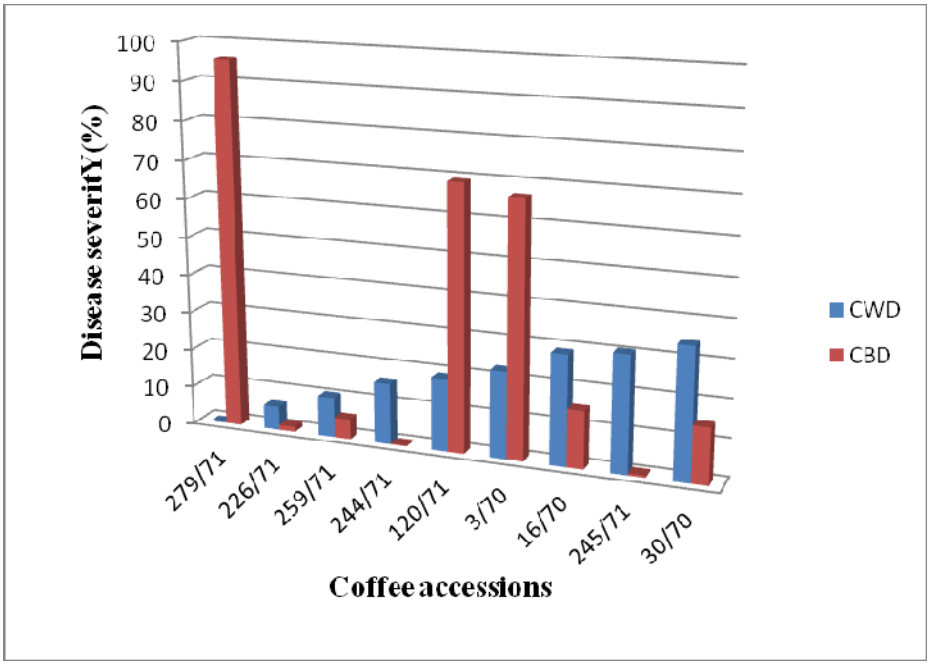

Figure 4 . Reaction of modrreately resistant coffee wilt disease (CWD) accession to CBD Set I. Jima Agricultural Reserch Center (JARC), Ethiopia, 2011/2013.

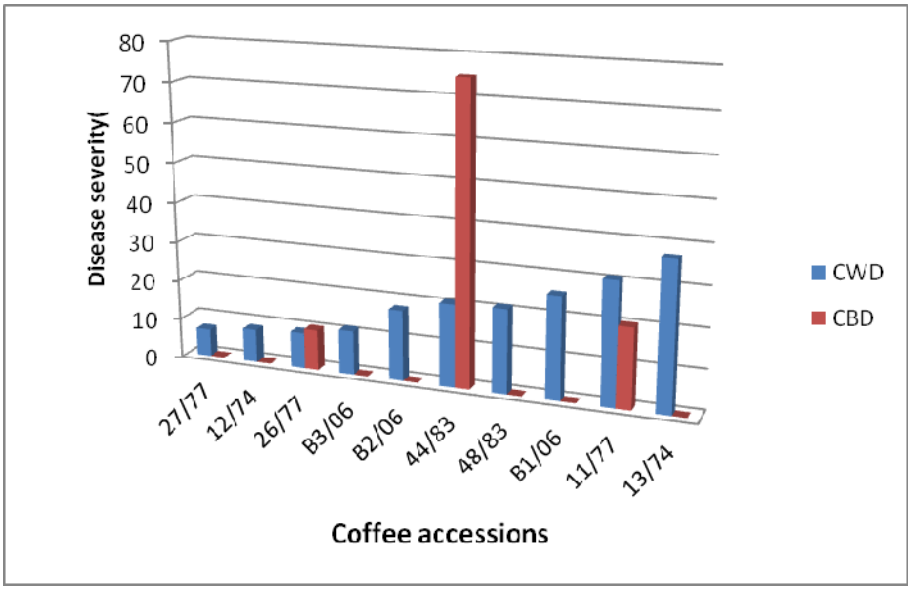

Figure 5 . Reaction of modereately resistant coffee wilt disease (CWD) accession to CBD Set II. Jimma Agricultural Research Center (JARC), Ethiopia , 2011/2013.

5. SUMMARY AND CONCLUSION

Coffee is a commodity of interest worldwide and one of the most economically important crops in the tropics and sub-tropics. Coffee exports constitute an important source of foreign exchange earnings for over 50 countries involved in its cultivation. More importantly, majority of the coffee farmers in the producing countries are small growers who primarily depend on coffee for their livelihood. Among the diseases affecting coffee production, coffee berry disease (*Colletotrichum kahawae)* coffee wilt disease (*Gibberella xylarioides*) and coffee leaf rust caused by the obligate parasitic fungus *Hemileia vastatrix* are the most important ones. Considering the economics of disease managements and also to discourage the chemical control measures, development and use of disease resistant varieties are the most effective and viable options for sustainable coffee production. The CBD resistance selection and screening national program so far released 31 CBD resistant varieties for different coffee growing agroecologies of Ethiopia. Out of these, the application of landrace development program with special emphasis of maintaing the typical quality of each specific region /locality *viz.*, Hararghe, Sidama/Yirgachefe, Wollega (Gimbi) resulted 11 speciality coffee varieties. Among the later, released group, varities 971 (Fayate) and 974 (odicha) are found resistant to coffee wilt disease in that, these two varieties were promoted to Sidama and Yirgachefe areas where CWD infestation is severe (Jefuka *et al*., 2013)

In Ethiopia coffee wilt disease is increasingly becoming more and more important, especially in garden, semi forest, forest and plantations coffees. The national incidence and severity of CWD in the country were 29.9 % and 3% respectively, in monetary terms it causes financial loss of more than 3.7 million USD. Current control method of CWD in Ethiopia includes eradication of diseased trees, mulching, the use of cover crops and protection by stem painting as a preventive measure. However these methods are tedious and expensive to implement. Searching for host resistance can serve as long term solution in managing coffee wilt disease.

This study was, therefore conducted under both laboratory and field conditions at Jimma Agricultural Research Center with objectives of evaluating coffee germplasm and identify resistant genotypes to coffee wilt disease. Hundred coffee accessions from conservation

block which shows field resistance of 80% and above were selected. The tested accessions comprised of Oromiya (54%), SNNPS (44%) and Amhara (2%). Inoculation was done in two batches with 30 seedlings per box using randomized complete block design with three replications using stem nicking, introducing a standard inoculum (2.3x 10^6 inoculum suspensions) of *G. xylariodes* isolate. In addition tested coffee accessions were evaluated visually for coffee berry disease (CBD) and coffee leaf rust (CLR) under field condition.

In seedling test of hundred coffee accessions against coffee wilt disease pathogen, significant variations were recorded among coffee accessions in reaction to CWD. Mean wilt (dead) percent seedlings varied between 0 - 98.8% and 2.3-97.5 % in set I and II respectively. For moderately resistant accessions mean wilt percent seedlings 0-33.8 and 2.3-36.6% were obtained for set I and set II, respectively. Nine accessions from batch I namely 279/71, 226/71, 259/71, 244/71, 120/71, 3/70, 16/70, 245/71, and 30/70) and 10 accessions from batch II including 27/77, 12/74, 26/77, B3/06, B2/06, 44/83, 48/83, B1/06, 11/77 and 13/74 showed low percentages of seedling death indicating moderate resistant as compared to resistant standard resistant check catimor J-19.

The clustering patterns of 55 and 51 coffee accessions were generated from means of three CWD parameters (Disease severity, Incubation period and AUDPC). These accessions were grouped in to three clusters in both set of experiment. In the first batch, the first cluster comprises of 44 accessions (80%) and the second cluster consists of ten accessions, (18.2%) and third cluster contain one accession (1.8%). In batch II accessions grouped in three clusters, the first cluster comprises 40 accessions (78.1%) and the second cluster consists of 5 accessions (9.8%) and the third cluster contains six Accessions (11.8%). In both set of experiment the first clusters are characterized by susceptible reaction to CWD, cluster three in batch one express resistant reaction, where as cluster two in batch one and cluster two and three in batch two express moderately resistant reaction to CWD.

Most of the accessions which were resistant in the field found susceptible under artificial inoculations. Correlation between mortality of trees in the field and artificial inoculation of seedlings were not significant. Whereas an attempt to quantify the relative resistance among *C.arabica* accessions using incubation period (time taken by plants from appearance of first symptoms to death) and AUDPC was found significant p=0.05

The field evaluation result showed that the two diseases coffee berry disease and coffee leaf rust were observed in conservation block of Jimma Agricultural Research Center. The disease assessment result indicated that coffee berry disease varied between 0-95.6 and 0-83.1 in batch I and batch II respectively.The mean coffee leaf rust varied from 0-34.4 in batch I and 0-13% in batch II. Among moderately resistant coffee accessions of Set I 244/71, 245/71 226/71 and 259/71 shows 0.23, 0.6, 1.33 and 5.22 CBD infection which is acceptable level of resistance under natural infection. Coffee accessions 30/70, 16/70, exhibited 14.8, and 15.14 % CBD infection, respectively. Three accessions 279/71, 120/71, and 3/70 showed 95.6, 69.3 and 66.7 % CBD infections. From set II B1/06, B2/06, B3/06, 12/74, 13/74, 48/83 and 27/77 shows <1% CBD infection. Three accessions from set II 26/77, 11/77, and 44/83 exhibited 10.3, 20.2 and 75 % CBD infection. Four accessions from Bale (B3/06, B2/06, 48/83, B1/06), three accessions from Gedeo (27/77, 12/74, 13/74) and four accessions from Bench maji (226/71, 259/71, 244/71, 245/71) expressed CBD infection less than five percent. Two accession from Jimma Zone (279/71 and 3/70) exhibited high level of CBD infection.

The results of pathogenecity tests proved that there is important diversity in conservation block of Jimma Agricultural Reserch Center in reaction to *G. xylarioides* infection. The origin of moderately resistant accessions againest CWD in both set of experiment was from Gedeo, Bale, Bench maji, Jimma, and Sidama zones. However, susceptible reaction was observed in coffee accessions collected from Kaffa, Iluababor, West Gojam and West Wellega which alert the National Coffee Reserch Center to do additional selection work targeted to coffee wilt disease resistance.

In this study, resistant, moderately resistant and highly susceptible accessions have been identified which can be used by the breeder to understand the type of resistance which is necessary for developing CWD resistant variety for *C.arabica*. For *C. robusta* moderate broad sense of heritability (0.329) and low narrow sense hertibility (0.112) was reported, which shows CWD resistance is heritable, but transmission of from parents to progenies only about 33% (Musoli, *et al*.,2009).

It is obvious that not all the moderately CWD resistant accessions have all desired qualities of commercial varieties. Therefore, the CWD tolerant accessions must undergo

preliminary field evaluation, after second round inoculation, where they have to be evaluated for yield, quality and resistance to other diseases such as coffee leaf rust, coffee berry disease and other minor disease. Further evaluation in different agroecological areas in wilt devastated gardens or farms (sick plot) so as to validate their performance and adaptation to different localities (multilocation trials) are recommended.

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7. APPENDICES

Appendix 1. Analysis of variance table for percent wilt of seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Batch I)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Acessions	54	84492. 70145	1564. 67966	20. 97	<.0001
Error	110	8209. 06000	74. 62782		
Corrected Total	164	92701. 76145			

Appendix 2. **Analysis of variance table for incubation periods (days) after inoculating seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Batch I)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Accessions	54	124402. 9091	2303. 7576	5. 34	<.0001
Error	110	47490. 6667	431. 7333		
Corrected Total	164	171893. 5758			

Appendix 3. **Analysis of variance table for area under disease progress curve after inoculating seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Bach I)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Accessions	54	540543. 7188	10010.0689	20. 88	<. 0001
Error	110	52723. 2733	479.3025		
Corrected Total	164	593266. 9921			

Appendix 4. Analysis of variance table for percent wilt of seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Batch II)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Accessions	50	77456. 04902	1549. 12098	13. 52	<.0001
Error	102	11689. 01333	114. 59817		
Corrected Total	152	89145. 06235			

Appendix 5. **Analysis of variance table for incubation periods (days) after inoculating seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Batch II)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Accessions	50	172013.8039	3440.2761	6.45	<.0001
Error	102	135974.6667	533.2340		
Corrected Total	152	307988.4706			

Appendix 6 . **Analysis of variance table for area under disease progress curve after inoculating seedlings of arabica coffee accessions inoculated with *G. xylarioides* isolate (Bach II)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Accessions	50	483356.8047	9667.1361	14.24	<.0001
Error	102	69247.9800	678.9018		
Corrected Total	152	552604.7847			