ADDIS ABABA UNIVERSITY,

INSTITUTE OF BIOTECHNOLOGY

Mapping of Novel Quantitative Trait Loci (QTL) for *Fusarium* Wilt Resistance in Chickpea (*Cicer arietinum* L.) and Analysis of the Genomic Diversity of *Fusarium oxysporum* f. sp. *ciceris* in Ethiopia

PhD Dissertation

Dagnachew Bekele Besha

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Dagnachew Bekele Besha

A Thesis Submitted to the Institute of Biotechnology, School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (PhD) in Biotechnology

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DECLARATION

I declare that the thesis hereby submitted for the Degree Doctor of Philosophy (PhD) in Biotechnology to Addis Ababa University, Institute of Biotechnology is my own work and has not previously been submitted by me or anybody else at another university.The materials obtained from other sources have been duly acknowledged in the thesis.

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most economically important food legumes cultivated in different parts of the world. Ethiopia is the largest producer, consumer and exporter of chickpea in Africa. However, several biotic and abiotic stresses restrict its potential productivity. Among the biotic stresses, fungal diseases are the major yield limiting factors throughout chickpea producing countries. *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc), is one of the most dominant and destructive pathogen threatening chickpea production in Ethiopia. Breeding for host plant resistance is the most cost efficient and eco-friendly strategy to control the disease. Nevertheless, chickpea breeding for *Fusarium* wilt resistance is regularly challenged with high pathogenic variability and limited availability of good resistance sources. So far only few efforts have been made to investigate the genetic diversity and geographic distribution of Foc pathogen in Ethiopia for designing effective breeding and integrated disease management strategies. For this disease, no report is available that encompass the breadth of major and minor chickpea producing areas of the country. In this study four sets of experiments were executed with the main objectives to: investigate the intensity and association of *Fusarium* wilt/root rot disease of chickpea under diverse biophysical factors in Ethiopia; identify new resistance sources and map a novel wilt resistant quantitative trait loci (QTL) in chickpea; analyses the genomic diversity, pathogenic variability and geographic distribution of Foc pathogen in the country; and develop rapid and reliable disease diagnostic assay for accurate disease diagnosis.

In 2015 and 2016 cropping seasons, geo-referenced field surveys were conducted covering a total of 62 major chickpea growing districts located in 19 diverse agro-ecological zones of Ethiopia, and a total of 217 diseased plant samples

were collected for pathogen identification and genomics study. Among these, from 51 representative farmers' fields, three 1 x 1 meter quadrat were surveyed along a diagonal transect to investigate the intensity and association of *Fusarium* wilt/root rot disease of chickpea under diverse biophysical factors in Ethiopia. Data on major biophysical factors were recorded, and pathogen was isolated based on the established morphological and cultural characteristics. For identification of new *Fusarium* wilt resistance sources, a total of 315 wild introgression lines and 47 recombinant inbred lines (RILs) were evaluated for *Fusarium* wilt resistance in sick plot at Debre Zeit Agricultural Research Center. To map *Fusarium* wilt resistance QTL in chickpea, total of 108 F_2 hybrids were generated by crossing *Fusarium* wilt resistant variety Dera and *Fusarium* wilt susceptible genotype JG 62, and genotyping-by-sequencing identified 1,659 single nucleotide polymorphisms (SNPs) that distinguish the two parental lines. A total of 166 representative *Fusarium* isolates collected from different part of the country were sequenced using whole genome sequencing (WGS) with Illumina HiSeq 4000 platform to investigate the genomic diversity, pathogenic variability and geographic distribution of Foc pathogen. For rapid and accurate detection of Foc pathogen directly from symptomatic chickpea plants, broad specificity PCR primers were designed based on the alignment of selected Benchmarking Universal Single Copy Orthologs (BUSCO) genes present and highly conserved in the genomes of a set of 66 *Fusarium* isolates. Moreover, a culture-independent broad-range18S amplicon survey was conducted to characterize chickpea-associated eukaryotic communities.

The result indicated that *Fusarium* wilt disease was widely distributed in all growing areas of the country. Across all surveyed sites, Foc was the predominant species encountered among fungi cultured from plant tissue, representing 69.4 % of total isolates. Diseases pressure was significantly ($P < 0.05$) associated with heavy black soils, Desi type chickpea, early planting, flowering and plant maturity. The highest mean percent diseases incidence per m^2 (45.65%) was recorded in the Amhara region, West Gojam zone, where heavy clay soils predominate. Wild introgression lines and advanced recombinant inbred lines showed significant genetic diversity for *Fusarium* wilt resistance and yield related traits that can be exploited to improve the agronomic value of the chickpea crop. In the present study 20 *Fusarium* wilt resistant RILs with high yield and desirable agro morphological traits were identified. For *Fusarium* wilt resistance QTL mapping, a total 836 high quality SNP markers were

assigned to six genetic linkage groups, each corresponding to separate chromosomes, with a total map size of 274.9 cM and 3.12 cM average distance between mapped markers. Major QTL explaining 55.28 % of the observed phenotypic variation was identified on chromosome 4 at 44.29 cM with a logarithm of odds (LOD) score of 13.8. Interestingly, Nei's genetic diversity analysis based on 196, 495 SNPs split test isolates into 20 distinct clusters irrespective of their regions of origin and geographical location. Among these,16 distinct clusters were *Fusarium oxysporium ciceris* (Foc) isolates. Phylogenetic analysis based on 1,052 highly conserved BUSCO genes also divided test isolates into six distinct *Fusarium* species, and 16 sub-groups (Foc isolates). Consistent with these results, pairwise average nucleotide identity (ANI) analysis based on 3,695 highly conserved BUSCO genes split test isolates in to six distinct *Fusarium* species, using 95 % ANI (ANI_{95}) as the lower species boundary. Besides, dendrogram built based on virulence data split Foc isolates into four distinct virulence groups confirming the existence of high pathogenic variability between Foc isolates in Ethiopia irrespective of their geographical origin. Mantel correlation estimate showed very weak correlation between geographical distance and genetic distances of *Fusarium* isolates in Ethiopia with $P = 0.280$ *and* $R^2 = 0.0006$. The results the PCR diagnostic assay showed that, on test with *Fusarium* specific PCR primer (EOG09331-PTT), 97.5 % of diseased plants with typical symptoms (39 out of 40 plants) gave uniformly strong amplification with the identity of amplicons confirmed by Sanger sequencing. However, some symptomatic plants yielded inconsistent results as PCR based disease diagnosis using organism-specific DNA amplification is unable to assess the presence of all other microbes that might better inform diagnosis. To address these issues, microbial community composition were surveyed using 18S amplicon sequencing. The result nominated *Phytophthora medicaginis* as alternative pathogens in some fields where *Fusarium* wilt was suspected. Such analyses represent a potentially powerful alternative to traditional plant disease diagnostics. Without the constraints of culturability and the bias of endpoint PCR, amplicon sequencing can provide powerful insights into disease dynamics.

In conclusion, the novel major QTL and associated genetic markers identified in the present study offer molecular tools for breeding wilt resistant against Ethiopian Foc isolates. This study indicated the presence of high genetic diversity and pathogenetic variability between *Fusarium* isolates in Ethiopia. Therefore, designing

effective country wide breeding and integrated disease management strategies against Foc pathogens is key to break the recurrent disease cycle in the country. The results of the present study provide detailed information and appropriate framework to develop effective breeding and integrated disease management strategies to combat *Fusarium* wilt disease of chickpea in Ethiopia.

Key words: ANI, BUSCO gene, Chickpea, *Fusarium*, Genotyping-by-sequencing, PCR, SNPs, 18S

DEDICATION

This Doctoral Dissertation is dedicated to my beloved mother, Beletu Tegegn, who is hard worker, wise and kind to all; undefeated by the vagaries of poverty and women oppressive rural cultural life, she has raised us with determination, affection and great discipline. She is my icon of high integrity, ethics and humanity.

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List of Acronyms and Abbreviation

AB	=	Ascochyta blight
AFLP	=	Amplified Fragment Length Polymorphism
ANI	=	Average Nucleotide Identity
ANIb	=	Average Nucleotide Identity Algorithm using BLAST
ANIm	=	Average Nucleotide Identity using MUMmer algorithm
ANOVA	=	Analysis of Variance
ASV	=	Amplicon Sequence Variant
BEAST	=	Bayesian Evolutionary Analysis of Sample Tree
BLAST	=	Basic Local Alignment Search Tool
BUSCO	=	Benchmarking Universal Single Copy Orthologs
CaLG	=	Chromosome Linkage Group
cM	=	Centimorgan
CSA	=	Central Statistics Authority
DADA2	=	Divisive Amplicon Denoising Algorithm 2
DArT	=	Diversity array technology
DARwin	=	Dissimilarity Analysis Representation for window
DF	=	Degree of freedom
DFW	=	Days to 50 % flowering
DM	=	Number of days to maturity
DZARC	=	Debre Zeit Agricultural Research Center
EB	=	Emulsion Buffer
EIAR	=	Ethiopian Institute of Agricultural Research
EtdFoc	=	Ethiopian *Fusarium isolates* collected by Dagnachew
EthFoc	=	Ethiopian *Fusarium isolates* collected by Sultan

FAOSTAT	=	Food and Agricultural Organization of the United Nations statistic data base
Foc	=	*Fusarium oxysporum* f. sp. *ciceris*
FOL	=	*Fusarium lycopersicum*
FW	=	*Fusarium* wilt
FWI	=	*Fusarium* wilt incidence
GA	=	Genetic Advance
GAB	=	Genomics Assisted Breeding
GAM	=	Genetic Advance as Percentage of Mean
GBS	=	Genotyping -By-Sequencing
GC	=	Guanine-cytosine
GCV	=	Genotypic Co-efficient of Variations
gDNA	=	Genomic DNA
GPS	=	Global Positioning System
GS	=	Genome Selection
GWAS	=	Genome-Wide Association Studies
H^2b	=	Broad sense heritability
HI	=	Harvest Index
HSW	=	Hundred Seed Weight
ICARDA	=	International Center for Agricultural Research in the Dry Areas
ICRISAT	=	International Crop Research Institute for Semi-Arid Tropics
ISSR	=	Inter Simple Sequence Repeats
ITS	=	Internal Transcribed Spacer
LG	=	Linkage Group
LOD	=	Logarithm of the odds
LSD	=	Least Significant Difference

MABC	=	Marker Assisted Back Crossing
MAF	=	Minor allelic frequency
MAFFT	=	Multiple Alignment using Fast Fourier Transform
MAGIC	=	Multiparent Advanced Generation Inter-cross
MAS	=	Marker assisted selection
MEGA	=	Molecular Evolutionary Genetics Analysis
Mbp	=	Million base pair
mtDNA	=	mitochondrial DNA
NCBI	=	National Center for Biotechnology Information
NPP	=	Number of pods per plant
NSP	=	Number of seeds per pod
NWP	=	Number of Wilted Plants
PBM	=	Plant Biomass
PCoA	=	Principal Coordinate Analysis
PCR	=	Polymerase chain reaction
PDA	=	Potato dextrose agar
PH	=	Plant height
PVC	=	Phenotypic Co-efficient of Variations
PVE	=	Phenotypic Variation
PWI	=	Percent wilt Incidence
QC	=	Quality control
QTL	=	Quantitative trait loci
RAD-Seq	=	Restriction site Associated DNA Sequencing
RAPD	=	Random amplified polymorphic DNA
reVILs	=	reverse Introgresion Lines

RCBD	=	Random Complete Block Design
rDNA	=	Ribosomal DNA
RFLP	=	Restriction fragment length polymorphism
RILs	=	Recombinant Inbred Lines
SAS	=	Statistical Analysis Software
S.E.	=	Standard error
SCAR	=	Sequences Characterized Amplified Regions
SNNP	=	Southern Nations, Nationalities, and Peoples
SNP	=	Single nucleotide polymorphisms
SSD	=	Single Seed Descent
SSR	=	Simple Sequence Repeats
TEF	=	Translation elongation factor 1-α
T_m	=	Melting temperature
UCD	=	University of California, Davis
UPGMA	=	Unweighted pair group average method
USA	=	United States of America
USAID	=	United States Agency for International Development
VE	=	Error Variance
VG	=	Genotypic variance
Vp	=	Phenotypic variance
WGS	=	Whole Genome Sequencing
XlnR:	=	Xylanase gene transcriptional activator
Xyl4	=	Xylanase gene
YLD	=	Grain Yield
χ^2	=	Chi square

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background and Justification

Chickpea (*Cicer arietinum* L.) is one of the most economically important food legumes cultivated in different parts of the world. It is a cheap source of high quality proteins in the diets for millions of families, particularly in developing countries for those who can not afford animal protein for balanced nutrition (Jendoubi et al., 2017). Chickpea fixes atmospheric nitrogen in soils and thus highly valued in the cropping systems for its overall impacts on soil health and fertility. Chickpea is grown in more than 55 countries around the world on 13.72 million hectares of land producing 14.25 million tons with average productivity of around 1.04 ton ha^{-1}(FAOSTAT, 2019).

India is the world leading chickpea producing country, annually producing around 9,937,990 tons on 9,547,030 hectares, with average productivity of around 1 ton ha^{-1} (FAOSTAT, 2019). In Africa, Ethiopia is the largest producer, consumer and exporter of chickpea, annually producing around 435,193 tons on about 208,838 hectares of land (FAOSTAT, 2019). It is mainly grown in Oromiya, Amhara, Tigray and Southern Nations, Nationalities People (SNNP) regions, at an altitude of 1400-2300 meter above sea level (CSA, 2018), with annual rainfall ranging between 700 and 2000 mm. Under optimum growing conditions chickpea productivity ranges between 3.5 to 5 ton ha^{-1} (Roorkiwal et al., 2018). However, in Ethiopia several biotic and abiotic stresses restrict its national average yield to around 2.08 ton ha^{-1}(FAOSTAT, 2019).

Chickpea sensitivity to diverse abiotic and biotic stresses has increased mainly due to lack of stable resistance varieties for different stresses and use and reuse of limited number of genotypes in production system (Muehlbauer and Sarker, 2017). Among the abiotic stresses, drought, heat stress, salinity, water logging occurring at various stages of the crop cycle are the major yield limiting factors in arid and semi-arid regions of the major chickpea producing countries including Ethiopia (Singh et al., 2008). Drought and heat stresses alone can reduce chickpea yields by up to 70 % (Varshney et al., 2014a).

Globally several biotic stresses are threatening chickpea production and productivity (Tarafdar et al., 2017, 2018). Above 172 pathogens have been reported that infect chickpea (*C. arietinum* L) world wide, however only a few of them have a potential to devastate the crop (Nene et al., 1996). The persistent chickpea diseases in the world with wide geographic areas includes *Fusarium* wilt, Ascochyta blight, dry root rot, botrytis gray mold, collar rot, black root rot, phytophthora root rot, pythium root and seed rot and stunt, while others are sporadic in occurance (Nene et al., 2012). In Ethiopia, the major diseases of chickpea include *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ciceris*), Ascochyta blight (*Ascochyta rabiei*), dry root rot (*Rhizoctonia bataticola*), black root rot (*Fusarium solani),*wet root rot (*Rhizoctoniasolani*) and collar rot (*Sclerotium rolfsii*) (Beniwal et al., 1992; Damte and Ojiewo 2016;Yimer et al., 2018). Among the soil borne diseases, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is the most dominant and devastating root infecting chickpea pathogen in Ethiopia (Yimer et al., 2018). Virus diseases such as the chickpea chlorotic stunt virus, bean leaf roll virus; beet western yellow virus, soybean dwarf virus, the pea seed-borne mosaic virus are also economically important in many

parts of chickpea growing areas of Ethiopia (Berhanu et al., 2005; Abraham et al., 2009).

Foc is one of the most widely distributed diseases of chickpeain the world (Patil et al., 2015). Recently, the disease is wide spread in most chickpea growing areas in Asia, Africa, Southern Europe and the Americas, but it has not yet been reported in Australia (Jimenez Diaz et al., 2015). In different countries, this disease can cause yield losses approaching 100 % in highly infested fields (Halila and Strange 1996; Navas Cortes et al., 2000; Sharma et al., 2016; Upasani et al., 2017).

Foc is pathogenic to multiple species of the genus *Cicer*, of which chickpea is the only cultivated member (Dean, 2012) causing highly catastrophic vascular wilt disease. It is mainly soil borne but also persistent as a seed borne pathogen (Haware et al. 1978; Pande et al. 2007; Jimenez Diaz et al., 2015). The fungus can survive in the soil for more than six years (Haware, 1990b). The disease can appears during all plant growth stage, but more common in the early seedling stage and at maturity (Nene et al., 1978; Beniwal et al. 1992). Three weeks after sowing, seedlings affected with this disease collapse, retaining almost normal green color (Nene et al., 1978). The Foc pathogen directly enters the xylem tissues and spreads rapidly up through the vascular system and become a systemicdisease in the host plant and also directly infect the seed. Subsequently, translocation of water and nutrients is severely prevented by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, usually followed by death of the whole plant (Cho and Muehlbauer, 2004).

Based on the disease symptoms two pathotypes, causing yellowing and wilting symptoms have been identified. The yellowing pathotype induces progressive foliar yellowing with vascular discoloration and late plant death, while the wilting pathotype causes fast and severe chlorosis, flaccidity, vascular discoloration and early plant death (Jimenez-Fernandez et al., 2013). So far eight distinct physiological races of Foc (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been reported (Jimenez Gasco et al., 2004b; Dubey et al., 2012; Sharma et al., 2014). Race 0 and 1B/C are classified as yellowing pathotypes, while races 1A, 2, 3, 4, 5 and 6 are classified as wilting pathotypes (Jimenez Gasco and Jimenez Diaz 2003). However, in different parts of the world recently change in the race scenario of Foc has been reported (Dubey et al., 2012; Sharma et al., 2014). In Ethiopia, both pathotypes and five Foc pathogenic races (races 0, 1, 2, 3 and 4) were reported (Tadesse et al.,1998b; Shehabu, 2008)

Due to the soil born nature of the pathogen, chemical control of the disease is not feasible. Cultural practices, especially extended crop rotations, can reduce disease incidence, but their efficacy is variable and implementation is complicated under smallholder conditions where chickpea is a vital component of diets and is a preferred cash crop (Nene and Reddy 1987; Trapero Casas and Jimenez Diaz,1985). In Ethiopia, traditional cropping practices particularly repeated use of local cultivars, poor crop rotation practices and sowing by broadcast method favor the inoculum build up in the soil and thereby the occurrence of the disease (Getachew and Woldeyesus, 2012).

Several studies have demonstrated use of resistant varieties is one of the most practical and cost effective strategy for control and management of *Fusarium* wilt

disease in chickpea (Malik et al., 2006; Nikam et al., 2007). In different parts of the world, high resistance have been identified through targeted disease resistance breeding efforts and effective field, greenhouse and laboratory procedures for resistance screening have been developed (Pande et al., 2006; Gaur et al., 2014; Mirzapour et al., 2014; Chobe et al., 2016). However, chickpea breeding for *Fusarium* wilt resistance regularly challenged with high pathogen variability, the existence of different physiological races and the undesirable agronomic traits in most resistance sources. For instance, in India, most of the resistant varieties have been found to be susceptible due to frequent breakdown of the resistance with different pathogenic races in different regions (Sharma et al., 2014, 2019).

In Ethiopia, although some moderately resistant chickpea varieties have been recommended for the management of the disease, their performance was not uniform in different partsof the country. This may be due to the variability of the pathogen in different parts of the country. Although cultivation of varieties possessing durable resistance to specific races of the pathogen is the most economical disease management strategy (Jalali and Chand, 1992, Sharma et al., 2014), the genetic diversity and geographical distribution of the of the pathogen in Ethiopia have not been yet studied.

Therefore, understanding the genetic diversity and geographical distribution of Foc pathogen in the country is prerequisite for the development of effective breeding strategy and for efficient use of available wilt resistant genotypes. Monitoring the genomic diversity and geographical distribution of Foc pathogens through use of advanced genomic technologies will greatly help to better understanding pathogen

diversity in the country. Integration of advanced genomic tools and experience in breeding program, particularly QTL mapping and marker assisted backcross breeding has been known to take a shorter time and enable to undertake a more precise breeding for *Fusarium* wilt resistance (Li et al., 2015).

QTLs that confers high level of resistance to different physiological races of the pathogen have been mapped (Sharma et al., 2005; Sharma and Muehlbaur 2007; Sabbavarapu et al., 2013; Li et al., 2015), and currently several genomic resources/technologies are available to further speed up the breeding process to develop wilt resistant chickpea varieties (Thudi et al., 2011, 2016b; Varshney et al. 2014b). Therefore, integration of genomic technologies in Ethiopia chickpea improvement program is indispensable to combat the ever increasing production and productivity challenges.

1.2. Relevance of the Present Study

Major challenges posed by various biotic and abiotic stresses must be addressed in order to keep enhanced chickpea production, productivity and contribute to livelihood improvement in Ethiopia. Several studies demonstrated that Foc is the most dominant root infecting chickpea pathogen in Ethiopia annually causing 30-100 % yield loss in different parts of the country (Beniwal et al.,1992; Bejiga et al. 1996; Tadesse, 1998a; Shehabu et al., 2008, Asrat, 2017; Yimer et al., 2018).

Recent studies also indicated disease incidenceand distribution in farmers field has shown a considerable increase in Ethiopia (Damte and Ojiewo 2016; Yimer et al., 2018). However, the genetic diversity and geographic distribution of Foc isolates in Ethiopia were not studied and yet not known.

Different studies have demonestrated that use of resistant varieties with durable resistance to specific races is the most economical wilt disease management strategy in chickpea (Jalali and Chand, 1992; Malik et al., 2006; Nikam et al., 2007; Sharma et al. 2019). Successful chickpea breeding program for *Fusarium* wilt resistance requires significant genetic variation for wilt resistance among the breeding lines, efficient evaluation and selection method for wilt resistance and desirable plant types for commercial production (Salimath et al., 2007). However, due to limited availability of *Fusarium* wilt resistance sources both in Ethiopia chickpea improvement program, and also in global germplasm collections (Pande et al., 2006), identification of chickpea genotypes with high level of wilt resistance is highly required. Besides, the performance of the available moderately wilt resistant varieties were also not uniform in different growing areas of the country suggesting the existence of different pathogenic races in different parts of the country.

In Ethiopia the significance of the disease in different parts of the country have not well studied to initiate effective breeding strategy for host plant resistance and integrated disease management measures. All previous studies were highly restricted only to the major chickpea producing areas of the country (Beniwal et al., 1992; Tadesse 1996;1998a; Shehabu et al., 2008; Abera et al. 2011; Damte and Ojiewo 2016; Yimer et al., 2018). So far, no reports are available that encompass the breadth of major and minor chickpea producing areas of the country. Besides, identification and mapping of QTLs for wilt resistance are major prerequisites to implement effective marker assisted backcrossing to incorporate wilt resistance genes in farmers and market preferred chickpea varieties in Ethiopia. The classical biological

pathotyping techniques alone are not enough for a reliable identification and characterization of fungal pathotypes and populations (Jamil et al., 2000). Therefore, development of rapid, efficient, reliable and reproducible DNA-based diagnostics assays that identify Foc pathogen and its pathogenic races are among the top priorities for applying effective integrated wilt disease management measures.

1.3. Research Objectives

1.3.1. General objective

To develop *Fusarium* wilt resistant and high yieldingchickpea varieties through integration of advanced genomics technologies

1.3.2. Specific objectives

I. To understand the extent and association of *Fusarium* wilt of chickpea with diverse biophysical factors in Ethiopia
II. To analyse the genetic diversity of chickpea (*Cicer arietinum* L.) advanced breeding lines for identification of high yielding new *Fusarium* wilt resistance sources
III. To identify and map quantitative trait loci (QTL) for *Fusarium* wilt resistancein chickpea (*Cicer arietinum* L.) through high density polymorphic DNA markers
IV. To study the genomic diversity, pathogenic variability and geographic distribution of *Fusarium* wilt pathogen of chickpea (*Fusarium oxysporum* f. sp. *ciceris*) in Ethiopia
V. To develop rapid and reliable molecular disease diagnostic assay for identification of chickpea wilt pathogen (*Fusarium oxysporum* f. sp. *ciceris*)

CHAPTER TWO

2. LITERATURE REVIEW

2.1.The Evolution of Genomics Assisted Breeding

Since the domestication of the first plants 13,000 to 11,000 years ago, mankind has tried to develop different food crops to satisfy his needs. The hybridizations and selection pressure applied over these years has resulted in the domestication of wild varieties into hundreds of thousands of breeds, forming the basis of our current crops (McCouch, 2004). However, this selection process has reduced the genetic basis of the plants used for food production (Tester and Langridge, 2010).

Besides, displacement of native crops by major crop, and intensification of agricultural production through the green revolution significantly reduced genetic and trait diversity within major crop species (Pingali, 2012). This narrow genetic base directly endangers food security as crops worldwide become susceptible to different biotic and abiotic stresses (Gur and Zamir, 2004).

However, the production and productivity of major food crops globally increased for the last century (Fischeretal., 2009). This has been mainly achieved through conventional plant breeding (Kole et al., 2015). For the last 70 years, when the world population tripled from around 2.5 billion in 1950 to more than 7.7 billion in 2020, the agriculture land has increased only by 11% (FAO, 2018). This low increase in land used for agriculture is mainly due to improved crops and agricultural techniques achieved through conventional plant breeding. According to FAO (2018), one of the major challenges for the coming years will be producing 70 % more food for an additional 2.3 billion people by 2050.

Besides, currently global agriculture is facing a serious threat from reduced genetic diversity within major crop species and climate change. Increasing food prices and greater global food insecurity are the outcomes of the very slow rate of increase in agricultural productivity as well as the fast population growth rate (FAO, 2018). Therefore, in the future producing more additional foods to feed the ever increasing population of the world will be one of the major challenges in plant breeding.

To overcome and respond to these ever increasing challenges, conventional plant breeding has made great progress in the last century. However, labor intensity, time consumption, low efficiency, and environment dependence, etc., are among the major barriers that impede conventional plant breeding. Nowadays, particularly in developed countries the focus of plant breeding has gradually switching from phenotype based to genotype based selection due to advances in molecular biology and highthroughput genotyping technology.

Modern plant breeding depends on the revolution that have brought advances in biotechnology, genomic and molecular marker development and application (Moose and Mumm 2008). In the early 1980s, modern plant breeding era began by integrating new biotechnological approaches with the landmark reports of producing transgenic plants using Agrobacterium (Bevan et al., 1983; Fraleyet al., 1983; Herrera-Estrella et al., 1983). Since then, genetic maps relying on molecular markers and allowing correlating genetic linkage between markers and quantitative traits developed (Edwards et al., 1987; Paterson et al., 1988; Xu and Crouch, 2008).

As a result, marker assisted selection (MAS) has improved breeding efficiency to some extent and prevailed in breeding programs for decades (Cooper et al., 2014). Several MAS strategies have been developed including marker assisted backcrossing for introgression of major genes or quantitative trait loci (QTL), enrichment of favorable alleles in early generations, and selection for quantitative traits using markers at multiple loci (Gupta et al., 2010). However, earlier molecular marker technologies are laborious and time consuming as compared withthe next-generation sequencing technology that allows detecting large numbers of highly polymorphic markers such as SNPs in a short time-frame (Yang, Tao et al., 2012).

Over the last two decades, with rapid development of whole genome sequencing technologies, the breeding paradigm is switching from a marker based system to a genomic base system, and marker assisted breeding is being replaced by genomics assisted breeding (GAB)(Cooper et al., 2014). The development of whole genome sequencing technologies has greatly improved GAB by providing a way to generate large amounts of DNA markers rapidly. Most of these DNA markers are single nucleotide polymorphisms (SNP) which are generated by different sequencing technologies. Nowadays, many crops have had these SNPs made available from sequencing projects.

The ultimate goal of GAB is to find the best combinations of alleles, optimal gene networks, and specific genomic regions to facilitate crop improvement (Cooper et al., 2014). GAB is especially useful for the improvement of complex traits due to its advantages of high accuracy, direct improvement, short breeding cycle, and high selection efficiency. Moreover, integration of genomics tools and conventional

breeding triggers new breeding strategies, like QTL mapping and gene pyramiding, which greatly accelerates the breeding.

2.2.The Importance of Legume Crops

Legumes are important higher plants with major impact on agriculture, human and livestock nutrition and environment (Gupta et al., 2014). Globally, legumes are known for their good nutritional values. For instance, in different parts of the world grain legumes, such as chickpea, broad bean, field pea, common beans, lentil,pigeon pea and cowpea constitute the major portion of dietary protein needs of human (Gupta et al., 2014). Other grain legumes such as soybean and peanut are a major source for vegetable oil for the world.

Legumes also play a significant role in the farming system (Haque and Jutzi, 1984). The most important role of legumes in the farming system is the fixation of atmospheric nitrogen through their symbiotic relationship with *Rhizobium* spp. that lead to two consequential role, capacity to increase soil fertility and accumulate high levels of protein in the plant tissue (Mannetje et al., 1980). Different studies showed that at farm level nitrogen fixation from annual legumes ranges from 15 to 120 kg of N ha^{-1} (Pearson et al., 1995; Dakora and Keya, 2000).

Previous studies proved that legumes enhance nutrient availability for subsequently grown cereals by producing exudates like citrate and malate that solubilize soil minerals including fixed forms of soil phosphorus (Fageria et al., 2008; Kirkegaard et al., 2008). Legumes also play important roles as break crops in rotation with cereals as they can break the life cycles of cropspecific pathogens of cereals as non-host crops (Kirkegaard et al., 2008). Therefore, to keep the farming system healthy and

sustainable, legumes should occupy a significant portion of area under cereals in crop rotation (Pearson et al., 1995).

However, most of the grain legumes are known in their low yield and productivity. The adaptability and productivityof legumes are limited by several biotic and abiotic stresses, including fungaland viral diseases, insect pests, drought, heat, frost, chilling, water-logging, salinityand mineral toxicities (Dita et al., 2006). For instance, *Fusarium* wilt, blights and different viral diseases often cause severe losses. Likewise, pod borers and sucking pests also cause serious problems in crop management.

Although conventional plant breeding and enhanced management strategies have attempted to address few constraints, most legume crops are still suffering with major biotic and abiotic constraints, particularly in situations where the existing genetic resourceslack the required traits. Therefore, advanced breeding techniques and management strategies arerequired to increase resistance and or tolerance against various bio-stresses, which is a major challenge in legume improvement programs.

2.3. The Chickpea Crop

Chickpea (*Cicer arietinum* L.) is an important cool season grain legume that is cultivated as food and fodder in different parts of the world (Gaur et al., 2014). It is a self-pollinated diploid species having chromosome number of 2n = 2x = 16 (Upadhyaya et al., 2008) with comparatively a small genome size of 738 Mbp (Varshney et al., 2013a). The cultivated chickpea (*C.arietinum)* is the only domesticated species under the genus *Cicer*, family Fabaceae and subfamily Papilionoideae (Gaur et al., 2014).

The genus *Cicer* comprises 44 species, including the cultivated annual (*C. arietinum* L.), 8 wild annuals and 35 wild perennials (Singh and Diwakar, 1995). The 8 wild annuals are *C. reticulatum, C.echinospermum, C. bijugum, C. judaicum, C. pinnatifidum, C. chorassanicum, C. yamashitae* and *C. cuneatum.* Like the cultivated chickpea (*C. arietinum)*, wild species are predominantly self-pollinated and diploid (2n =16), with a genome size of 738 Mbp (Singh and Diwakar, 1995). However, cultivated chickpea differs from its wild relatives in its erect plant growth habit, altered phenology, seed coat texture and reduced seed dormancy (Penmetsaet al., 2016). These are traits that were highly favored by humans during crop domestication (Hammer, 1984) and refereed as '*domestication syndrome*'

2.3.1. Origin and divergence

The cultivated chickpea (*Cicer arietinum* L.) is one of the ancient crops originated in the fertile crescent between 10,000 and 12,000 years ago (Redden and Berger, 2007). Due to the presence of its immediate wild progenitor (*Cicer reticulatum*) and closely related wild species (*Cicer echinospermum* and *Cicer bijugum*), modern day south eastern Turkey and the adjoining northern region of Syria were believed as the most probable center of origin for cultivated chickpea (van der Maesen, 1987). Earlier studies on the cytogenetic relationships of different species confirmed that the wild *Cicer reticulatum as* immediate wild progenitor of the cultivated chickpea (Ladizinsky and Adler, 1976).

Previous studies showed that the estimated divergence time between *Cicer arietinum* and *Cicer reticulatum* was around 9,000 to 12,000 years ago (Von Wettberg et al.,

2018), whereas the estimated divergence time between *Cicer reticulatum* and *Cicer echinospermum was* at 95,000 to 127,000 years ago (Redden and Berger, 2007).

The phylogenetic relationships among cultivated chickpea and wild annual species have been studied using different molecular markers (Iruela et al. 2002; Sudupak et al. 2002; Rao et al. 2006; Choudhary et al. 2012a) and the results showed that the annual species grouped in to 4 phylogenetic groups. The cultivated chickpea (*Cicer arietinum*), its immediate progenitor of *C. reticulatum* and its closely related sister wild species (*C. echinospermum*) formed one group, whereas *C. bijugum*, *C. pinnatifidum* and *C. judaicum* formed another group, and *C. chorassanicum* was grouped with *C. yamashitae*.

In the process of evolution, chickpea has emerged into two distinctgroup asdesi and Kabuli types mainly based on seed features such as size, shape, coat thickness and color. Seeds of Desi are smaller in size, darker in color and with thicker seed coats, whereas the Kabuli type is associated with light colored, larger sized seeds and with thinner seed coats.

Desi and kabuli types chickpea also differ in stem pigmentation during vegetative stage and in flower colors. Pink to purple pigmented petals are typical of desi chickpea, whereas non-pigmented white petals are characteristic of kabuli chickpea. Besides, at very low frequencies additional variants exist for color of seed coats such as green, black and blue flower colors (Pundir et al.,1985). As suggested by Kumar et al.(2000), three genetic loci, C, B and P, conditioning flower color in chickpea, where recessive alleles at either C or B confer white petal color, whereas the recessive condition at P results in blue petals.

The longstanding proposal by Ladizinsky and Adler, (1976) which suggests the desi type represents the early domesticated form while kabuli being a subsequently derived type still well supported with the high similarity of desi type seed to that of wild *C. reticulatum*. In spite of the widely held view that the desi and kabuli types are distinct subgroups within cultivated chickpea, different molecular studies have been equivocal in supporting such a distinction (Roorkiwal et al., 2014).

Therefore, the origin of the kabuli type is currently ambivalent (Penmetsa, et al., 2016). Besides, molecular diversity studies indicated that wild relatives of chickpea have high genetic diversity compared to its cultivated species *C.arietinum* and supports the conclusion that chickpea has a narrow genetic base (Choudhary et al., 2012a; Von Wettberg et al., 2018).

2.3.2. Distribution of chickpea in the world

After domestication, chickpea was spread throughout the Middle East and Mediterranean regions. It was probably diffused from where it was originated and first domesticated (the Middle East) to different continents of the world by the Phoenicians, and subsequently spread to India, Europe and later reached Africa, Latin and Central American countries (Singh and Diwakar, 1995; Joshi et al., 2001; Redden and Berger, 2007).

Chickpea reached the Indian sub continent approximately 4,000 years ago and has been cultivated continuously there since then, with Indian traditional landrace chickpea varieties undergoing diversification separately from those elsewhere (Redden and Berger, 2007). Similarly, chickpea was introduced to Ethiopia between

2,000 and 3,500 years ago and has been grown continuously and independently (Asnake *et al*, 2020; Joshi et al., 2001; Redden and Berger, 2007). Ethiopia is now considered as the secondary center of diversity for chickpea (Asnake et al., 2020; van der Maesen, 1987). Later, in the past century, chickpea cultivation was established in countries with industrial agricultural practices predominate, including the Pacific coastal regions of North, Central, South America, and Australia (Redden and Berger, 2007).

2.3.3. Major economic importance

Chickpea is cheap source of high quality proteins in the diets for millions of families, particularly in developing countries for those who cannot afford animal protein for balanced nutrition. For instance, chickpea serves as a main source of dietary protein for more than 80 % of the Indian population who has vegetarian consumption tradition (Jendoubi et al., 2017). It is an excellent source of carbohydrates (64%), high quality protein (23%), fat (5%) and crude fiber (6%) (Jabran et al., 2012). It also contains 340 mg of phosphorus, 190 mg of calcium, 140 mg of magnesium, 7 mg of iron and 3 mg of zinc in 100 g of seed (Jabran et al., 2012). The awareness of health benefits of chickpea has led to considerable increase in its worldwide production, consumptionand trades.

Chickpea is highly valued in the cropping systems, particularly in rotation with cereals, for its overall impacts on soil health and fertility. Chickpea fixes atmospheric nitrogen in soils and thus improves soil fertility and saves fertilizer costs in subsequent crop rotation. Under normal condition, chickpea fixes around 60 kg/ha of atmospheric nitrogen (Abi-Ghanem, et al., 2012). However, chickpeas varieties had a

greater effect on variation in proportion of nitrogen fixed than did the symbionts strain (Abi-Ghanem, et al., 2012). Therefore, it plays an important roles in increasing the productivity of the succeeding crops in a rotation, and significantly contributing to the sustainability of the production systems.

In different parts of the world, chickpea often used as break crops in rotation with cereals as it can break the life cycles of crop specific pathogens of cereals. Moreover, its residue is also very rich in digestible crude protein content compared to cereals and often used as an important animal feed to increase livestock production and productivity (Menale et al., 2009). It can also be grown as a second crop using residual moisture and supplements additional income for smallholder producers.

2.3.4. Global production and productivity

Globally, chickpea is grown in more than 55 countries annually with average productivity around 1,000 kg/ha. For instance, in 2018/19 cropping season, chickpea was planted on an area of 17.82 million hectares of land produced17.19 million metric ton with average productivity of 965 kg ha^{-1} (FAOSTAT, 2018). In 2018, the world top ten chickpea producers were India, Australia, Turkey, Russia, USA, Ethiopia, Myanmar, Mexico, Pakistan and Canada respectively. India alone produced 11.38 million metric ton and contributed 66.20 % to global production (FAOSTAT, 2018). The major world consumers and importing countries are India, Pakistan and Bangladesh (Jondobi et al., 2017). Countries such as Australia, Mexico and Russia are not chickpea consumers but major world exporters. About 80 % of the area all over the world is under the Desi type and around 20 % is under the Kabuli type (Gaur et al.,2014). The Desi type is grown mostly in Indian subcontinents, Ethiopia, Mexico

and Iran while the Kabuli type is grown in Southern Europe, Northern Africa, Afghanistan, Pakistan and Chile (Upadhyaya et al., 2008; Kassie et al., 2009).

2.3.5. Production and productivity trend in Ethiopia

In Africa, Ethiopia is the largest producer, consumer and exporter of chickpea, annually producing around 435,193 tons on about 208,838 hectares of land (FAOSTAT, 2019). It is mainly grown in Oromiya, Amhara, Tigray and Southern Nations, Nationalities People (SNNP) regions, at an altitude of 1400-2300 meter above sea level (CSA, 2018), with annual rainfall ranging between 700 and 2000 mm. Under optimum growing conditions chickpea productivity ranges between 3.5 to 5 ton ha^{-1} (Roorkiwal et al., 2018). However, in Ethiopia several biotic and abiotic stresses restrict its national average yield to around 2.08 ton ha^{-1}(FAOSTAT, 2019).

In Ethiopia, chickpea is mainly grown in Oromiya region (West, East, South West, and North Shewa, West Harerge, Arsi and Bale zones), Amhara region (East and West Gojam, North and South Gonder, North and South Wollo and North Shewa zones) and in few districts of Tigray and Southern Nations, Nationalities People (SNNP) regions at an altitude of 1,400-2,300 meter above sea level (m.a.s.l.), where annual rainfall ranges between 700 and 2000 mm (CSA, 2018). In Ethiopia, chickpea is mostly produced by smallholder farmers with residual moisture on vertisols after harvesting cereals (Damte and Ojiewo, 2016).

For the last two decades, in Ethiopia chickpea productivity doubled from below 0.89 ton/ha in 2000 to 2.14 tons/ha in 2018 (Fig 2.1). However, this is still much lower than its genetic potential, 3.5 to 5 ton ha^{-1} that can be expected under prevailing

conditions (Roorkiwal et al., 2020). This is mainly because of several abiotic and biotic factors, including susceptibility of landraces to drought, water logging and poor cultural practices; low or no protection measures againstweeds, diseases and insect pests, and inherently low grain yielding potential of landraces (Damte and Ojiewo, 2016, Yimer et al., 2018).

Fig 2.1.Chickpea production and productivity trend in Ethiopia from 2000 to 2018

2.4. Major Chickpea Production Constraints

Chickpea (*Cicer arietinum* L.) is predominantly cultivated in the arid and semi-arid regions of the world on marginal lands under rain fed conditions. The crop is therefore, vulnerable to various abiotic and biotic constraints occurring under these situations. Inmost tropical and sub-tropicalregions where limitations imposed by different biotic andabiotic stresses are severe, the full yield potential of legume crops is rarely attained (Buddenhagen and Richards, 1988).

2.4.1. Major abiotic stresses

Among the abiotic stresses drought, salinity, water logging, high temperature and chilling frequently limit growth and productivity of chickpea. Its production in marginal areas is considered to be constrained by low rainfall. Chickpea is commonly

grown under rainfed conditions during the post rainy season on progressively declining residual soil moisture which eventually exposes the crop to the terminal drought stress.

In chickpea, depending on the genotypes planted, the severity and timing of drought occurrence, yield losses because of drought range from 30 % to 100 % (Leport et al., 1999). Water supply to chickpea crops between flowering and maturity stages was significantly related to yield, and water stresses experienced only during flowering caused up to 50 % loss of potential pods (Whish et al., 2007). Besides, heat stress, salinity, water logging occurring at various stages of the crop cycle are the major yield reducers in arid and semi-arid regions of the major chickpea producing countries (Singh et al., 2008).

Chickpea, like many other legumes, is sensitive to soil salinity. As chickpea is usually grown under residual soil moisture, the salinity stress is often combined with terminal drought. Soil salinity is a major abiotic constraint to chickpea productivity in many parts of India, Pakistan, Iran, West Asia, North Africa and Australia.

2.4.2. Major biotic stresses

The major biotic stresses that leads to high yield reduction and instability in chickpea are those caused by fungal, bacterial and viral diseases, insect pests and parasitic nematodes (Ranalli and Cubero,1997). In different parts of the world, although chickpea crop is attacked by more than 172 pathogens (Nene et al., 1996), only a few of them have a significant impact on production globally.

Fusarium wilt caused by Foc, dry root rot (*Rhizoctonia bataticola*), black root rot (*Fusarium solani*), wet root rot (*Rhizoctonia solani),* and collar rot (*Sclerotium rolfsii*) are the important root diseases of chickpea in areas where the growing season is dry and warm (Gaur et al., 2014), whereas *ascochyta* blight (*Ascochyta rabiei*), and botrytis grey mold (*Botrytis cineria*) are the important foliar diseases in the areas where the growing season is cool and humid.

Among these, *Fusarium* wilt caused by Focand *Ascochyta* blight caused by *Ascochyta rabiei* are the major yield limiting factors in several chickpea growing countries (Dubey et al., 2007). *Fusarium* wilt is one of the most important diseases affecting chickpea worldwide. It is widespread in most chickpea growingareas in Asia, Africa, southern Europe and the Americas, but it hasnot yet been reported in Australia (Cunnington et al., 2007). The pathogen causing *Fusarium* wilt is soil borne and can affect all stages of plant growth and development but higher incidence at flowering and podding stage. The severity of the disease is maximum under high temperature and drought conditions. Annually 10–90 % losses occur due to chickpea wilt disease (Sharma and Muehlbauer, 2007).

Ascochyta blight isanother important foliar disease of chickpea particularly under cool, humid weather conditions and capable of causing complete (100%) yield losses under favorable conditions (Pande et al., 2005; Sharma and Ghosh, 2016). Ascochyta blight is a highly devastating foliar disease of chickpea in West and Central Asia, North Africa, North America, Australia and in Ethiopia (Pande et al*.,* 2005). It occurs mainly is in areas where cool, cloudy and humid weather occurs during the crop season. The spread of the disease is more with cool (15-25^{o}C) and humid weather (>150 mm rainfall) that prevails during the crop season (Pande et al., 2005).

Besides, pod borer (*Helicoverpa armigera*), several viral diseases including chickpea chlorotic stunt virus, bean leaf roll virus, beet western yellow virus, soybean dwarf virus and the Pea seed borne mosaic virus, rust (*Uromyces ciceris arietini*), Phytophthora root rot (*Phytophthora medicaginis*), cutworm (*Agrotis* sp.) and leaf miner (*Liriomyza cicerina*) are also important in different chickpea growing areasalong with economically important viral diseases in chickpea (Berhanu et al., 2005; Abraham et al., 2009; Damte et al., 2016). Therefore, chickpea breeding for different abiotic and biotic stresses are very important to maintain chickpea production and productivity in marginal areas.

2.5. *Fusarium* Wilt of Chickpea

Fusarium wilt is one of the most destructive disease of chickpea in the world. In severe out breaks, the disease often cause 70 % to 100 % crop loss (Halila and Strange, 1996). *Fusarium oxysporum* Schlechtend.: Fr. (FO) is a complex species comprising a wide variety of biological forms including pathogenic, endophytic, saprophytic and beneficial strains (Leslie and Summerell, 2006). The plant pathogenic strains affect a wide variety of economically important plants mainly causing vascular wilt, yellows, root rot, bulb rot and damping (Lievens et al., 2008; Michielse and Rep 2009).

Pathogenic strains usually show a high level of host specificity and infect only a single or a few plant species. Therefore, based on host genotypes that they infect, so far more than 120 formae speciales have been reported and few of them are further classified into physiological races (Gordon and Martyn, 1997; Baayen et al., 2000; Agrios, 2005).

2.5.1. The Species *Fusarium oxysporum*

The genus *Fusarium*is a large cosmopolitan genus whose members can cause wilts, blights, rots, and cankers of many horticultural, field, ornamental, and forest crops in both agricultural and natural ecosystems (Farr et al., 1989). *Fusarium oxysporum* was one of the speciesfirst described by von Schlechtendahl in 1824, and later amended by Synder and Hansen in 1940 (Nelson et al., 1983). As described by Dean et al. (2012), *Fusarium oxysporum* is ranked fifth among the most notorious plant pathogens following *the Magnaporthe oryzae, Botrytis cinera, Puccinia spp. and Fusarium graminearum.* The plant pathogenic strains affect a wide variety of economically important plants mainly causing vascular wilt, yellows, root rot, bulb rot and damping (Michielse and Rep 2009), while the nonpathogenic strains are defined as the strains for which no host plants have been identified yet (Lievens et al., 2008).

Fusarium oxysporum vascular wilts are initiated mainly as root infections, in which the chlamydospores present on plant debris or in soil germinate and mycelia penetrate into plant roots through natural openings. The mycelium advances into root cortex reaching xylem vessels through the pits and continues to grow and produce several microconidia. These microconidia are carried upward by xylem sap, where they germinate and invade more xylem vessels. This creates severe water stress, resulting in wilt symptoms as the xylem vessels are clogged by mycelium, conidia, gels and gum that are produced by plant defenses (Agrios, 2005).

Identification of *Fusarium oxysporum* is traditionally based on the combination of diagnostic symptoms on the host and the presence of the fungus in theaffected tissues (Baayen et al., 2000). However, this classical approach is becoming increasingly

challenging because more than one forma specialis may infect aparticular host, along with nonpathogenic strains, which are common soil andrhizosphere inhabitants (Edel et al., 2000).

2.5.2. *Fusarium* wilt pathogen of chickpea

Fusarium wilt, caused by the asexual soil and seed borne fungus (*Fusarium oxysporum* (Schlechtend.:Fr.) f. sp. *ciceris* (Padwick) Matuo & K. Sato) is one of the most destructive disease of chickpea in the world (Haware and Nene, 1982). Though *Fusarium* wilts of chickpea was first reported in India by Butler in 1918, its etiology was not correctly determined until 1940 by Padwick (Cunnington et al., 2007).

The fungus was first named as *Fusarium orthoceras Appel and Wollenw. var. ciceris* by Padwick in1940, and later Sen Gupta and Chattopadhyay renamed the pathogen *Fusarium oxysporum* Schl. f. sp. *ciceris* (Padwick) Snyder and Hansen (Haware, 1990a). This was accepted as the correct name of the pathogen until revised and renamed as *Fusarium oxysporum* f. sp. *ciceris* by Holliday in 1980 (Jalali and Chand,1992). Currently the accepted classification for the *Fusarium* wilt pathogen of Chickpea (*Fusarium oxysporum* f.sp. *ciceris)* is: Kingdom: Fungi, Division/Phylum: Ascomycota, Class: Sordariomycetes, Order: Hypocreales, Family: Nectriaceae, Genus:*Fusarium*, Species: *oxysporum,* and forma specialis: *ciceris* (Leslie and Summerell, 2006).

Foc is an asexually reproducing root inhabiting fungus, which survive inactive in soil by means of chlamydospores.The fungus can survive in soil and chickpea debris by means of chlamydospores for at least 6 years (Haware et al., 1996). Foc causes wilt

only in chickpea plants (Lievens et al., 2008). Among other legumes, lentil, field pea and pigeonpea are symptomless carriers of the chickpea wilt fungus (Haware and Nene, 1982). Hence, infection of symptomless dicotyledonous weeds can enhance survival of the pathogen in fallow soils. Therefore, infested soil is a mainsource of primary inoculum for the development of *Fusarium* wilt epidemics in chickpea.

Infected seeds are also a source of primary inoculum of the disease. Plants grown from infected seeds wilt faster than infected plants that originated from healthy seeds sown in infested soil (Haware et al., 1978). Infected seeds play an important role in the long distance dispersal of the pathogen and in its introduction into Foc free soils and geographic areas (Pande et al., 2007). Short distance spread of the pathogen can also occur by dispersal of infested soil or chickpea debris through human activity, machinery, water, or wind.

Morphological characterization of Foc is mainly based on shape and type of macro and micro conidia, the structure of the micro conidiophore and the formation of chlamydospores (Leslie and Summerell, 2006). Macro and micro conidia appear generally spare, straight to curved or oval to cylindrical in shape, measuring 2.5-3.5 x 5-11 µm on solid medium (Fig 2.2). The macroconidia are straight to slightly curved, slender, thin walled usually with three or four septa, a foot shaped basal cell and a tapered and curved apical cell (Ebbole and Sachs, 1990). The microconidia are ellipsoidal and either have no septum or a single one formed from phialides in false heads by basipetal division. Microconidia are predominantly uninucleate and germinated poorly and variably, which are important in secondary infection (Ebbole and Sachs, 1990).

Chlamydospores in soil are the primary inoculum for *Fusarium* wilt in chickpea, their germination being stimulated by seed and root exudates of hosts and non-hosts. Foc enters host through roots and itenters in germinating seeds and growing seedlings directly without need of wounds soon after sowing in infested soil. Invasion takes place mainly through the cotyledons and zones of the epicotyl and hypocotyl at the junction of or close to cotyledons, and to a lesser extent in the zone of root elongation and maturation (Stevenson et al., 1997). It then grows in the intercellular spaces of the root cortex to reach the central root cylinder and enter into the xylem vessels, and causes systemic infection by progressive vascular damage, wilting and finally death of the plant. Further colonization by the pathogen takes place by means of hyphal growth and microconidiacarried in the vessels by transpiration stream, as well as by lateral mycelia spread to adjacent vessels from infected ones (Jimenez-Fernandez et al., 2013).

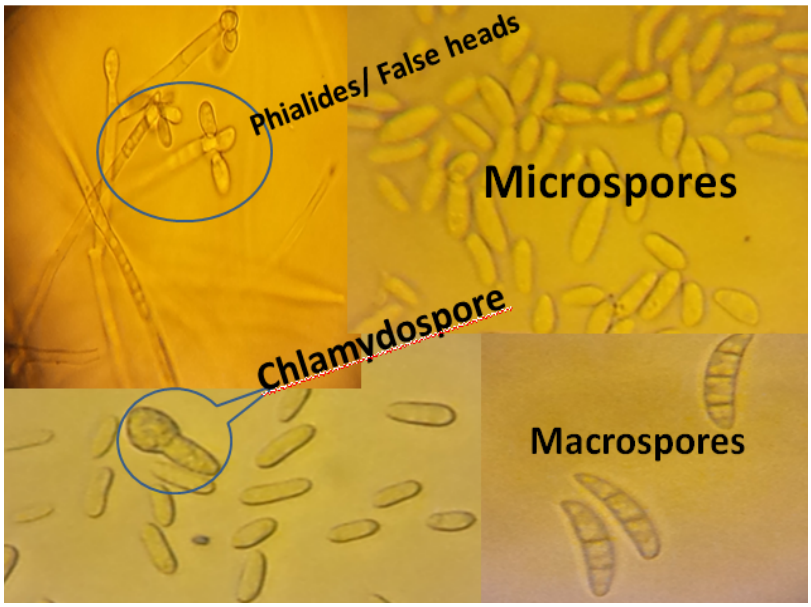


Fig 2.2. Different types of spores produced by *Fusarium oxysporum*

2.5.3. Genetic diversity and geographic distributionof Foc

Foc exhibits great genetic diversity. Once the inoculum is established in the soil, it survive in the soil for several years and during these periods, it passes through different environmental stresses and biological competition, which lead to the existence of variations.These variations in the virulence within isolates has led to the evolution of pathogenic races. So far Foc has been classified into two pathotypes and eight physiological races (Jimenez Gasco et al., 2004b; Dubey et al., 2012).

The two pathotypes, yellowing and wilting have been differentiated by the disease symptoms induced in the plant (Jimenez Gasco et al., 2001, 2004b). The yellowing pathotype produces progressive yellowing of leaves and vascular discoloration, followed by plant death within 40 days after inoculation of the pathogen, while the wilting pathotypes induces severe chlorosis and flaccidity, vascular discoloration, and the plant dies within 20 days after inoculation (Jimenez Gasco et al., 2004b). Generally, the yellowing Foc pathotype is less aggressive than the wilting pathotypes (Navas-Cortes et al., 2000), but differences in aggressiveness to chickpea cultivars can also occur among races within a pathotype.

Based on disease reactions on a set of differential chickpea cultivars, eight pathogenic races of Foc (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported from different parts of the world (Jimenez Gasco et al., 2001; Shehabu et al., 2008; Dubey et al. 2012; Bayraktar and Dolar, 2012; Al-Taae et al., 2013). However, recently change in the race scenario of Foc has been reported in India (Dubey et al., 2012, 2014; Sharma et al., 2013, 2014).

Among these, the six races (race 1A, 2, 3, 4, 5, and 6) induce wilting syndrome, whereas races 0 and 1B/C induce the yellowing syndrome (Jimenez Gasco et al., 2001). These two pathotypes can be distinguished unambiguously by RAPD and different DNA fingerprinting assays (Kelly et al.,1994,1998; Jimenez-Gasco et al., 2001, 2004a).

Among all races, race 1 is more wide spread and has been reported in India, California, Morocco, Spain, Mediterranean region and in Ethiopia (Jimenez Gasco et al., 2001; Shehabu et al., 2008). The widespread distribution of race 1 can be attributed to the seed transmission through large exchange of germplasm that took place more in last one decade. Likewise, race 6 also has been reported in California, Spain Israel and Morocco, and recently in India (Jimenez-Diaz et al., 1989; Kelly et al., 1994; Dubey et al., 2012; Sharma et al., 2014).

In Ethiopia, 5 different races, race 0, 1, 2, 3 and 4 and other two unknown races were reported (Tadesse et al. 1998b, Shehabu et al., 2008). Similarly, races 2 and 3 were reported in Turkey (Bayraktar and Dolar, 2012). However, recently change in the race scenario of Foc has been reported in different parts of the world (Dubey et al., 2014; Sharma et al., 2014). The occurrence of new race of Foc in different countries mainly appeared to be a recent introduction through seed transmission and pathogen genetic variability from accumulation of mutations over time.

Besides, races 1 and 6 have similar virulence pattern on chickpea differentials (Halila and Strange, 1996) and high genetic similarities(Jimenez-Gasco et al., 2001). It seems these two pathogenic races were not yet evolutionarily separated from each other to

have developed diagnostic and separate pathogenicity test. The occurrence of multiple and new races of Foc in different parts of the world suggests the continuous changes in Foc population. Therefore, the identification of pathogenic races of Foc is important for disease resistance breeding and for the efficient use of available Fusarium wilt resistant cultivars in chickpea.

Monitoring pathogenic variability of fungus based on DNA markers greatly helped in understanding pathogen diversity. In past, different kinds of molecular markers were employed to characterize Foc isolates, for instance, random amplified polymorphic DNA (RAPD) (Honnareddy and Dubey et al., 2006), restriction fragment length polymorphism (RFLP) (Barve et al., 2001), amplified fragment length polymorphism (AFLP) (Sharma et al., 2009), inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) (Dubey SC, 2008, Singh et al., 2008).

However, the marker systems are put to limited use because of lack of reliability and resolution, and they are labour intensive and not amenable for high throughput genotyping (Sharma et al., 2014). Though, characterization of Foc isolates using different molecular markers greatly helped in understanding the genetic and pathogen variability, the marker systems are labor intensive, lacks reliability and resolution, and they are not suitable for high throughput genotyping (Sharma et al., 2014).

Besides, previous studies reported that, despite their extensive pathogenic variability and geographic distribution, the eight physiological races of Foc displayed little genetic diversity, and were found to have identical sequences for the genes encoding translation elongation factor 1-α (TEF1-α), b-tubulin, histone 3, actin, and calmodulin

(Jimenez-Gasco et al., 2002). Similarly, the internal transcribed spacer region of the rDNA (ITS), mitochondrial regions, a xylanase gene (xyl4) and its transcriptional activator (xlnR), and microsatellites based genetic diversity analysis showed striking genetic similarity among the Foc races (Demers et al. 2014; Dubey et al., 2014).

2.5.4. Typical symptoms and disease diagnosis

In infected plants, *Fusarium* wilt disease symptoms can develop at any plant growth and development stages depending on the environmental conditions in which cultivation occurs (Jimenez-Diaz et al., 2015). In highly susceptible genotypes, early wilting symptoms such as flaccidity of individual leaves followed by a dull green discoloration, desiccation and collapse of the entire plant often observed within 25 days after sowing (Jimenez-Diaz et al., 2015; Al-taae et al., 2013). However, late wilting symptoms are usually appears six to eight weeks after sowing, mainly from early flowering to podding stages.

The typical symptoms of late wilted plants includes drooping of the petioles, rachis and leaflets, followed by yellowing and necrosis of foliage (Jimenez-Diaz et al., 2015). Initially, drooping is observed in the upper part of the plant but within few days it occurs on the entire plant. Symptoms may affect only a few branches of a plant resulting in partial wilt. Relatively,early wilting causes more yield loss than late wilting. Seeds from late wilted plants are lighter, rougher and duller as compared with healthy plants (Haware and Nene, 1980; Navas-Cortes et al., 2000).

Early good diagnosis is a first step to ensure efficient management of *Fusarium* wilt in chickpeas. Roots of affected seedlings and plants show no external root

discoloration if they are uprooted before being severely affected or dried. However, when the roots and stem are split vertically a dark brown discoloration of xylem tissues can be seen. This distortion occur in the vascular tissues of affected roots and stems as a result of cavity formation between phloem and xylem, xylem and medulla, and phloem and cortical parenchyma. These together with formation of optically dense gels and occlusions in xylem vessel, contributes to retarded vascular flow of water and nutrients as well as development of morphological symptoms (Jimenez-Diaz et al., 1989).

Careful examination affected plants and roots for the absence of external root symptoms and presence of dark brown discoloration in xylem tissues of roots and stem are very critical and essential for appropriate disease diagnosis (Jimenez-Diaz et al., 2015). Transverse sections of the infected roots examined under the microscope show the presence of hyphae and spores of the fungus in the xylem, thereby confirming the diagnosis of vascular wilt (Hossain et al., 2013). Therefore, maximum care should be taken to avoid the possible confusion oftypical *Fusarium* wilt symptoms with leaf yellowing, wiltingand phloem discoloration that are common in viruses infected chickpea plants (Nene et al., 1978; Kaiser et al., 1993). Pods from the wilted plants look normal but seeds are generally smaller, lighter, more wrinkled and discolored than those from healthy plants.

Besides, leaf yellowing and necrosis are often displayed inchickpea plants infected with other root fungi such as *Fusarium solani* f. sp. *pisi*, *Fusarium solani* f. sp. *eumarti* (Trapero Casas and Jimenez-Diaz, 1985). Moreover, maximum care must be taken when confirming initial diagnosis by isolation in pure culture because

endophytic, non-pathogenic strains of *Fusarium oxysporum* are frequently isolated even from upper stem tissues of symptomatic chickpeas (Trapero Casas and Jimenez-Diaz, 1985, Jimenez-Fernandez et al., 2011b; Malcolm et al., 2013).

Proper identification of races of the pathogen in a given area of chickpea production is important for the breeding program as well as for the efficient use of available sources of resistance. Determination of races of this pathogen is simple but costly in time, facilities, and resources. Previously, specific primers and polymerase chain reaction (PCR) assays that identify Foc and each pathogenic races 0, 1A, 5, and 6 were developed by Jimenez-Gasco et al (2001). Therefore, more advanced methods for the rapid, efficient, reliable, and reproducible identification of the pathogen and its pathogenic races are needed.

2.5.5.Disease control and management measures

Management of *Fusarium* wilt disease of chickpea is difficultto achieve and no single control measureis fully effective (Landa et al., 2004). It is a monocyclic disease in which developmentis driven by the pathogen's primary inoculum.Therefore, disease management measures should betargeted to exclusion of the pathogen as well as by reducing the amount and efficiency of the initial inoculum (Jimenez-Diaz et al., 2015). These mainly include use of resistant varieties and healthy planting material, avoid chickpea planting into high risk soils, reduction or elimination of the inoculum in soil and application of good crop management practices.

Host resistance is one of the key components for the disease management within the plant disease tetrahedron(Agrios, 2005). Previous reports have demonstrated that the

use of successful resistant varieties is the only economical and practical solution to control *Fusarium* wilt of chickpea (Malik et al., 2006; Nikam et al., 2007). However, due to the evolution of variability in the pathogen and breakdown of the resistance, most of the resistant varieties have been found to be susceptible after some years. This is because the fungal population is highly variable and carries a wide range of genes for virulence (Chongo et al., 2004).

Cultivation of varieties possessing resistance to specific races of the pathogen prevalent in a region is the most economical disease management strategy (Jalali and Chand, 1992). Therefore, development and use of resistant cultivars as part of integrated disease management is the key for successful production of chickpea. However, the deployment of resistant cultivars has not been to required level because of undesirable agronomic characteristics in some developed materials. Furthermore, the high pathogenic variability in Foc populations may limit the effectiveness and extensive use of available resistance (Bayraktar and Dolar, 2012).

Use of infected planting material can lead to introducing the pathogen into pathogen free soils or production areas. Therefore, the importance of checking the health of that material through certification programs, phytosanitary inspection and quarantine legislation should be considered. Proper selection of the planting site optimizes the use of wilt pathogenfree planting material in non-infested soils (Jimenez-Diaz and JimEnez-Gasco, 2011). Beside, seed borne inoculum can be eradicated by seed dressing (Haware et al., 1978).

2.6. Chickpea Breeding for Major Production Constraints

2.6.1. Major breeding methods in chickpea

The development of resistant varieties to major biotic and abiotic constraints is one of the most important objectives of chickpea breeding programs. Through conventional breeding approaches, several chickpea varieties were released, which have significantly contributedto improved production and productivity of the cropwith good resistance to major biotic and abiotic stresses (Gaur et al., 2012a).

During the early phase,mass or pure line selections from landraces or introduced genotypes were used to develop improved varieties.Later, hybridization is invariably being used to increase genetic variability in different chickpea breeding programs and most varieties have been developed through effective crossing (Gaur et al., 2012a, Jendoubi et al., 2017).

Single or multiple crosses often used to create genetic variability for different traits of interests. Backcross breeding is commonly used to incorporate one or few traits, from a germplasm line or a wild relatives into a well-adapted variety. Most chickpea breeding programs have been used extensively intraspecific crosses between Desi and Kabuli parents with different genetic backgrounds (Hossain et al., 2010). These types of crosses have consistently produced high yielding progeny and have been the source of many new cultivars (Gaur et al., 2010; Gaur et al., 2012a).

Several efforts have been made to use interspecific crosses for enhancing genetic variability and introgression of useful genes, particularly for resistance to biotic and abiotic stresses (Ahmad et al., 2005; Gaur et al., 2010) into the cultivated species

Cicer from closely related wild *Cicer* species. Mutation breeding has also been used in chickpea improvement for creating variability and in different countries several varieties have been developed through breeding (Gaur et al., 2012a).

Since chickpea is self-pollinated, the development of pure line cultivars requires fixing genes in breeding lines.Various modifications of pedigree and bulk methods were used in handling segregating generations in different countries (Gaur et al., 2012a). In early segregating generations, selection is done for simple traits such as disease resistance and seed traits. However, due to high environmental effects single plant selection for yield is effective at later stages and generally starts from F_4 or F_5 (Gaur et al., 2012a). In Ethiopia, for past three decades, different early generation segregating genotypes introduced from abroad and developed through crossing were rigorously evaluated in sickplot for the development of *Fusarium* wilt/root rot resistant varieties. However, there were only a little success in developing few moderately resistant varieties.

Therefore, these days there is a need to enhance precision and efficiency of selections in the segregating generations for higher and rapid genetic gains. Precision in selection for resistance/tolerance to stresses can be improved by screening under controlled environmental conditions or at hot spot locations and through use of advanced molecular breeding approaches. Marker assisted selection (MAS) is also considered for improving the precision and efficiency of conventional plant breeding methods. MAS would be useful for pyramiding of resistance genes from different sources and for combining resistance to two or more stresses for instance resistance to *Fusarium* wilt and resistance to pod borer.

Although so far there is no any effort to develop and integrate modern genetic tools in Ethiopia chickpea breeding program, globally remarkable progress has been made in chickpea in developing novel genetic tools, such as molecular markers, genetic maps, and genome profiling techniques to identify genomic regions, quantitative trait loci (QTL) and genes governing traits of interest (Varshney et al., 2009; Upadhyaya et al., 2011). These new advances in genomics provide exciting opportunities to researchers and breeders to utilize these new technologies for improving and stabilizing chickpea yield for the benefit of farmers and consumers.

2.6.2. Breeding achievement for major abiotic stresses

Terminal drought is the most important abiotic constraint to chickpea production, and development of early maturing varieties has been the most effective strategy for escape from terminal drought. Excellent progress has been made in the development of early maturing chickpea cultivars, which can escapeterminal drought and heat stresses (Gaur et al., 2005). For instance, ICCV 2 was the first landmark variety released in India that matures in about 85 days, and it is perhaps the world's earliest maturing variety of kabuli chickpea.

High root biomass has been found to be associated with drought tolerance (Saxena et al., 1993; Krishnamurthy et al., 1996; Ali et al., 2005) and a positive relationship has been established between root biomass and seed yield under drought conditions (Kashiwagi et al., 2006). Large root system and smaller leaf area have been suggested to determine drought tolerance, the former is being important for absorption of available soil moisture while the later helps to reduce the amount of water loss through transpiration (Saxena, 2003). Besides, a large genetic variation for heat

tolerance has been identified in chickpea (Upadhyaya et al., 2011; Krishnamurthy et al. 2011) and a heat tolerant breeding materials have been released for cultivation. Few varieties with tolerance to moderate levels of salinity have been developed in India and Australia (Maliro et al., 2004).

2.6.3. Breeding achievement for major biotic stresses

Chickpea is susceptible to several diseases and insect pests, however only few have a significant impact in production system. The most important biotic constraints include *Fusarium* wilt, Ascochyta blight, dry and wet root rots, black root, pod borer and etc. In different countries several germplasm accessions were evaluated for *Fusarium* wilt resistance, and some kabuli and desi accessions were identified as a good sources of resistance (Sharma et al., 2019).

Ascochyta blight, caused by *Ascochyta rabiei,* is also a highly devastating foliar disease of chickpea in Ethiopia. Considerable effort has been made in identifying resistant germplasm and breeding for resistance. However, lack of highly resistant germplasm and a highly variable pathogen have rendered the development of different varieties having high level and durable resistance (Pande et al., 2005). Millan et al. (2006) reviewed progress on identification of markers for Ascochyta blight resistance QTLs and molecular markers are available for major QTLs involved in Ascochyta blight resistance. Therefore, it is possible to use marker-assisted breeding for pyramiding Ascochyta blight resistance genes. Though higher levels of resistance were observed in some wild species, development of cultivars resistant to pod borer remained a challenge due to non-availability of sources with high levels of resistance (Sharma et al., 2005).

2.7. Chickpea Breeding for *Fusarium* Wilt resistance

Chickpea breeding for *Fusarium* wilt resistance is one of the most practical, cost efficient and eco-friendly breeding goal to control the disease (Malik et al.,2006; Nikam et al., 2007; Jimenez-Diaz et al., 2015; Jendoubi et al., 2017). However, cultivated chickpea gene pool has a narrow genetic base for *Fusarium* wilt resistance and other yield related morphological traits (Pande et al. 2006). Successful chickpea breeding program for *Fusarium* wilt resistance requires significant genetic variation for wilt resistance among the breeding lines, efficient evaluation and selection method for wilt resistance and desirable plant types for commercial production (Singh, 1987; Salimath et al., 2007).

Due to limited availability of good disease resistance sources in global chickpea germplasm collections (Pande et al. 2006), identification of chickpea genotypes with high level of wilt resistance is highly required. This can be achieved either looking for good resistance source from wild relatives (Singh et al. 2008; Munoz et al. 2017) or creating variability through effective crossing techniques and exhaustive evaluation of broader chickpea genotypes from different background for *Fusarium* wilt resistance (Sharma et al. 2012b; Pande et al., 2012).

In different countries, numerous sources of high resistance have been identified from different sources (from wild relatives, through hybridization, gene pyramiding etc), and effective field, greenhouse and laboratory procedures for resistance screening have been developed (Pande et al., 2006; Mirzapour et al., 2014; Gaur et al., 2014; Chobe et al., 2016). Several wilt resistant varieties have been developed in different chickpea producing countries, and are being utilized in resistance breeding programs

(Fikre et al., 2018; Sharma et al., 2012; 2019). Different efforts have been made to use interspecific crosses for enhancing genetic variability and introgression of useful genes from wild *cicer* spp. into the cultivated species.

So far, breeding efforts for *Fusarium* wilt have contributed to significantly reduce the disease effect on the chickpea. However, chickpea breeding for *Fusarium* wilt resistance regularly challenged with high pathogen variability, the existence of different physiological races in different growing areas, frequent break down of the resistance and the undesirable agronomic traits in some developed wilt resistance varieties (Sharma et al., 2019).

2.8. Utilizationof Genomics Technologies in Chickpea Breeding

Though conventional breeding methods are still most frequently used to develop new genotypes, recent advances in chickpea genomics have brought more opportunities to explore unique genomic characteristics andinitiate genomics assisted breeding for improvement of its adaptation to diverse abiotic and biotic stresses (Jain et al., 2013; Varshney et al., 2013a).The last decade has witnessed the development of extensive genetic and genomic resources in chickpea (Hiremath et al., 2012; Kudapa et al., 2014; Agarwal et al., 2016; Varshney et al., 2016, 2017, 2018; Mashaki et al., 2018; Roorkiwal et al., 2020).

Currently, different genomic tools are being employed in different chickpea breeding programs to improve and accelerate breeding precision and efficiency (Gaur et al., 2014;Varshney et al., 2018; Roorkiwal et al 2020). Integration of modern genomic resources with conventional breeding efforts willhelp in the delivery of climate

resilient, diseases and insect pests resistant chickpea varieties in comparatively less time and expected to contribute substantial improvements in chickpea production and productivity (Roorkiwal et al., 2020).

The availability of diverse molecular markers, high density genotyping assays, draft genome assemblies and large scale resequencing created big opportunity for application of genomics assisted breeding in chickpea (Varshney et al., 2013a, 2019a, 2019b, Thudi et al., 2016a; Rasheed et al., 2017; Roorkiwal et al., 2018a, Roorkiwal et al., 2020). Particularly, the information revealed by the draft genome sequence of chickpea (Varshney et al., 2013a) further advanced efforts on the development of genomic resources and their applications in chickpea improvement (Roorkiwal et al., 2020).

In recent few years, next generation sequencing technologies such as Illumina (Solexa) sequencing, whole genome sequencing, targeted sequencing, amplicon sequencing, exome sequencing, RNA sequencing etc enabled the development of a wide variety of molecular markers in chickpea (Thudi et al., 2011, 2014; Jaganathan et al.2015; Kale et al., 2015; Varshney et al., 2018). So far, above 2,000 SSR markers, 15,000 diversity array technology (DArT) platform and millions of SNP markers have been developed and available for chickpea improvement programs (Varshney et al., 2016, Roorkiwal et al., 2020). The revolutionin next generation sequencing technologies has enabled sequencing to be performed at a higher depth using wholegenome resequencing (Roorkiwal et al., 2020).

Moreover, the re-sequencing of over 400 chickpea accessions from 45 countries identified key candidate genes that were under selection and those associated with agronomically important traits (Varshney et al., 2019a). Particularly, 50,590 high quality non redundant SNPs obtained from whole genome re-sequencing were used to develop a high throughput SNP genotyping plat form *'Axiom.Cicer SNP Array'* (Roorkiwal et al., 2018a), which is now routinely used for trait mapping and QTL detection to advance breeding applications in chickpea (Roorkiwal et al., 2020).

2.9. QTL Mapping in Chickpea

Most agronomically and economically important traits in chickpea are quantitative traits that are controlled by multiple QTL. Discovery of QTL and underlying causal genes/alleles is of great importance to marker assisted breeding. Linkage mapping is a classical method towards the genetic dissection of the genetic basis of quantitative trait loci.

So far, several QTLs have been mapped for traits related to diseases resistance, yield and yield related morphological traits, flower color, flowering time, double pod and growth habit (Cho et al., 2002; Rajesh et al., 2002; Cobos et al., 2005; Lichtenzveig et al., 2006; Sharma and Muehlbauer, 2007; Cobos et al., 2009; Varshney et al. 2014a; Roorkiwal et al., 2018; Sivasakthi et al., 2018).

Different genomic resources have been deployed in chickpea to locate and tag the molecular markers linked to wilt disease resistance for different pathogenic races (Gaur et al., 2012b; Gaur et al., 2014; Garg et al., 2018). Foc resistance genes for races 0,1,2,3,4 and 5 were reported on linkage group 2 (LG02) organized in two

adjacent resistance gene clusters (Rubio et al., 2003; Sharma and Muehlbauer 2007; Gowda et al., 2009; Halila et al., 2009; Cobos et al., 2009).

One of the two resistance genes for race 0 was found in LG5 (Cobos et al., 2005). Sabbavarapu et al. (2013) reported two major QTLs on CaLG06 for race 1. Recently important quantitative trait loci (QTLs) for *Fusarium* wilt resistance were reported using SNP and SSR markers (Garg et al., 2018). Except for races 1B/C and 6, the genetics of resistance for these six races were reported as monogenic, digenic or quantitative, (Singh et al., 1987; Kumar, 1988; Tullu et al., 1999; Rubio et al., 2003; Tekeoglu et al., 2000; Sharma et al., 2004; 2005; Kumar, 2015). The genetic control of resistance for races 1B/C and 6 are yet not reported (Garg et al., 2018).

For race 1, chickpea resistance is governed by recessive genes. Based on the allelic constitution at the two duplicate loci, wilting can happen early at seedling stage or late after flowering stage (Bayraktar et al., 2012). The dominant allele at both the loci gives early wilting; recessive allele in homozygous form at either of these two loci gives late wilting, and recessive alleles at both the loci (h1h1h2h2) confer complete resistance. For instance, the susceptible cultivars JG62 has dominant allele at both the loci (H1H1H2H2) and show early wilting, whereas resistant cultivar WR 315 has recessive alleles at both loci (h1h1h2h2) and completely resistant to race-1(Patil et al., 2014).

Likewise, two or three independent genes were found to confer resistance to race 2 (Kumar, 1998; Gumber et al., 1995), however, resistance to race 3 has been found to be monogenic and resistance to race 4 is recessive and digenic (Tullu et al., 1999).

Race 5 resistance is controlled by a single gene (Tekeoglu et al., 2000). For race 0 resistance is controlled by two genes (Rubio et al., 2003; Halila et al., 2009; 2010).

Sequencing based trait mapping is being performed by sequencing the whole population or by sequencing the pooled samples belonging to extreme bulks for the trait of interest (Varshney et al., 2019b). Low to high density sequencing approaches are being used to generate high density genetic maps and enhance the resolution of trait mapping in chickpea. In recent years, the GBS approach has been used to detect genome wide SNPs in chickpea to understand allelic diversity and population structure, and develop high density linkage maps, QTL analysis, GWAS and GS. GBS approach has been widely used for linkage mapping and QTL detection of ascochyta blight resistance (Deokar et al., 2019), heat tolerance (Paul et al., 2018), seed iron and zinc concentrations (Upadhyaya et al., 2016) and seed quality (Verma et al., 2015).

The GBS approach was also used for enhancing marker density within the 'QTL-hotspot' region, which harbours multiple QTLs for drought tolerance. Recently, GBS was used for high-resolution association analysis and GWAS integrated with QTL mapping and transcript profiling in germplasm lines and mapping populations to detect molecular signatures regulating photosynthetic efficiency in chickpea (Roorkiwal et al., 2020). The RAD-Seq technology was used to construct a highdensity genetic map using an intraspecific population and provided novel insights into the frequency of recombination and hotspots across the chickpea genome (Deokar et al., 2014).

Although many QTLs for different traits were identified, the markers were not close enough for their effective use in molecular breeding. Genome wide physical maps have been used in several species to effectively integrate genomic tools for marker assisted breeding, high-resolution mapping and positional cloning of genes and QTL (Millan et al., 2015). In addition, physical maps will also enable desirable genomesequencing and comparative genomics. Despite these advantages, a genomewide physical map has not been developed for chickpea (Roorkiwal et al., 2020). However, efforts are underway to develop genome wide physical map. The integration of genetic and physical map is expected to enhance genetics and genomics research and breeding applications in chickpea (Roorkiwal et al., 2020).

2.10. Genomic Assisted Breeding for *Fusarium* Wilt Resistance

Genomics offers tools to address the challenge of increasing food yield, quality and stability of production through advanced breeding techniques. Application of DNA markers to facilitate marker assisted selection (MAS) for crop improvement have proved successful in crossbreeding (Varshney et al., 2014b). Advances in plant genomics provide further means to improve the understandings of crop diversity at species and gene levels, and offer DNA markers to accelerate the pace of genetic improvement (Varshney et al., 2014b).

Genetic mapping and QTL analysis, via bi-parental or association mapping populations, have accelerated the dissection of genetic control of agricultural traits, potentially allowing MAS, QTL, and association mapping studies or direct calculation and genomic selection (GS) of high value genotypes to be made in the context of breeding programs (Mannur et al., 2019).

Integrating genomic technologies in chickpea breeding for *Fusarium* wilt resistance can accelerate the development of resistant varieties. Different genomic resources have been deployed in chickpea to locate and tag the molecular markers linked to *Fusarium* wilt disease resistance for different pathogenic races (Sabbavarapu et al. 2013; Gaur et al., 2014; Li et al., 2015). The first molecular markers linked to *Fusarium* wilt in chickpea was reported by in 1997 (Guar et al., 2012). Since then, several QTL genomic regions that confers high level of resistance to different physiological races of the pathogen have been mapped (Li et al., 2015; Sabbavarapu et al., 2013).

Currently several genomic resources are available to further speed up the breeding process to develop wilt resistant chickpea varieties (Hiremath et al. 2012; Nayak et al. 2010; Thudi et al., 2011, 2016b; Varshney et al. 2014b). Excellent progress has been made in understanding the genetic control of *Fusarium* wilt resistance and mapping *Fusarium* wilt resistance genes (Millan et al., 2006). Besides, integration of genomics tools in chickpea breeding programs such as marker-assisted selection (MAS) is very promising for crop improvement (Guar et al., 2012). The effectiveness of MAS depends on the strength of the linkage between the marker and the gene locus controlling the trait of interest, the localization in a saturated genomic area and the high level of polymorphism (Singh et al., 2008; Millan et al., 2015).

Through marker assisted back crossing (MABC) successful efforts have been reported in developing superior chickpea genotypes with good level of disease resistance (Mannur et al., 2019; Varshney et al., 2014c). Molecular markers closely linked with some of the genes conferring resistance to various *Fusarium* wilt races have been

identified and used for pyramiding resistance genes for these races (Guar et al., 2014). Moreover, use of MABC for improvement of elite chickpea cultivars for *Fusarium* wilt resistance has been successfully demonstrated by Varshney et al. (2014c). MAS facilitates the pyramiding of different resistance genes in the same genotype, resulting in faster line development and variety release (Collard et al., 2008; Millan et al., 2015).

Numerous MAS strategies have been developed including marker-assisted backcrossing or introgression of major genes or quantitative trait loci (QTL), enrichment of favorable alleles in early generations, and selection for quantitative traits using markers at multiple loci (Varshney et al. (2014c). Over the last two decades, the rapid development of whole-genome sequencing and marker development technologies enabled the use of high-density single nucleotide polymorphism (SNP) markers to analyze the whole genome at very low cost (Varshney et al., 2014c).

Whole genome and transcriptome sequencing bridges between the genotype and phenotype, and leads to a new revolution in plant breeding, especially for complex traits. Therefore, integration of genomics tools and conventional breeding triggers new breeding strategies, like gene pyramiding and genome selection (GS), which greatly accelerates the breeding.

CHAPTER THREE

The Intensity of Chickpea *Fusarium* Wilt and Its Association with Major Biophysical Factors and Identification of New Resistance Sources for Breeding

Abstract

Fusarium wilt/root rot complex are among the major challenges to chickpea production throughout Ethiopia. Previous studies have surveyed economically important chickpea diseases in Ethiopia, but were restricted to the major chickpea growing areas and thus were unable to provide a broad geographic view of disease pressure. In the present study, we focus on understanding the intensity of wilt/root rot disease pressure and its correlation with major biophysical factor in Ethiopia, and identification of new resistance sources for targeted breeding. In 2015/2016, geo-referenced field survey was conducted in 51 districts representing 17 chickpea growing zones, revealing that diseases were widely distributed in all surveyed areas. Across all surveyed sites, *Fusarium oxysporum* f. sp. *ciceris* (Foc) was the predominant species encountered among fungi cultured from plant tissue, representing 69.4 % of total isolates. The remaining isolates were other root rot pathogens including *Rhizoctonia bataticola, Fusarium solani, Sclerotium rolfslii, Rhizoctonia solani* and unidentified fungi. *Fusarium* wilt and root rot diseases incidence were significantly ($P < 0.05$) associated with clay soils (vertisols), Desi type chickpea, early planting, flowering and plant maturity.The highest mean percent diseases incidence per m^2 (45.65%) was recorded in the Amhara region, West Gojam zone, where typical vertisols predominate and chickpea production is regularly threatened by high diseases pressure. To tackle these diseases, from a total of 315 wild introgression lines evaluated in sick plot, 10 (3.18 %) and 5 (1.59 %) were highly resistant and moderately resistant with mean disease incidence of 0 % and 18.8 %, respectively. Similarly, among 47 advanced recombinant inbred lines (RILs) evaluated in sick plot, 20 genotypes were highly resistant with high yield and yield-related morphological traits. Therefore, these genotypes can be utilized in any chickpea breeding program as a novel wilt resistance sources to develop resistant varieties. In conclusion, the results of this study highlight factors associated with chickpea wilt and root diseases, identified new *Fusarium* wilt resistance sources and in doing so provide a framework for integrated disease management, while also implicating agricultural environments where breeding strategies for disease resistance might have greatest impact.

Keywords: *Fusarium* wilt, root rot complex, *Cicer arietinum*, chickpea, Disease Resistance

3.1. Background and Justification

Despite its large cultivation area and considerable economic importance, chickpea's productivity in Ethiopia is declining (CSA, 2018). Decreasing productivity derives in large part from constraints of diverse biotic and abiotic stresses that are exacerbated by insufficient crop management practices. Among fungal diseases impacting chickpea are *Fusarium* wilt, dry root rot, black root rot, wet root rot, collar rot and *Ascochyta* blight, caused respectively by *Fusarium oxysporum*, *Rhizoctonia bataticola, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii and Ascochyta rabiei* (Beniwal et al., 1992; Ahmed et al., 2006; Navas Cortes et al., 2008).

According to Yimer et al. (2018), in major chickpea growing areas of Ethiopia the dominant root infecting pathogen is *Fusarium oxysporum*f. sp. *ciceris (*Foc). Due to the soil borne nature of this pathogen, chemical control is generally ineffective (Jimenez Diaz et al., 2015). Breeding for host plant resistance is the most cost-efficient and eco-friendly strategy to control the disease (Jimenez-Diaz et al., 2015; Jendoubi et al., 2017).

However, the deployment of resistant varieties has not been extensively used due to lack of stable resistance source with desirable yield and yield-related morphological traits in cultivated chickpea with a narrow genetic base for traits' improvement. Different cultural practicescan reduce disease incidence to certain extent, however their efficacy is variable and implementation is uneven under smallholder conditions where chickpea is a vital component of diets and a preferred cash crop (Nene and Reddy 1987; Trapero Casas and Jimenez Diaz, 1985).

Among studies examining chickpea diseases in Ethiopia, most were restricted to the main chickpea producing areas of the country (Beniwal et al., 1992; Tadesse 1996; Tadesse et al. 1998a; Shehabu et al. 2008; Abera et al. 2011; Damte and Ojiewo 2016; Yimer et al., 2018). No reports are available that encompass the breadth of major and minor producing areas.

Understanding the magnitude of disease pressure,country-wide and identification of stable resistance sources are major prerequisites to initiate effective disease resistance breeding strategy and integrated disease management measures to combat the disease. Therefore, the main objective of the present study wasto enumerate *Fusarium* wilt disease pressure throughout chickpea growingareas, correlate disease pressure with major biophysical factors,and to identify new wilt resistance sources for targeted disease resistance breeding.

3.2. Materials and Methods

3.2.1. The Extent of *Fusarium* wilt of chickpea in Ethiopia

3.2.1.1. Study areas and data collection

In 2015/2016, global positioning system (GPS) based field surveys were conducted in Oromiya, Amhara and SNNP regions, including 51 districts (weredas) in 17 diverse chickpea growing zones (Fig. 3.1). Surveyed chickpea growing areas, altitudinal ranges and the proportion of soil types are given in Table 3.1. Chickpea fields were selected at intervals of 5 to 10 km, with geo-referenced fields in surveyed areas. In each of 51 representative farmers' fields, three 1 x 1 meter quadrat were surveyed along a diagonal transect following the right diagonal sampling design (Delp et al., 1986). Smallholder farms are typically less than 0.22 ha (CSA, 2016) and thus

quadrat samples were taken at 4 to 5 meter intervals alonga diagonal transect. All plants in each quadrat were counted, and each plant within the quadrat was scored for typical *Fusarium* wilt and root rot diseases symptoms.

Data on major biophysical factors, including planting date, soil type, chickpea variety, phenological stage of the crop at sampling, rotation history and field management practices employed by the farmers were recorded. According to Yimer et al. (2018), soil samples were assigned visually based on the main soil color as heavy black (dark/deep black soil); light black (an intermediate black and brown colored soil) and light (bright brown or reddish brown soil). The physical and chemical properties of soil samples, particularly soil pH and texture classes were determined through laboratory analysis at Debre Zeit Agricultural Research Center in Ethiopia (Table 3.2) according to Eyasu 2016.

3.2.1.2. Pathogens isolation and identification

From each field, three quadrat samples were collected for pathogen isolation and identification. From each quadrat, ten diseased plants were used to identify associated fungi based on morphological symptomatology. Samples were surface sterilized with a 10 % aqueous solution of Clorox® (0.6 % sodium hypochlorite, NaOCl) for one minute and rinsed extensively with sterile distilled water for 2 to 3 minutes. Treated tissue was cut into small pieces and directly plated onto potato dextrose agar containing chloramphenicol (120 mg/L). After one week of incubation at 25-28^{o}C, individual fungal colonies were selected and purified to homogeneity by repeated sub-culturing of single spores (Leslie and Summerell, 2006).

Pathogen identification was based on established morphological and cultural characteristics (Leslie and Summerell, 2006; Sharma et al., 2012a; Rasu et al., 2013; Sneh et al., 1991). Moreover, accurate pathogen identification, whole genome sequences were obtained for all samples categorized as *Fusarium* based on the above morphological and cultural characteristics, and the result is deposited to the National Center for Biotechnology Information (NCBI) under Bio Project PRJNA412392.

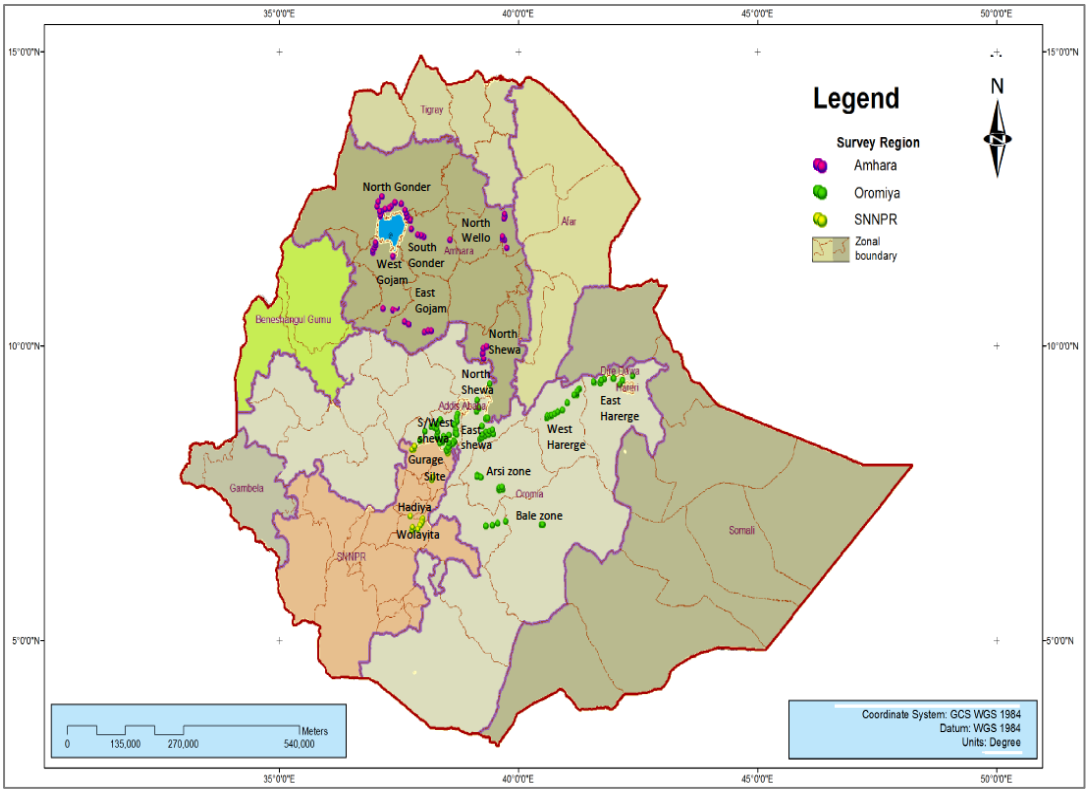


Fig 3.1. Surveyed chickpea producing areas in Ethiopia

Table 3.1. Altitudinal range, seed type and soil characteristics within surveyed zones.

Regions/ Zones	Number of districts	Altitudinal range (masl[a])	Chickpea type (%)		Soil type (%)		
			Desi	Kabuli	Clay	Clay loam	Loam
N/Gonder	4	1810-2180	80.0	20.0	53.3	26.7	20.0
N/Shewa	3	1714-2664	75.0	25.0	30.0	35.0	35.0
W/Gojam	2	1802-2082	78.7	21.3	88.9	11.10	0.00
E/Gojam	2	1822-2457	75.8	24.2	66.7	33.3	0.0
S/Gonder	2	1800-2766	80.0	20.0	60.0	20.0	20.0
N/Wollo	3	1784-1914	85.7	14.3	0.0	57.1	42.9
E/Shewa	5	1978-2556	70.4	29.6	70.4	25.9	3.7
S/W/Shewa	7	1787-2421	71.4	28.6	78.5	17.9	3.6
N/Shewa	3	2479-2913	75.0	25.0	100	0.0	0.0
W/Harerge	3	1714-2340	53.3	46.7	80.0	6.7	13.3
E/Harerge	4	1978-2556	84.6	15.4	23.0	38.5	38.5
Arsi	2	2331-2595	83.3	16.7	100	0.0	0.0
Bale	3	1996-2611	75.0	25.0	37.5	37.5	25.0
Wolayita	2	1712-2071	57.1	42.9	0	0	100
Gurage	2	1796-2142	66.7	33.3	66.7	16.7	16.6
Silte	2	2037-2076	76.3	23.7	0.0	50.0	50.0
Hadiya	2	1892-1935	64.3	35.7	0.0	0.0	100.0

[a]*masl is meters above sea level. N= North; W= West; E =East and S= South*

Table 3.2. Soil samples physical and chemical properties and major soil group in surveyed areas

Soil color[a]	Soil pH (range)	Soil type (Texture)	Percent Clay	Major soil group[b]
Heavy black	6.3 - 7.4	Clay	57.6	TypicalVertisols
Light black	6.5 - 7.3	Clay-loam	55.6	Vertisols
Light soil	6.4 - 7.1	Loam	19.6	Leptosols and Nitosols

[a]*Soil color was assigned visually based on the dominant soil color; Heavy black is dark/deep black soil; light black is an intermediate black and brown colored soil and light is bright brown or reddish brown soil.* [b]*Major soil group assigned according to Eyasu (2016) and Reeuwijk et al (1998)*

3.2.2. Disease phenotyping

3.2.2.1. Plant materials

In this study, a total of 315 wild introgression lines and 47 recombinant inbred lines (RILs) were used under different trials along with three disease resistant checks Dera (highly resistant), Arerti (moderately resistant) and Habru (moderately susceptible) and the universally susceptible check JG62. Wild introgression lineswere received from feed the future innovation laboratory for climate resilient chickpea project,University of California, Davis, USA. These lines were developed throughcrossing wild chickpea species, *C. reticulatum* and *C. echinosprmum* with early maturing Indian chickpea genotype, ICCV-92029 at University of California, Davis. Checks and RILs were received from Ethiopian Institute of Agricultural Research, Debre Zeit Research Center, Chickpea breeding program. RILs were derived from multi-parent intraspecific crosses (MAGIC) using eight diverse Kabuli chickpea varieties released in Ethiopiafor differentpurposes (Table 3.3).

Table 3.3. Lists of best kabuli varieties used as a crossing parents for MAGIC RILs Development, their origin and major desirable morphological traits

Variety Name	Origin/Source	Major desirable morphological traits
Arerti	ICARDA[a]	High yield & multiple disease resistance
Habru	ICARDA	High yield and disease resistance
Ejere	ICARDA	Good seed size and disease resistance
Teji	ICARDA	Good seed size and disease resistance
Shasho	ICRISAT[b]	High yield and good seed size
Chefe	ICRISAT	Good seed size and disease resistance
Yelibe	ICRISAT	Good seed size and drought tolerance
Acos Dubie	Mexico, Latin America	Extra big seed size

[a]International Center for Agricultural Research in Dry Areas, [b]International Crop Research Institute for Semi-Arid Tropics

3.2.2.2. Experimental design and data collection

In 2018 cropping seasons, a total of 315 wild introgression lines (F_4 generation) and 9 RILs (F_5 generation) were evaluated for wilt disease resistance at Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center sick plot along with the disease resistant checks (Dera, Arerti and Habru), and the susceptible check (JG-62). Each wild introgression lines was planted in a one meter long single row with spacing of 10cm between plants and 30cm between rows. However, RILs and checks were plantedafter every ten rows. In 2019 main season, 47 advanced RILs (F_6 generations) were evaluated for *Fusarium* wilt disease resistance in a sick plot with 1.2m x 2m plot size in 3 replications with spacing of 30 cm between rows and 10 cm between plants.

For all trials wilt disease data was recorded on 15, 30, 45 and 60 days after planting and percent disease incidence was estimated according to pande et al., 2012. In parallel, RILs were evaluated for yield and yield related morphological traits at Debre Zeit Agricultural Research Center experimental field using RCBD design with 1.2m x 2m plot size in 3 replications, and with spacing of 30 cm between rows and 10 cm between plants. Data on days to 50 % flowering (DFW) and plant height in cm (PH) were during flowering stage, and number of days to maturity (DM), number of pods per plant (NPP), number of seeds per pod (NSP), hundred seed weight in gram (HSW), grain yield tons per hectare (GYD) and average plant biomass in gram (PBM) were collected at harvest.

3.2.3. Data analysis

3.2.3.1. Association of biophysical factors with disease intensity

In total, 153 quadrats were analyzed, representing 3 quadrats per field. Percent wilt incidence (PWI) was analyzed according to Pande et al. (2012). The number of wilted plants per meter square (NWP/m^2) and percent wilt disease incidence per meter square (PWI/m^2) were organized into class boundaries for each variable classes (Table 3.4), according to Fininsa and Yuen (2001). Chi-square and logistic regression analysis were employed according to Landau and Everitt (2004) and using the Statistical Analysis Software package version 9.2 (SAS Institute, 2008) to investigate the associations of different biophysical factors (predictor variables) with *Fusarium* wilt incidence. The predictor variables are independent variables such as soil type, chickpea type, planting time, growth stage and crop rotation practices used in regression analysis.

Table 3.4. Classes boundaries used to analyze the number of diseased chickpea plants with wilt/root rot and percent disease incidence per m^2 for each variable classes.

Independent Variables	Variable classes	Number of diseased plants per m^2		Percent wilt incidence per m^2	
		<5	>5	< 20 %	> 20 %
Soil type					
	Clay	27	59	33	53
	Clay loam	10	15	17	8
	Loam	25	17	24	18
Chickpea type					
	Desi	31	93	47	77
	Kabuli	23	6	24	5
Planting time[a]					
	Early	9	20	9	20
	Intermediate	24	67	38	53
	Late	21	12	24	9
Growth stage					
	Vegetative	21	10	22	9
	Flowering	24	92	21	95
	Full podding	2	4	1	5
Previous crops					
	Pulse	10	31	9	32
	Cereals	52	51	47	56
	Others	3	6	3	6

[a]*Early planting is all planting in August; Intermediate is planting in the first three weeks of September, and late planting is planting in the last weeks of September and afterwards.*

3.2.3.2. Disease phenotyping

Disease incidence data for wild introgression lines, RILs and checks was analyzed according to Pande et al (2012) as follows.

$$Disease\ incidence\ \% = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants}\ x\ 100$$

Based on the disease incidence, test genotypes catagorized according to their to *Fusarium* wilt disease. Genotypes showed less than 10 % were considered as resistant, 10.1-20 %, moderately resistant, 20.1-40 % moderately susceptible and above 40 % were considered as highly susceptible.

All other dataon yield and yield related morphological traits of RILs were subjected to analysis of variance (ANOVA) according to Gomez and Gomez (1984) using the Statistical Analysis Software package version 9.2 (SAS Institute, 2008). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were computed according to Singh and Chaudhury (1985) as follows:

$$\text{GCV \%} = \sqrt{\frac{VG}{x}} \text{X } 100$$

$$\text{PCV \%} = \sqrt{\frac{VP}{x}} \text{X } 100$$

Where V_G =genotypic variance, V_P = phenotypic variance, x= samples mean

Broad sense heritability (H^2_b) and genetic advance (GA) was measured as given by Allard (1960).

$$H^2_b = [V_G/(V_G + V_E/r] * 100$$
$$GA = K\sqrt{Vp} * H^2_b$$
$$GAM = (GA/x) * 100$$

Where H^2_b =broad senense heritability in single location, V_G = genotypic variance, V_P = phenotypic variance, V_E = error variance, r = number of replications. GA = Genetic Advance, K = constant, which is equal to 2.06 at 5 % selection intensity, GAM= Genetic advance as percentage of mean, x = samples mean

Cluster analysis was performed using DARwin computer program software Version 6 (http://darwin.cirad.fr) using dissimilarity indix of mean euclidean distance,following the hierarchical clustering procedure with the unweighted pair group average method (UPGMA). The robustness of the tree topology was estimated from bootstrap value analysis with 1,000 replications. Correlation coefficient and principal component analyses were computed using the Statistical Analysis Software package version 9.2 (SAS Institute, 2008).

3.3. Results

3.3.1. Pathogen identification

Across all surveyed sites, Foc was the predominant fungus encountered in cultured plant tissue, representing 69.40 % of total isolates (Fig.3.2). The ubiquity of *Foc* is reflected in its isolation from all surveyed fields in Amhara, Oromiya and SNNPR regions (Fig.3.3), where it occurred in 94.4 %, 90.5 % and 72.2 % of sampled quadrats, respectively. As described in Fig.3.2, each field was represented with three quadrat samples, and if a pathogen is observed in at least one quadrat, therefore, it is considered present in that field. The remaining 30.6 % of isolates were classified as other root rot pathogens (Fig. 3.2), including *Rhizoctonia bataticola, Fusarium solani, Sclerotium rolfslii, Rhizoctonia solani* and unidentified fungi, which were isolated from 61.1 %, 52.4 % and 33.3 % of the surveyed fields in Amhara, Oromiya and SNNP regions, respectively (Fig.3.3).

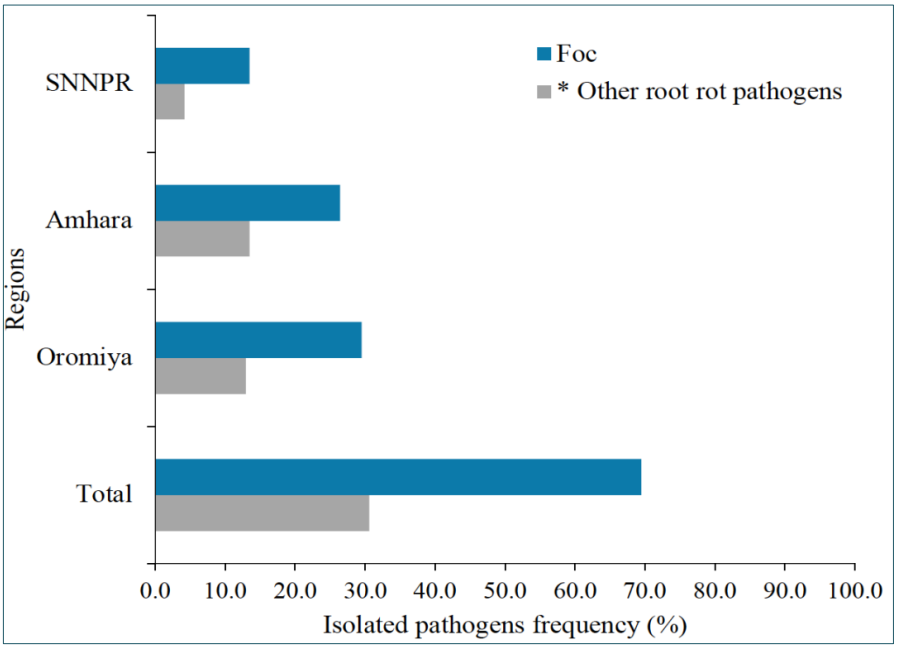


Fig 3.2. Percentage of isolated Foc and root rot pathogens frequency in surveyed regions **Rhizoctonia bataticola*, *Fusarium solani*, *Sclerotium rolfsli*, *Rhizoctonia solani* and other unidentified pathogens

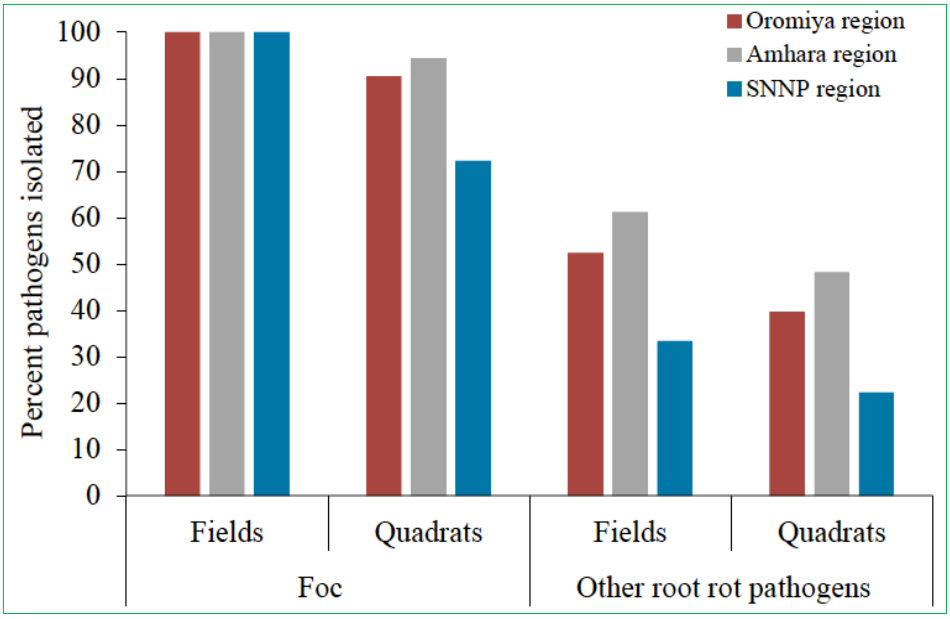


Fig 3.3. The proportions of Foc and other root rot pathogens isolated from farmers' fields and in each quadrat within the field

Whole genome DNA sequencing confirmed the predominance of *Fusarium oxysporum* in the collection. Draft genome assemblies were obtained for 166 Etd Foc strains that had been classified as *Fusarium* based on morphological criteria. Curatorial analyses at NCBI as well as our internal efforts (deposited to NCBI as Bio Project PRJNA412392) identified as *Fusarium* as the genus for 99 % of the strains and with confident identification 141 (85 %) as *Fusarium oxysporum ciceris* (Foc) with average genome size of 53.50 Mbp (Fig.3.4).

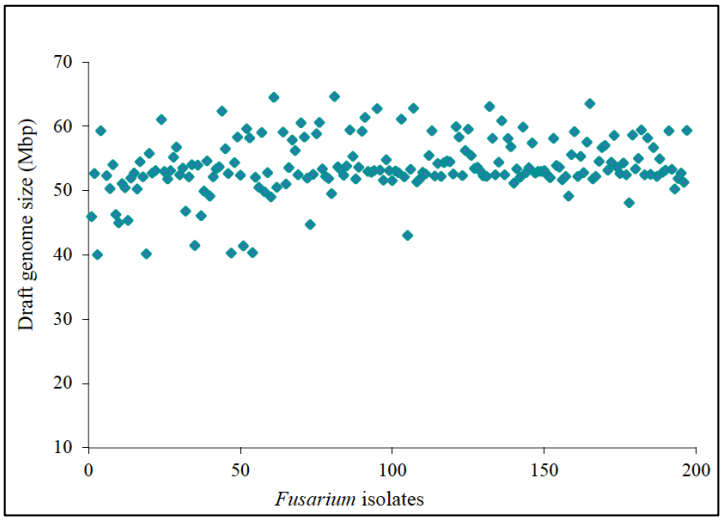


Fig 3.4. Draft genome size (Mbp) of 195 *Fusarium isolate*s collection in Ethiopia

3.3.2. Association of biophysical factors with disease intensity

Soil samples physical and chemical properties divided soils into three textural classes: clay, clay loam and loam, each with distinct soil properties (Table 3.4). Vertisols were the dominant substrate, although with variable clay content. Interestingly, analysis of agronomic and soil types (textural classes) data revealed significant association between these biophysical factors and the extent of wilt disease incidence in chickpea (Table 3.5). Planting date, chickpea seed type and growth stage were significant ($P < 0.01$) in their association with number of diseased plants and disease incidence in surveyed areas. Soil type and crop rotation practices were also significantly ($P < 0.05$) associated with disease, but to a lesser extent.

Table 3.5. Likelihood ratio chi-square analysis and the association of major biophysical factors with *Fusarium* wilt/root rot disease in chickpea

Predictor Variables	Variable classes	Diseased plant/m^2				Disease incidence/m^2		
		df	χ^2	S.E[a]	P	χ^2	S.E[a]	P. value
Soil type	Clay Clay-loam Loam	2	9.2	0.02	0.010[b]	8.71	0.02	0.013[b]
Chickpea type	Desi Kabuli	1	29.64	0.07	0.000[b]	20.08	0.05	0.000[b]
Planting date	Early Intermediate Late	2	14.48	0.03	0.001[b]	13.05	0.02	0.000[b]
Growth stage	Flowering Podding Vegetative	2	23.85	0.05	0.000[b]	31.11	0.05	0.000[b]
Previous crops	Pulses Cereals Others	2	8.84	0.02	0.012[b]	7.41	0.02	0.025[b]

Df=degree of freedom, [a] S.E. is standard error, [b] Likelihood ratio chi-square probability

Comparison of each biophysical factor variable classes with the reference variable class indicated that high *Fusarium* wilt and root rot disease incidence per m^2 (Table 3.6) and highnumber of diseased plants per m^2 (Table 3.7) were both significantly

associated with soil type, plant type, sowing date and plant phenology (Tables 3.6 and 3.7). Thus, *Fusarium* wilt/root complex was 2.14 to 12.22 times higher depending on whether the crop was grown in clay soil versus loam soil, whether the planted genotype was Desi versus Kabuli chickpea, early versus late planting and plant phenology at sampling (vegetative, flowering or podding) (Table 3.6). The same factors impacted the density of diseased plants, with similar directionalities (Table 3.7).

Table 3.6. Logistic regression analysis and the association of the predictor variables with *Fusarium* wilt and root rot disease incidence per m^2

Predictor Variables	Variables Classes	Parameter Estimate	S.E	Wald	P value	Odd ratio[c]
Soil type	Clay	0.76	0.38	3.96	< 0.05	2.14
	Clay-loam	-0.47	0.53	0.77	0.38	0.63
	Loam	R^g				
Chickpea type						
	Desi	2.06	0.53	15.41	< 0.001	7.86
	Kabuli	R^g				
Planting time						
	Early	1.78	0.56	10.09	< 0.001	5.93
	Intermediate	1.31	0.45	8.72	< 0.005	3.72
	Late	R^g				
Growth stage						
	Flowering	2.40	0.46	26.90	< 0.001	11.06
	Podding	2.50	1.17	4.62	< 0.05	12.22
	Vegetative	R^g				
Previous crop						
	Pulses	0.575	0.801	0.52	0.47	1.78
	Cereals	-0.518	0.734	0.50	0.48	0.60
	Others	R^g				

[c]*Exponentiation of the parameter estimate, R^g variable class used for comparison as a reference group*

Table 3.7. The association of the predictor variables with number of diseased plants/m^2

Predictor Variables	Variables Classes	Parameter Estimate	S.E	Wald	P value	Odd ratio[c]
Soil type	Clay	1.17	0.39	8.92	< 0.05	3.21
	Clay-loam	0.79	0.52	2.36	0.13	2.20
	Loam	R^g				
Chickpea type						
	Desi	2.44	0.50	23.56	< 0.001	11.50
	Kabuli	R^g				
Planting time						
	Early	1.36	0.54	6.32	< 0.05	4.89
	Intermediate	1.59	0.43	13.41	< 0.001	3.89
	Late	R^g				
Growth stage						
	Flowering	2.09	0.48	21.73	< 0.001	8.05
	Podding	1.44	0.95	2.29	0.13	4.20
	Vegetative	R^g				
Previous crop						
	Pulses	0.44	0.80	0.30	0.58	1.55
	Cereals	-0.71	0.73	0.94	0.33	0.49
	Others	R^g				

[c]*Exponentiation of the parameter estimate,* R^g *Variable class used for comparison as a reference group*

3.3.3. Disease intensity in diverse growing areas

As described above, *Fusarium* wilt and root rot diseases are ubiquitous throughout chickpea cultivated regions in Ethiopia. Average disease incidence was highest throughout Amhara (30.98 %) and Oromiya (22.83 %) regions, and lower in SNNP (15.18%), although there was significant variation among zones in each region (Fig3.5). Interestingly, chickpea farms in SNNP have a lower rate of clay soils and increased use of improved Kabuli seed types (Table 3.1).

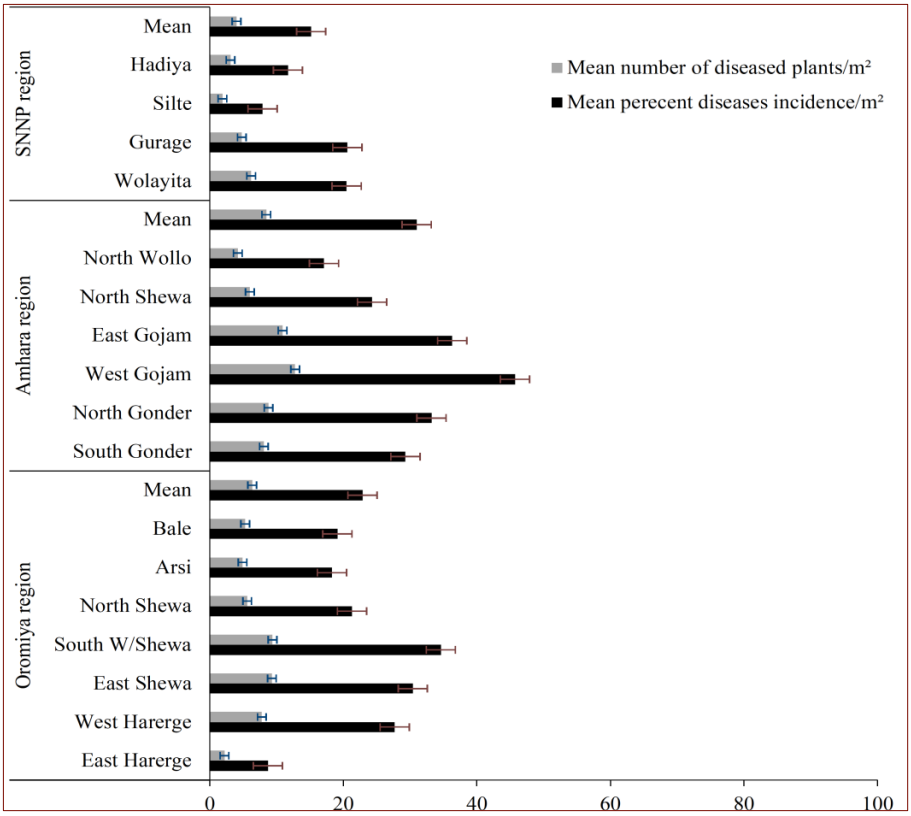


Fig 3.5. Mean number of diseased plants and percent disease incidence per m^2.

3.3.4. Identification of new resistance source

Among the 315 wild introgression lines screened in sick plot, 10 (3.18 %) and 5 (1.59 %) genotypes were resistant and moderately resistant to wilt/root rot disease with mean disease incidence of 0 % and 18.8 %, respectively (Table 3.8 and Fig 3.6). However, 20 and 280 genotypes were moderately susceptible and susceptible with mean disease incidence of 32.3 % and 95.67 %, respectively (Fig.3.6). Similarly, from 9 RILs (F_5 generation) screened for *Fusarium* wilt/root rot resistance in sick plot, 5 (55.56 %) and 3 (33.33%) resistant and moderately susceptible. The resistant check (Dera) and the susceptible check (JG-62) were exhibited 0 % and 100 % wilt incidence respectively. However, Arerti and Habru remained moderately resistant for wilt/root rot with 10 to 20 % disease incidence.

3.3.4.1. Wild introgression lines

Table 3.8. Wild introgression lines with high level of resistance tochickpea wilt disease in sick plot

Family /Lineage	UC Davis ID Number	Mean FWI %	Level of host resistance
1. ICCV96029 x Egill_065	1550	0.00	Highly resistant
2. ICCV96029 x Egill_065	1553	0.00	
3. ICCV96029 x Egill_065	1581	0.00	
4. ICCV96029 x Egill_065	1610	0.00	
5. ICCV96029 x Egill_065	1703	0.00	
6. ICCV96029 x Bari2_072n2	236	0.00	
7. ICCV96029 x Gunas_063	348	0.00	
8. ICCV96029 x CudiA_152	957	0.00	
9. ICCV96029 x Derei_072	1413	0.00	
10. ICCV96029 x Oyali_084	2413	0.00	
11. ICCV96029 x Besev_079	836	17.0	Moderately
12. ICCV96029 x CudiB_022C	1063	17.0	resistant
13. ICCV96029 x Bari3_100	519	20.0	
14. ICCV96029 x Derei_070	1307	20.0	
15. ICCV96029 x Egill_065	1551	20.0	

FWI=Fusarium wilt incidence

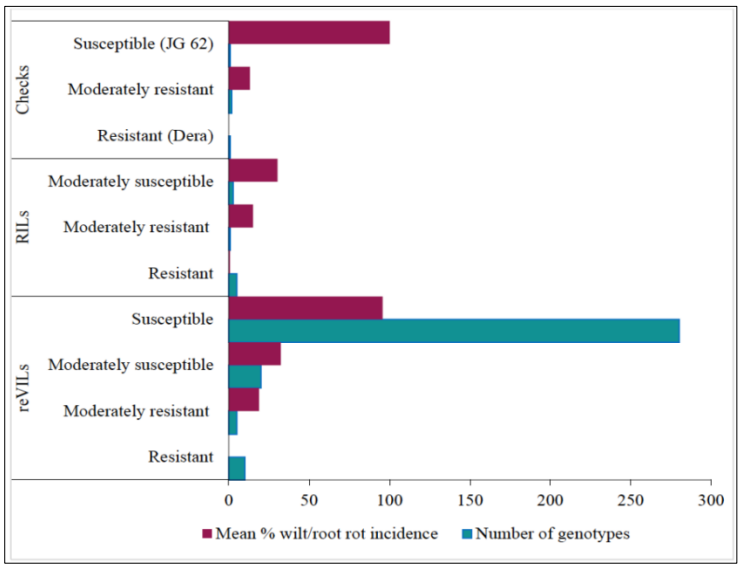


Fig.3.6.The response of wild reverse introgression lines (reVILs), recombinant Inbred Lines (RILs) and checks forwilt diseases in Debre Zeit sick plot

3.3.4.2. Recombinant inbred lines (RILs)

RILs (F_6) response for *Fusarium* wilt disease ranges from highly resistant (0 %) to highly susceptible (100 %) **(**Table 3.9**)**. The resistant check (Dera) and susceptible check (JG-62) exhibited 0 % and 100 % disease incidence, respectively.

Table 3.9. Analysis of variance based on the performance of 50 test genotypes for *Fusarium* wilt diseases in sick plot

Source of variation	Mean FWI %	Source of variation	*df*	Values
Range	0 - 100 %	Mean Squares		
Grand mean	28.85			
R^2	0.96	Genotypes	49	2868.64**
C.V %	25.78	Error	98	55.34
		SD		7.44
		LSD (0.05%)		12.05

FWI=Fusarium wilt incidence, *df = degree of freedom*, ****significant at 0.01 probability level, SD = Standard deviation, LSD=Least significant difference***

Dendrogram built based on disease response in sick plot split test genotypes into 4 distinct groups (Fig 3.7). Group-I represented with 12 moderately wilt resistant test genotypes with mean wilt incidence of 15.36 %. Interestingly, group-II was the largest group containing 20 wilt resistant genotypes with mean wilt incidence of 3.83 % (Table 3.10). The resistant check (Dera) with 0 % disease incidence was clustered in group-II along with wilt resistant RILs. However, RILs in group-III and group IV, each with 9 test genotypes, exhibited mean wilt incidence of 37.5 % and 84.44 % respectively. The wilt susceptible check (JG-62) with wilt incidence of 100 % was also clustered in group-IV along with the susceptible RILs.

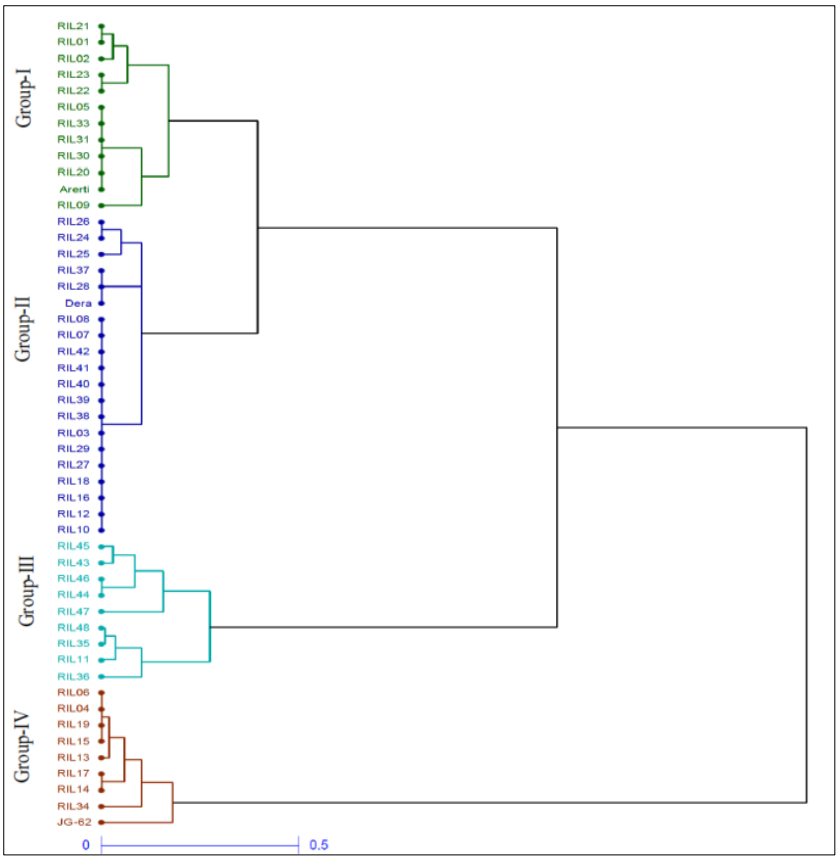


Fig.3.7. UPGMA dendrogram of 50 RILsbased on disease response in sick plot

Based on the above analyses, 20 wilt resistant genotypes were identified in group-II (Table 6), with mean wilt incidence ranging from 0 % to 5 %. Among these, RIL28, RIL37, RIL26 and RIL24 showed very high level wilt resistance with mean incidence of 0.0%, 0.0 %, 1.67 % and 1.67 %, respectively (Table 3.10). These RILs also gave high mean grain yield, each with above 3.3 tons/ha. Therefore, these high yielding and wilt resistant RILs that can be deployed in chickpea improvement programs as a new sources of *Fusarium* wilt resistance to generate high yielding and wilt resistant varieties.

Table 3.10. Mean trait performance of 20 high yielding and Fusarium wilt resistant genotypes in group-II

Genotypic ID	DFW	DM	NPP	NSP	HSW	PBM	HI (%)	GYD	FWI(%)
RIL-28	41.33	121.7	37.0	1.20	36.4	28.8	34.80	3.30	0.00
RIL-37	43.00	124.7	34.7	1.30	36.9	26.6	34.35	3.38	0.00
Dera	50.33	124.7	29.0	1.10	35.5	23.3	37.27	2.63	0.00
RIL24	39.33	121.0	28.3	1.17	33.9	22.9	43.66	3.33	1.67
RIL26	40.33	124.0	30.0	1.20	35.6	23.3	44.15	3.43	1.67
RIL25	40.00	123.0	28.0	1.23	34.7	20.7	45.71	3.15	3.33
RIL03	44.33	116.7	27.7	1.23	33.2	24.2	42.85	3.45	5.00
RIL07	42.00	119.0	29.3	1.23	34.5	26.7	39.48	3.51	5.00
RIL08	40.00	124.0	30.0	1.23	33.4	26.7	38.32	3.40	5.00
RIL10	39.00	112.0	30.0	1.20	32.5	27.9	37.26	3.47	5.00
RIL12	42.00	121.0	24.0	1.20	34.3	26.7	37.68	3.35	5.00
RIL16	43.33	123.0	27.7	1.20	35.4	26.9	39.75	3.56	5.00
RIL18	40.00	124.0	29.7	1.23	35.4	27.0	38.13	3.44	5.00
RIL27	41.67	124.7	30.0	1.20	35.5	28.8	37.65	3.60	5.00
RIL29	40.00	123.7	32.0	1.10	35.9	26.7	36.61	3.25	5.00
RIL38	40.33	124.0	24.0	1.10	37.3	26.3	33.73	2.94	5.00
RIL39	43.00	120.0	22.7	1.30	37.6	25.8	34.19	2.94	5.00
RIL40	40.00	123.7	15.3	1.20	35.4	25.0	36.73	3.06	5.00
RIL41	42.33	120.7	29.0	1.20	35.6	24.2	34.04	2.74	5.00
RIL42	42.00	117.3	25.0	1.10	35.9	28.8	34.34	3.28	5.00
Mean	41.72	121.6	27.7	1.20	35.3	25.9	38.04	3.26	3.83

Days to 50 % flowering (DFW), Plant height in cm (PH), Number of days to maturity (DM), Number of pods per plant (NPP), Number of seeds per pod (NSP), Hundred seed weight in gram (HSW), Grain yield tons per hectare (GYD), Average plant biomass in gram (PBM), Harvest index (HI) and Fusarium wilt incidence (FWI).

3.3.5. RILs diversity for major yield related traits

The analysis of variance revealed highly significant differences ($P < 0.01$) among the RILs in days to 50 % flowering (DFW), plant height (PH), number of days to maturity (DM), number of pods per plant (NPP), 100 seed weight (HSW), grain yield in tons per hectare (GYD) and percent harvest index (HI %) (Table 3.11). Significant differences were ($P < 0.05$) observed in number of seeds per pod (NSP).This result suggests the presence of high genotypic diversity among RILs for yield and yield related morphological traits.

Table 3.11. Mean trait performances of 50 genotypes for major morphological traits.

Source of variation	df	Mean trait performance								
		DFW	PH	DM	NPP	NSP	HSW	GYD	PBM	HI
Grand mean		41.37	44.57	120.25	27.7	1.12	32.9	2.96	25.6	36.6
R^2		0.87	0.94	0.93	0.93	0.82	0.96	0.91	0.77	0.92
Mean Squares										
Genotypes	49	21.81 **	22.15 **	54.16 **	46.29 **	0.01 *	57.66 **	0.60 **	16.9 **	61.4 **
Error	98	1.58	0.72	1.92	1.71	0.01	1.13	0.04	2.58	3.00
SD		1.26	0.85	1.39	1.31	0.03	1.06	0.19	1.61	1.73
LSD (0.05%)		*2.04*	*1.37*	*2.25*	*2.12*	*0.05*	*1.72*	*0.31*	*1.04*	*2.80*

*Degree of freedom (df), *,**significant at 0.05 and 0.01 probability level respectively.*
Days to 50 % flowering (DFW), Plant height in cm (PH), Number of days to maturity (DM), Number of pods per plant (NPP), Number of seeds per pod (NSP), Hundred seed weight in gram (HSW), Grain yield tons per hectare (GYD), Average plant biomass in gram (PBM), Harvest index (HI), Standard deviation (SD), Least significant difference (LSD).

The correlation analysis also revealed grain yield was positively and significantly ($P < 0.01$) correlated with percent harvest index ($r = 0.64$) and with number of pods per plant ($r = 0.35$) (Table 3.12). Harvest index was positively and significantly ($P < 0.01$) correlated with number of days to 50 % flowering (DFW) and with number of days to maturity (DM), but negatively and significantly ($P < 0.01$) correlated with plant biomass (PBM).

Table 3.12. Correlation coefficients analysis of 50 test genotypes for major morphological traits.

Traits	DFW	PH	DM	NPP	NSP	HSW	GYD	PBM	HI %
DFW	1.00								
PH	0.10	1.00							
DM	0.23	0.06	1.00						
NPP	0.25*	0.16	0.23	1.00					
NSP	0.06	0.06	0.12	0.11	1.00				
HSW	0.11	0.27*	0.20	0.14	0.06	1.00			
GYD	0.12	0.14	0.22	0.35*	0.13	0.14	1.00		
PBM	0.35*	0.04	-0.2	0.02	0.10	0.38**	0.21	1.00	
HI%	0.41**	0.14	0.35*	0.25*	0.01	-0.19	0.64**	-0.60**	1.00

,significant at 0.05 and 0.01 probability level respectively. Days to 50 % flowering (DFW), Plant height in cm (PH), Number of days to maturity (DM), Number of pods per plant (NPP), Number of seeds per pod (NSP), Hundred seed weight in gram (HSW), Grain yield tons per hectare (GYD), Average plant biomass in gram (PBM), Harvest index (HI).*

High genetic advance as a percentage of the mean (GAM) for grain yield tons/ha (GYD), number of pods/plant (NPP), hundred seed weight in gram (HSW) and harvest index (HI) %, with high genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) (Table 3.13) revealed the presence of significant genetic diversity among RILs for major yield and yield related morphological traits. Very high broad sense heritability (H^2_b) obtained for all morphological traits, ranging from 84.73 % in PBM to 97.82 % in HSW.

Table 3.13. Estimates of genetic parameters for major morphological traits in 50 test genotypes

Traits	Mean	R^2	V_G	V_P	GCV	PCV	H^2_b	GAM
DF	41.37	0.87	6.74	7.27	6.28	6.52	92.70	12.45
PH	44.56	0.94	7.14	7.38	6.00	6.10	96.75	12.15
DM	120.3	0.93	17.41	18.05	3.47	3.53	96.45	07.02
NPP	27.67	0.93	14.86	15.43	13.93	14.20	96.30	28.16
NSP	1.19	0.82	0.00	0.00	4.45	4.72	88.89	8.64
HSW	32.92	0.96	18.80	19.22	13.17	13.32	97.82	26.84
GYL	2.96	0.91	0.19	0.20	14.73	15.11	95.00	29.57
PBM	25.63	0.77	4.77	5.63	8.52	9.26	84.73	16.15
HI (%)	36.59	0.92	19.46	20.46	12.06	12.36	95.11	24.22

Genetic variance (V_G), Phenotypic variance (V_p), Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Broad sense heritability (H^2_b), Genetic advance at 5% selection intensity (GA), Genetic advance as percentage of mean (GAM)

Besides, cluster analysis grouped test genotypes into 4 distinct clusters (Fig. 3.8). Cluster 1 is the largest cluster with 19 RILs followed by cluster 2 with 17 RILs, whereas cluster 4 is the smallest cluster with three checks and two RILs. Cluster mean analysis indicated that each cluster haspeculiar characteristics. RILs in cluster 4 were characterized by highest mean for number of days to 50 % flowering; percent harvest index andnumber of pods per plant, but with the lowest mean for grain yield, hundred seed weight, average plant biomass and number of seeds per pod.

RILs in cluster 3 were characterized by the lowest mean for number of days to maturity and percent harvest index, and with intermediate performance for the rests of the traits. Similarly, RILs in cluster-1 were characterized by highest mean for number of seeds per pod (NSP) and hundred seed weight (HSW) but with lowest mean for number of days to 50 % flowering and with intermediate performance for the rests of the traits. Cluster-2 also mainly represented by RILs with intermediate performance for most morphological traits under investigation, but with least number of pods per plant (NPP).

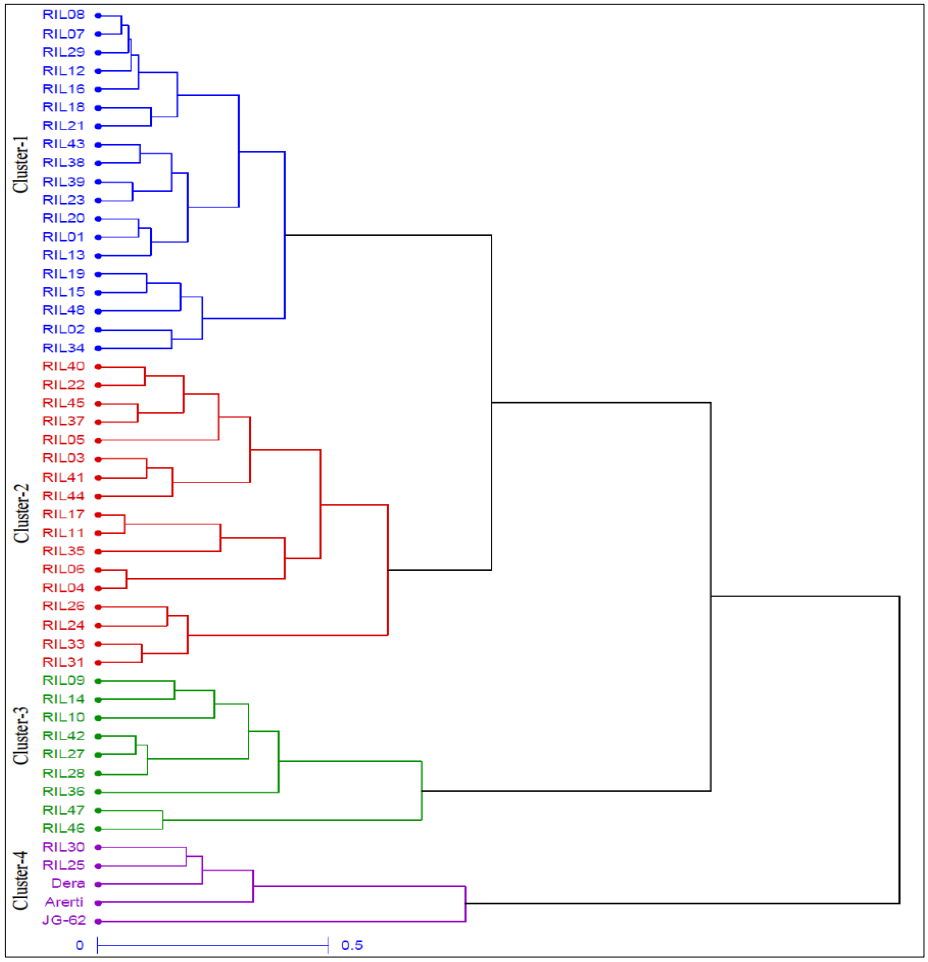


Fig.3.8. Dendrogram of 50 test genotypes based on yield related morphological traits

The principal coordinate analysis (PCoA) showed the first two principal Coordinate with eigen values greater than 1 accounted for 93.94 % of the total variation; with PCo-1 and PCo-2 contributing 58.26 % and 35.68 % respectively (Fig. 3.9).

Consistent with cluster analysis, the PCoA a split test genotypes into 4 distinct clusters. Test genotypes that distributed in the upper and the lower left hand quadrants around PCo-Y axis corresponded to RILs in cluster-1. Similarly, test genotypes in the upper and the lower right hand quadrants around PCo-Y axis corresponded to RILs in cluster-2. The two distinct clusters in the upper and the lower left and right hand quadrants grouped furthest to PCo-Y axis corresponded to RILs in cluster III and IV respectively.

The first principal coordinate (PCo-1) that accounted for 58.26 % of the total variation had high and positive eigen vectors for grain yield, harvest index %, number of days to maturity, number of days to 50 % maturity and number of pods per plant, whereas the second principal coordinate (PCo-2) that accounted for 35.68 % of the total variation had high and positive eigenvector for hundred seed weight and plant biomass.

A positive and high eigenvector for a given trait indicate that positive correlation between that trait and the given PCo, while high and negative eigenvector indicates negative correlation between the trait and a given PCo. Therefore, this study indicated that harvest index, grain yield, hundred seed weight, plant biomass, number of seeds per pod, plant height and number of pods per plant accounted for the highest proportion for the genetic diversity in the tested RILs.

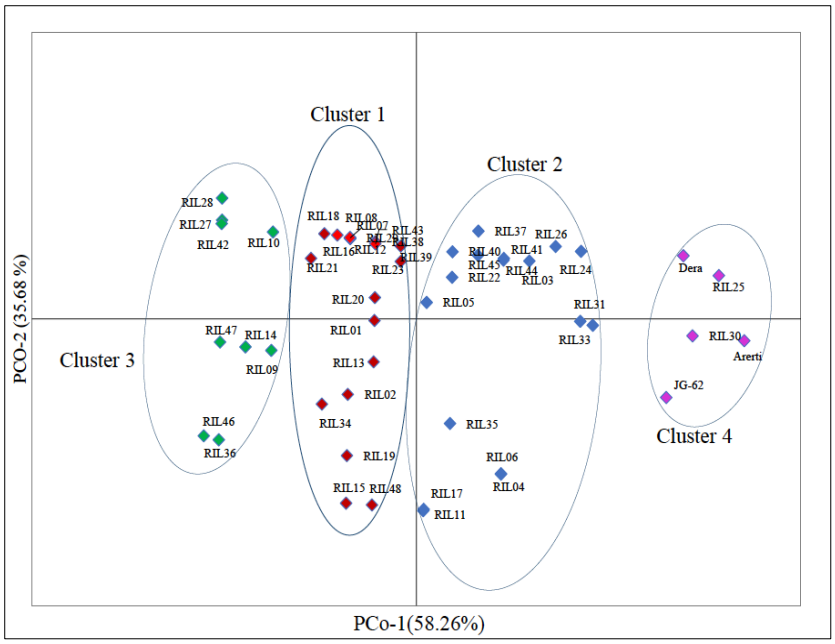


Fig 3.9. Principal coordinate analysis of 50 test genotypes based on major morphological traits.

3.4. Discussion

3.4.1. The association of biophysical factors with disease intensity

The present study indicates that despite difference in major biophysical factors, chickpea wilt diseases are widely distributed throughout Ethiopia. *Fusarium oxysporum* is considered to be the most damaging of chickpea's fungal root diseases, andboth morphological and genomic data confirm that *Fusarium oxysporum* is predominant country-wide. In agreement with the present report, several previous authors (Beniwal et al. 1992; Tadesse 1996; Tadesse et al., 1998a; Shehabu et al., 2008; Abera et al., 2011; Damte and Ojiewo 2016;Yimer et al., 2018) described wide distribution of wilt and root rot diseases in Ethiopia's major chickpea growing areas. Here we observed the highest average disease incidence in Amhara region, in West Gojam zone and particularly in North and South Achefer districts, where chickpeaproduction is regularly threatened by high disease pressure. In Oromiya region, disease pressure was highest in South West Shewa, East Shewa and Western Harerge zones. Despite ongoing efforts to disseminate improved chickpea

technologies and agronomic practices for *Fusarium* wilt disease in these areas (Fikre, 2014), fungal root diseases remain a serious and regular threat to chickpea production.

Interestingly, regions with the highest rates of disease were also those with the highest usage of Desi seed types. "Desi" and "Kabuli" seed are market classes, without a simple genetic basis (Penmetsa et al., 2016). Importantly, the local prevalence of Desi versus Kabuli genotypes reflects the intensity of agronomic practices. Desi-type seed in Ethiopia are traditional since ages and often landraces without modern genetic improvements. Landraces are culturally-preferred and prevalent on smallholder farms, where the adoption of new agricultural technologies are often low. By contrast, Ethiopian Kabuli-type varieties are lately adapted products and their availability is more frequently associated with organized seed systems (grower cooperatives and the seed industry). The current study identified SNNP as the region with the lowest rates of wilt and root rot diseases. Although Kabuli genotypes are abundant on SNNP farms, the higher acceptance of new agricultural technologies might better explain the lower rates of disease.

The surveyed zones lie within the tepid mid-highlands and cold highlands (i.e. 1, 500 to 3, 200 m.a.s.l) and characterized by relatively high rainfall and cool temperatures (FAO, 1986; Mesfin Abebe, 1998). Although rates of disease incidence in some of the surveyed areas in this report fall within the ranges of previous reports (Damte and Ojiewo 2016; Yimer et al., 2018), in general we report higher diseases incidences than prior studies. This suggests that wilt and root rot disease incidence and its negative impact on crop performance is an increasing threat to farmers' livelihood. A practical consequence of this situation is that farmers in major chickpea growing areas

are shifting from chickpea production to other crops, including grass pea. Root diseases are increasingly observed in minor and non-traditional chickpea growing areas of the country, particularly in East and West Harerge, Wolayita, Gurage, Arsi and Bale zones.

We also observed that soil type was a correlated factor, with clay soil (heavy black soil) having higher than loam soil (light soil). These observations agree with previous reports indicating high *Fusarium* wilt incidence on black soils, up to 75 % (Rachana 2002). Soils differ in their moisture holding capacity, which along with temperature is known to influence wilt disease development in chickpea (Bejiga et al., 1994; Chand and Khirbat 2009). Areas of the country with the longest history of chickpea cultivation, in Amhara and Oromiya, also have a greater percentage of clay soil (vertisols) (Eyasu, 2016). Thus, while soil type may be an important factor, interpreting these data is confounded by historical cultivation practices that are biased by soil type and may impact the prevalence of root-infecting fungi.

In contrast to soil type, which is largely a fixed feature of location, management practices can evolve over time and may offer avenues to counter root disease. Indeed, both crop rotation and late planting were correlated with decreased disease incidence. In the absence of rotation or when chickpea was grown after other pulse crops, disease was more severe. The *Fusarium* wilt pathogen can colonize the roots of other pulse crops without developing external symptoms (Jimenez Diaz et al., 2015), thus increasing inoculum density within the soil. Planting date, while significant in the current data, may be confounded by factors not recorded here, including yearly

differences in soil moisture and other disease pressures to which farmers respond by adjusting planting schedules.

The prevalence of wilt and root rot complex throughout chickpea growing regions of Ethiopia likely owes to the persistence of the pathogens in infested soils, and the long history of chickpea cultivation which derives from the great importance of chickpea as a cash and nutritional crop in affected areas. Special attention is required to break the recurrent wilt and root rot diseases cycle. There is a need for improved management practices, more accurate and sensitive pathogen detection technologies, and breeding genetic resistance into farmer-preferred local Desi genotypes using molecular methods. Taken together the results from this study provide a framework for integrated Fusarium wilt and root rot disease management, highlighting factors most frequently associated with diseases and nominating agricultural practices and environments where breeding strategies for disease resistance should be developed to minimize the ever increasing challenges of these diseases.

3.4.2. Identification of new resistance sources

In any plant breeding program new varieties are continuously required to meet producers and consumers increasing demands, and to protect crops from diverse biotic and abiotic stresses. To satisfy these demands, plant breeding always requires identification of new resistance sources against different biotic and abiotic stresses. In the present study, wild introgression lines and advanced recombinant inbred lines developed through inter crossing different chickpea varieties with diverse genetic background showed significant genetic diversity for yield and yield related traits that can be exploited to improve the agronomic value of the chickpea crop.

Besides, genotypic and phenotypic coefficient of variations, broad sense heritability and genetic advance as a percentage of the mean for grain yield, hundred seed weight, number of pods per plant, plant biomass and harvest index also indicated the existence of high genetic diversity among RIL for yield and yield related morphological traits. Similar previous study also reported the existence of high genetic diversity among advanced chickpea breeding lines (Hajibarat et al., 2014). This indicates the high possibility of creating significant genetic diversity in chickpea for yield and yield related morphological traits through effective breeding approaches. Previous studies reported that, genetic diversity analysis based on morphological traits is one of the strongest determinants of the agronomic value of crops (Jannatabadi et al.2014; Hajibarat et al., 2014).

Cluster and principal coordinate analyses based on major yield related morphological traits also divided RILs into 4 distinct diversity groups indicating significant genetic diversity among RILs. Previous studies also reported the existence of high genetic diversity inEthiopian chickpea breeding lines (Keneni et al., 2012). Moreover, advanced RILs evaluated for *Fusarium* wilt resistance also exhibited very high genetic variability for *Fusarium* wilt resistance suggesting the presence of diverse wilt resistance genes in the recombinant genotypes. Although excellent progress has been made in the development of chickpea genotypes with high levels of resistance to wilt disease (Gaur et al., 2012), the deployment of resistant cultivars has not been extensively used mainly due to the undesirable agronomic traits found in some developed resistance sources (Jendoubi et al., 2017).

In the presentstudy 20 *Fusarium* wilt resistant RILs with high yield and desirable agro morphological traits were identified. Among these, RIL28, RIL37, RIL26 and RIL24 revealed very high level *Fusarium* wilt resistance with high yield and yield related morphological traits. In agreement with the present findings, previous studies in different countries indicated the possibility of creating significant genetic diversity in chickpea for *Fusarium* wilt disease resistance, yield and yield related morphological traits through different crossing techniques and from wild relatives (Gaur et al., 2012; Hajibarat et al., 2014; Jannatabadi et al. 2014; Naveed et al.2015, Sharma et al., 2019). Similar studies also reported that in different countries RILs developed through intraspecific and interspecific crosses often used as good source of disease resistance (Gaur et al. 2014; Munoz et al. 2017; Jendoubi et al. 2017; Lincoln et al., 2018; Huynh et al., 2018).

The results of the present study suggested the high possibility of introducing significant genetic diversity in chickpea breeding program through pyramiding of diverse genes from different sources to generate novel *Fusarium* wilt resistant genotypes with high yield and yield related morphological traits. The high yielding and novel *Fusarium* wilt resistant genotypes identified in the present study can be utilized in chickpea improvement programs as a novel *Fusarium* wilt resistance sources to develop *Fusarium* wilt resistant varieties with high yield and yield related morphological traits. Additionally, the information generated on the genetic diversity of advanced chickpea breeding lines in Ethiopia helps for the classification of the breeding lines into different heterotic groups in the breeding programs.

CHAPTER FOUR

Genotyping-by-Sequencing Based Single Nucleotide Polymorphisms for Mapping of Novel *Fusarium* Wilt Resistance Quantitative Trait Loci in Chickpea (*Cicer arietinum* L.)

Abstract

Fusarium wilt is a major constraint to chickpea production in Ethiopia. Although traditional breeding efforts have contributed to reducing the effect of wilt disease, introduction of effective resistance into otherwise-preferred varieties and against geographically-disperse pathogen genotypes remains elusive. Genomics assisted breeding has potential to accelerate the introgression and combination of resistance traits into farmer-preferred varieties, but doing so depends on the identification of an effective set of explanatory genetic loci and of tightly linked markers. In the present study, 108 F_2 progeny were obtained by crossing the wilt resistant variety Dera with the wilt susceptible genotype JG62. Disease phenotyping in a *Fusarium* sick plot revealed the expected resistant and susceptible phenotypes of parental controls, while F_2 hybrids segregated for the parental phenotypes wilt responses: fully susceptible and highly resistant. Genotyping-by-sequencing identified 1,659 single nucleotide polymorphisms (SNPs) that distinguish the two parental lines and that segregate with minor allele frequencies > 0.25. After quality filtering, 82 F_2 hybrids and 836 SNP were used for linkage map construction and genetic analysis. Polymorphic markers were assigned to six genetic linkage groups, each corresponding to separate chromosomes, with a total map size of 274.9 cM and 3.12 cM average distance between mapped markers. A single QTL, explaining 55.28 % of the observed phenotypic variation, was identified on chromosome 4 at 44.29 cM with a logarithm of odds (LOD) score of 13.8. The corresponding genomic interval spans 6.3Mb (Ca4_22648775-28990767). This novel major QTL and associated genetic markers offer molecular tools for breeding wilt resistant against endemic Ethiopian *Fusarium* strains.

Keywords: Chickpea, *Fusarium*, Wilt, Genotyping-by-sequencing, QTL, Resistance

4.1. Background and Justification

Fusarium wilt is a major constraint to chickpea production in Ethiopia. Wilt disease management is complicated, especially in the vertisol production system (Rachana 2002). The effectiveness of crop rotation is reduced due both to the long-lived nature of fungal inoculum in soil and the fact that chickpea is an important cash crop, preferred and therefore frequently re-sown, by many farmers. Seed treatment with fungicides can be effective at reducing seed transmission of the pathogen and at promoting early plant establishment, but such measures are beyond the means of most smallholder farmers (Jendoubi et al., 2017). Host genetic resistance to *Fusarium* wilt is a potential solution, but farmer-preferred landraces and/or products lack genetic resistance traits (Gaur et al., 2012).

Polymorphism rates are notoriously low in cultivated chickpea germplasm (von Wettberg et al., 2018), and thus the frequency of molecular markers identified in genetic studies has typically been limiting to trait analysis. However, with the advent of a draft reference genome for chickpea (Varshney et al., 2013a) and low cost genotyping-by-sequencing methods (Elshire et al., 2011), it is feasible to combine polymorphism discovery and genotyping in a single step, and to generate molecular genetic maps with densities in excess of recombination frequencies. Such discovered polymorphisms have applications in modern molecular plant breeding for foreground and background selections (Scheben et al., 2017). However, to apply MAS, identification of best disease resistant sources with significant QTLs regions that confer wilt resistance and molecular markers that tightly linked to the QTLs regions are prerequisite (Sharma et al., 2016).

In Ethiopia, previous studies showed that the dominant races in Debre Zeit Agricultural Research Center sick plot were race 2,3 and race 4 (Shebabu et al., 2008). These races were distributed to major chickpea growing areas of the country threatening chickpea production and productivity (Shebabu et al., 2008). The present study mainly targeted to identify and map QTLs that confer high level of *Fusarium* wilt resistance in chickpea variety Dera. The identified QTL and the molecular markers tightly linked to this QTL will be used to develop wilt resistant varieties through marker assisted disease resistance breeding methods.

4.2. Materials and Methods

4.2.1. Mapping population development

The F_2 hybrids were generated by crossing two highly contrasting parents for *Fusarium* wilt resistance in Ethiopia; Dera and JG 62 as male and female parent respectively. Dera is Fusarium wilt resistant Kabuli type chickpea variety recently released in Ethiopia by EIAR (Ethiopian Institute of Agricultural Research), Debre Zeit chickpea breeding program. Whereas, JG 62 is Fusarium wilt highly susceptible Desi type chickpea originally obtained from ICRISAT and maintained and regularly used as *Fusarium* wilt susceptible check in different countries. From this crossing 108 F_2 hybrids were developed and used to map the Fusarium wilt resistance genes in the resistant variety Dera.

4.2.2. Disease phenotyping

In 2018 cropping season, 238 individuals from two parental lines (Dera and JG-62) and 108 F_2plants were evaluated for *Fusarium* wilt disease resistance in Debre Zeit Agricultural Research Center, *Fusarium* sick plot which is located at 8.40°′N, 38.58°′W. Each individual plant was planted in 1meter long single row with 10 cm

and 30 cm spacing between plants and rows respectively. Disease data was recorded on individual plant on 15, 30, 45 and 60 days after planting. Test genotypes that did not showed any disease symptoms were considered as resistant, whereas those individuals with wilt symptoms were regarded as susceptible.

6.2.3. Genomic DNA extraction

Young leaves from 108 F_2 individuals and two parental lines were collected for DNA extraction from the same plants grown in *Fusarium* wilt sick plot for disease resistance evaluation. The genomic DNA was extracted using a Qiagen DNeasy plant mini Kit from QIAGEN group, CA, USA, according to the manufactures protocol. DNA samples were quantified with standard Pico green and Qubit fluorometer methods and normalized for GBS (Genotyping-by-Sequencing) library preparation.

6.2.4. GBS library preparation and sequencing

Ten nanogram genomic DNA from each sample was restriction digested using Hind-III restriction enzyme. The digested product was ligated with uniquely barcoded adaptors using DNA ligase enzyme to construct the GBS libraries. The GBS libraries were sequenced using Illumina short read technology, on a HiSeq 4000 at Novogene Inc (Sacramento, CA).

6.2.5. Data analysis and QTL mapping

The responses of F_2 individuals to *Fusarium* wilt disease were checked for the expected Mendelian segregation ratios using a chi-squared test. GBS data were used following TASSEL v 4.0 reference based GBS analysis pipeline for genotyping. The chickpea draft genome sequence (Varshney et al., 2013a) was used as reference assembly and good quality reads were aligned and imputation of missing data was

carried out using FSFHap algorithm implemented in TASSEL v4.0. The imputed SNPs markers were further cleaned up by discarding all outliers which were monomorphic between the two parents, any marker with the minor allele frequency of less than 20% and markers that had more than 20 % missing value.

Besides, for each marker locus, observed genotype frequencies were checked for deviations from the expected 1:2:1 Mendelian segregation ratios using a chi-square goodness-of-fit test. After quality screening, 836 high quality SNPs markers were used for genetic map construction and QTL analysis using Rqtl. The map was constructed at LOD 3.0 with Mapdisto mapping function, and phenotyping data together with GBS SNP genotyping data were used for QTL analysis. The visualization of genetic maps was done using the software Map Chart (Voorrips et al., 2002).

4.3. Results

4.3.1. Disease phenotyping in sick plot

The crossing parents, Dera and JG62, performed as expected in sick plot. All Dera plants were 100 % resistant, and all JG62 plants were susceptible to wilt disease (Fig.4.1). Among 108 F_2 hybrids evaluated in sick plot, 44 (40.74 %) and 64 (59.26 %) individuals were resistant and susceptible for wilt disease respectively. After removing the outliers and individual plants with poor GBS data, 82 F_2 hybrids were selected for linkage mapping and QTL analysis. Among these, disease phenotyping showed that 42 and 40 F_2 hybrids were resistant and susceptible for wilt disease respectively. Chi-square analysis with 108 test individuals and selected 82 F_2 hybrids

showed significant deviation (*P<0.05*) from the expected 3:1 mendelian segregation ratio (Table 4.1).

Table.4.1. Chi square analysis for F_2 hybrids performance in *Fusarium* sick plot

Number of F_2 hybrids	Resistant	Susceptible	Total	χ^2 (3:1)
Phenotyped	44	64	108	67.6*
Selected for mapping	42	40	82	24.73*

**Significant deviation from the expected Mendelian segregation ratio*

4.3.2. Genotyping and SNP marker development

The two parental linesand all F_2 hybrids were sequenced sufficiently in a lane with about 833M reads, which is higher than normal. After quality filtered and outliers removed, from a total 1,939 SNPs markers, 1,659 SNPs with minor allelic frequency (MAF) > 0.25 were identified as markers distinguishing the two parental lines, Dera and JG62 (Fig.4.1).

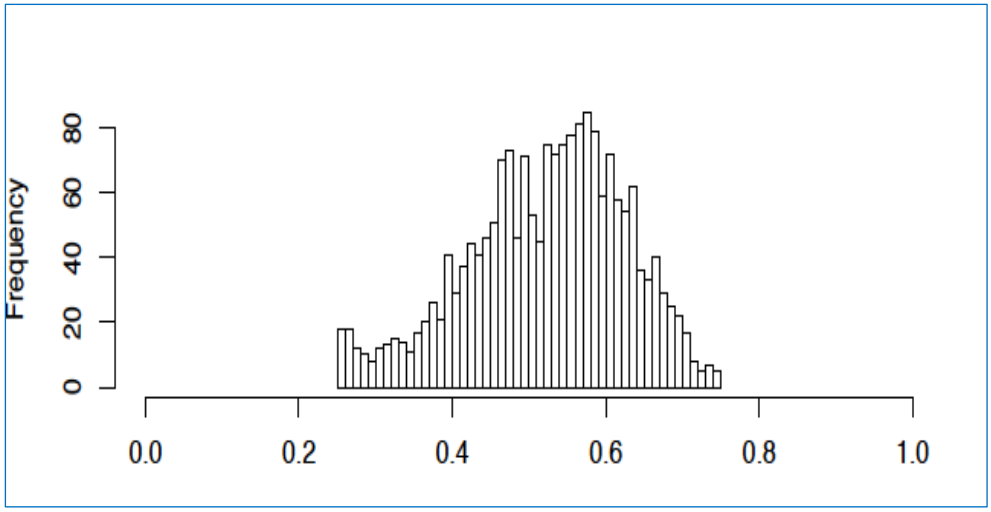


Fig. 4.1. Allele frequency with 1,659 imputed SNPs markers with minor allelic frequmcy (MAF) > 0.25

After quality filtered and outliers removed, the principal component analysis (PCA) based on 1,659 SNPs markers confirmed that a typical characteristic of a biparental hybrid F_2 mapping population (Fig. 4.2). In PCA, the two parental lines Dera and JG-62 were at the extreme with the F_2 hybrid scattered between them as expected.

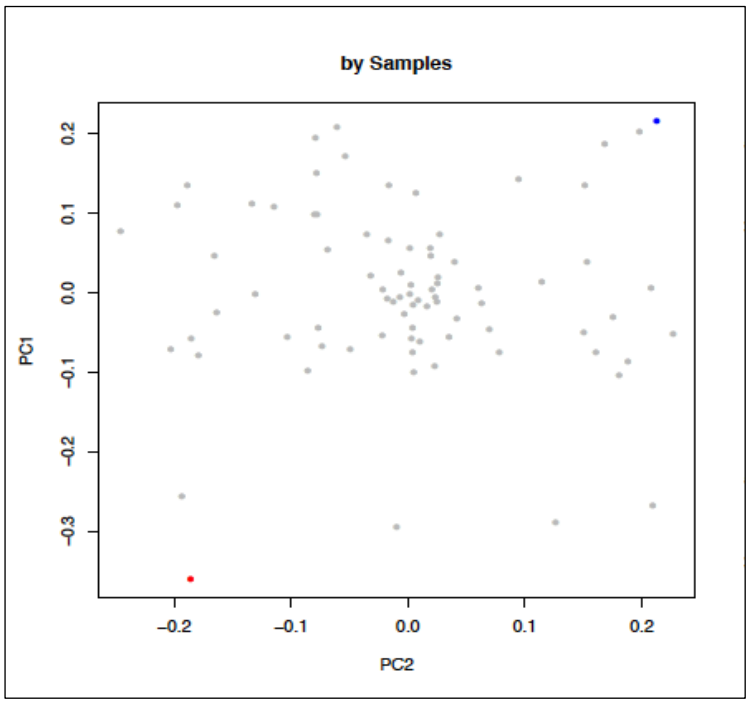


Fig.4.2. PCA analysis showing Dera in blue and JG-62 in red dot at the extreme, with the F_2hybrid samples in grey dot scattered between them.

The imputed SNPs markers were further cleaned up by discarding all outliers which were monomorphic between the two parental lines and markers that had more than 20 % missing value. Finally, a total of 836 high quality marker loci polymorphic between the two parental lines were used to construct genetic linkage map and QTL analysis.

4.3.3. Genetic linkage map construction

A total of 82 F_2 hybrids with 836 high quality SNP markers were used to construct genetic linkage map (Fig.4.3). The result showed that all markers were aligned on 6 linkage groups with a total map size of 274.9 cM, and with 3.12 cM average distance

between mapped markers (Table 4.2). The number of markers aligned per chromosome varies from 17 markers on chromosome 5 to 445 markers on chromosome 4 (Table 4.2). Therefore, chromosome 4 was the most polymorphic linkage group aligned with the largest number of SNPs markers (445).

The largest LG-1 contained 138 SNP markers and was 86.7 cM long, whereas the smallest LG-3 contained only 34 SNP markers and was 13.96 cM long. Chi-square analysis for the SNP markers in all chromosomes showed a good fit to the expected 1:2:1Mendelian segregation ratio (*df =2, p >0.05*). However, few aligned markers on chromosome 4 showed significant deviation from the expected segregation ratio (1:2:1 ratio) for F_2 hybrids (Table 4.3).

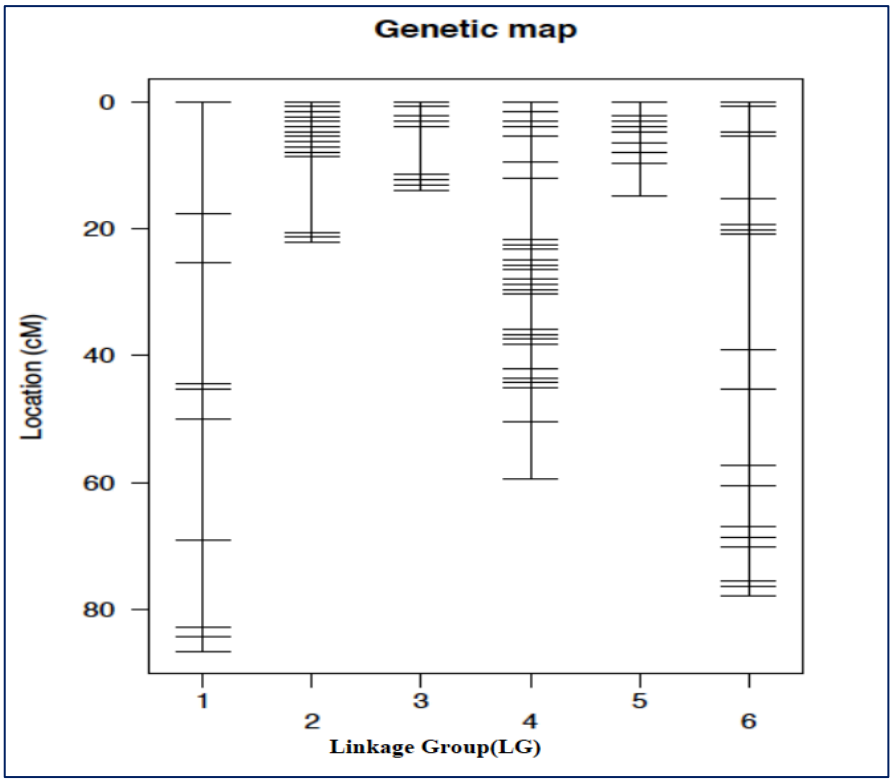


Fig. 4.3. Genetic/linkage map based on 836 high quality polymorphic SNP markers

Table 4.2. Linkage groups, number of aligned and mapped markers and average distance between mapped marker (cM) for each chromosome

Linkage group (Chromosome)	Markers aligned	Mapped markers	LG length (cM)	Distance between markers(cM)
LG-1	138	10	86.7cM	8.67
LG-2	78	15	22.16cM	1.48
LG-3	34	9	13.96cM	1.55
LG-4	445	27	59.37cM	2.20
LG-5	17	9	14.86cM	1.65
LG-6	124	18	77.85cM	4.33
Total	836	88	274.9cM	3.12

Table 4. 3. Segregation of mapped markers on chromosome 4 using 82 F2 individuals

Markers name	Position(cM)	χ^2 (1:2:1)
Ca4_3321643	0.00	0.13
Ca4_4139673	1.58	0.83
Ca4_4489791	3.15	2.25
Ca4_4911083	3.94	3.37
Ca4_5073318	5.48	5.32
Ca4_7684536	9.60	4.16
Ca4_8224176	12.05	5.32
Ca4_11218306	21.80	8.57*
Ca4_11346333	22.56	5.08
Ca4_12596626	23.34	5.38
Ca4_13588193	24.96	4.22
Ca4_13636367	25.72	4.37
Ca4_13593553	26.48	4.04
Ca4_13694702	28.03	5.11
Ca4_13830485	28.79	5.24
Ca4_14733816	29.57	3.07
Ca4_14865246	30.35	1.99
Ca4_16891814	35.97	8.38*
Ca4_17623284	36.72	10.13*
Ca4_17718536	37.47	8.33*
Ca4_17748985	38.22	8.21*
Ca4_20896684	42.01	13.95*
Ca4_22648775	43.54	10.13*
Ca4_28990767	44.29	13.95*
Sca242_398952	45.14	15.00*
Sca242_311328	50.52	25.57*
Sca398_350391	59.37	14.16*

**Markers showed significant deviation from the expected mendelian segregation ratio*

4.3.4. QTL analysis for *Fusarium* wilt resistance

In this study, one major QTL was identified for *Fusarium* wilt resistance in chickpea explaining 55.28 % of the observed phenotypic variation (PVE) (Fig 4.4 and Table 4.4). This large effect QTL was detected on chromosome 4 (LG-4) flanked by Ca4_22648775 (left) and Ca4_28990767 (right), spanning the genomic interval of 6.34 Mbp which good for small number of population. It is located close to the right flanking marker Ca4_28990767, at peak QTL marker position of 44.29 cM and LOD score of 13.8 (Table 4.4, Fig.4.4 and Fig.4.5).

The newly identified QTL is named as 'qFWLG4-1' following conventional nomenclature for newly identified QTL, with the initial letter 'q' representing 'QTL', followed by the trait name *Fusarium* wilt resistance (FW), linkage group (LG4) and a numeric number indicating the number of QTL identified on the same LG-4.

Table 4.4. Flanking markers for the detected QTL, peak position, LOD score, additive and dominanteffect and phenotypic variance explained.

Flanking markers	Position (cM)	LOD score	Additive effect	Dominant effect	PVE* (%)
Left: Ca4_22648775	43.54				
Right:Ca4_28990767	44.29				
Close:Ca4_28990767	44.29	13.8	49.9	12.4	55.28

**Phenotypic variance explained*

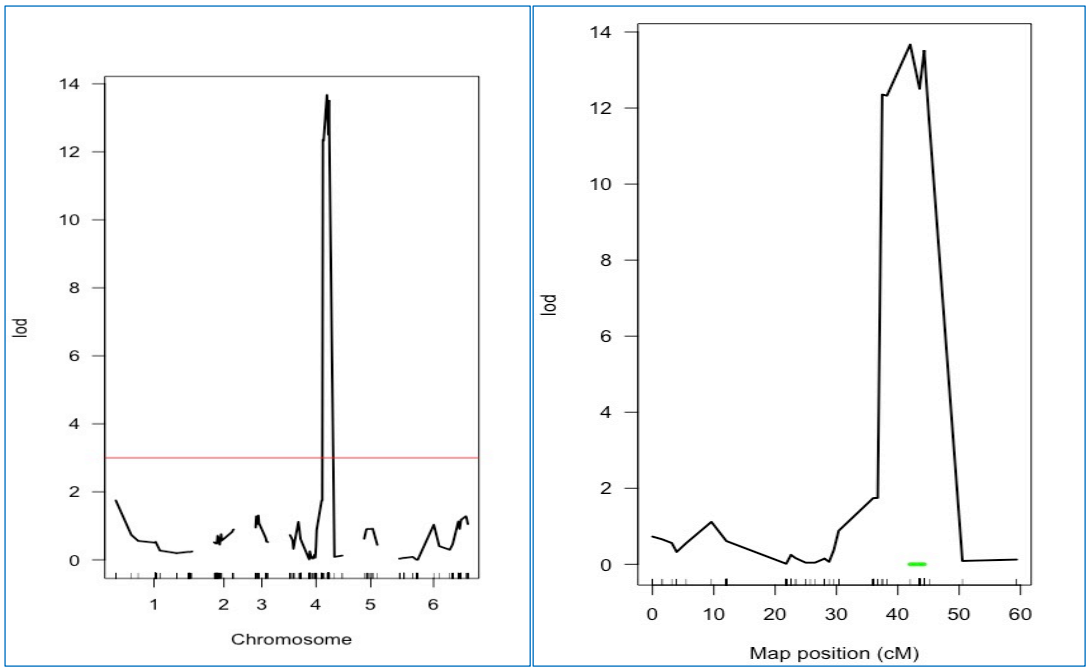


Fig.4.4. QTL detected on chromosome 4 for *Fusarium* wilt resistance in chickpea and map position (cM)

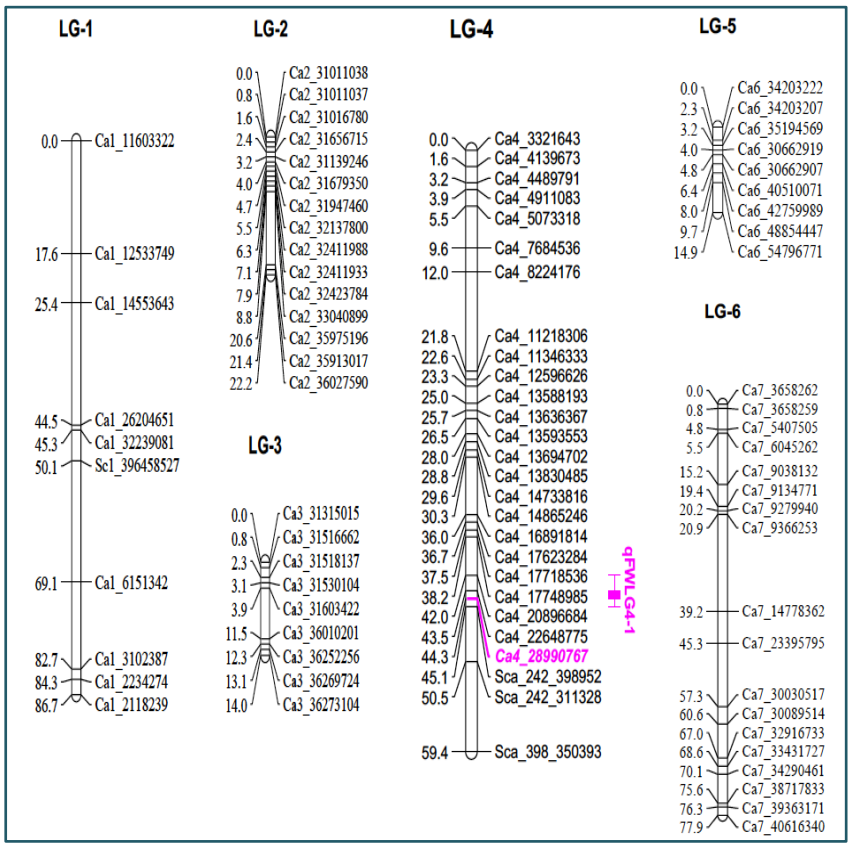


Fig.4.5. Linkage map and chromosomal position of QTL detected for Fusarium wilt disease in chickpea

At the marker locus where the significant QTL is detected (Ca4_28990767), the F_2 hybrids showed unusual mendelian segregation ratio of 1:2:2 (*AA = 15; AB = 35 and BB = 32)* (Fig.4.6). At this marker locus, all AA homozygote individuals were susceptible, and BB homozygote individuals were resistant to *Fusarium* wilt of chickpea. However, at this locus, from a total of 35 F_2 heterozygote individuals (AB), 12 (34.3 %) were resistant and 23 (65.7 %) were susceptible. At all marker locus, the susceptable parent (JG 62) was homozygous AA, and the resistant parent Dere was homozygous BB.

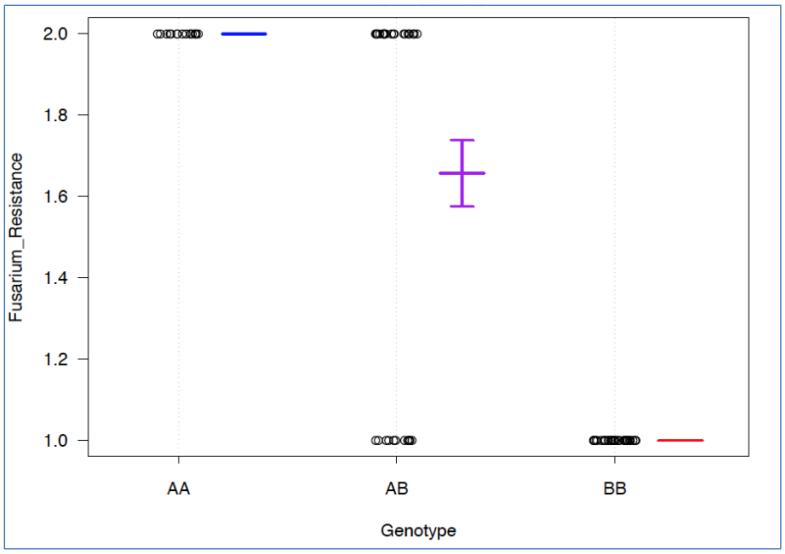


Fig.**4.6**. Genotypic ratio for*Fusarium* wilt resistanceat the marker locus (Ca4_28990767).
AA= JG62, BB= Dera, AB= F_2 hybrid, and Resistant = 1, Susceptible = 2,

4.4. Discussion

Recently with the availability of different genomic resources in chickpea (Garg et al., 2018; Varshney et al., 2012; Kujur et al., 2015), genomics-assisted disease resistance breeding which combine genome information with the trait phenotyping data is becoming the most effective approach to accelerate the development of disease resistant varieties (Saxena et al., 2016).

In the present study, the newly identified major QTL which explained 55.28 % of the observed phenotypic variation is a novel QTL for *Fusarium* wilt resistance in chickpea reported for the first time on chromosome 4 closely associated with Ca4_28990767 SNP marker at 44.29 cM using Kabuli type chickpea (Dera) as a resistant parent. Almost all previous studies used WR 315 (Desi type chickpea) as resistant parent (Sharma et al., 2009; Gowda et al., 2009; Sabbavarapu et al., 2013) and significant QTLs were reported on only LG02, LG05 and LG06 (Jendoubi et al., 2017). These suggested that the new QTL identified might be due to the new wilt resistant Kabuli type chickpea used in the present study.

The identified major effect QTL is an important target to facilitate the discovery of candidate wilt resistance genes in chickpea to develop wilt resistant variety through marker assisted selection. It can be transferred to different genetic backgrounds of users preferred and widely cultivated wilt susceptible chickpea varieties through marker assisted backcrossing (MABC) or marker assisted recurrent selection (MARS) in chickpea breeding programs. To further delineate the locus and fully understand the gene responsible for wilt resistance in Dera, it is recommended to get sufficient recombination. In the present study, the small population size limited to get sufficient recombination to delineate the locus. As a result, for this QTL the genomic interval spans 6.3Mb (Ca4_22648775-28990767). Genetic distances among markers are dependent on sufficient chromosome recombination which requires a large population size and precise disease phenotyping (Han et al., 2018; Ohlson et al., 2018; Chu et al., 2019). Therefore, further investigations and fine mapping with a larger population and high density markers are very important to effectively apply the present finding in different chickpea breeding programs.

CHAPTER FIVE

Genomic Diversity and Geographic Distribution of *Fusarium* Wilt Pathogen of Chickpea (*Fusarium oxysporum* f. sp. *ciceris*) in Ethiopia

Abstract

Foc is one of the most dominant pathogen critically challenging chickpea production and productivity in Ethiopia. Chickpea breeding for *Fusarium* wilt resistance regularly challengedwith high pathogenic variability and the existence of different pathogenic races. However, so far only few efforts have been made to understand the genetic diversity and geographic distribution of *Fusarium oxysporium ciceris* (Foc) isolates for designing effective country wide breeding strategies. Therefore, in the present study, a total of 217 Foc isolates were collected from diverse chickpea growing areas of the country to study the genomic diversity and geographical distribution of the pathogen. Among these, a total of 166 representative isolates were selected and sequenced using whole genome sequencing (WGS) with Illumina HiSeq 4000 platform. Based on diversity group, regions of origin and sequence qualities, 27 representative *Fusarium* isolates were selected and used for the pathogenicity test. The result indicated the presence of high genetic diversity and pathogenic variability between *Fusarium oxysporium* isolates in Ethiopia. Nei's genetic diversityanalysis based on 196, 495 single nucleotide polymorphic (SNPs) markers split test isolates into 20 distinct clusters irrespective of their regions of origin and geographical location. Among these,16 distinct clusters were *Fusarium oxysporium* isolates. Similarly, phylogenetic analysis based on 1,052 highly conserved BUSCO genes divided test isolates into six distinct *Fusarium* species. Consistent with this result, pairwise average nucleotide identity (ANI) analysis based on 3,695 highly conserved BUSCO genes split test isolates into six distinct *Fusarium* species, using 95 % ANI (ANI_{95}) as the lower species boundary. Mantel correlation estimate showed very poor correlation between geographical and genetic distances of *Fusarium* isolates in Ethiopia with $P = 0.280$ and $R^2 = 0.0006$. The pathogenicity test also confirmed the existence of high pathogenic variability among Foc isolates irrespective of their geographical origin.Taken together, the results of the present study suggest, despite the presence of high genetic diversity and pathogenic variability among Foc isolates in Ethiopia, closely related isolates were widely distributed throughout chickpea growing areas of the country irrespective of their geographical origin. Therefore, designing effective and country wide breeding strategies against all Foc pathogensis indispensable to break the recurrent disease cycle in the country.

Key words: ANI, BUSCO, Chickpea, *Fusarium*, WGS, SNPs, Diversity, Pathogenicity

5.1. Background and Justification

Foc pathogen is both seed and soil borne in nature. Once the inoculum is established in the soil, it is too difficult to eradicate as the chlamydospores survive in the soil for at least 6 years (Haware et al., 1996; Singh et al., 2007). During these periods, it passes through different environmental stresses and biological competition, which lead to the existence of variations. As a result, Foc exhibits great genetic diversity. So far it has been classified into two pathotypes and eight physiological races (Jimenez Gasco et al., 2001, 2004; Dubey et al., 2012).

Use of resistant varieties is one of the most practical and cost effective strategy for control and management of *Fusarium* wilt disease in chickpea. However, chickpea breeding for *Fusarium* wilt resistance regularly challengedwith high pathogenic variability and the existence of different physiological races (Jimenez Gasco et al., 2004b; Bayraktar and Dolar, 2012; Sharma et al., 2014). In different countries molecular markers, particularly SSR and ISSR (Dubey and Singh, 2008), AFLP (Sharma et al. 2009), RFLP (Barve et al, 2001) and RAPD (Honnareddy and Dubey, 2006) were used for identification of the genetic diversity among Foc isolates.

In Ethiopia, no efforts have been made to understand the genetic diversity and geographic distribution of Foc pathogens (Yimer, 2018). Detailed studies and characterization of Ethiopian Foc isolates is essential to design effective disease resistance breeding strategies and develop efficient country wide integrated disease management measures. Therefore, this study was mainly designed to understand the genetic diversity, geographical distribution and pathogenic variability of Foc isolates in Ethiopia.

5.2. Materials and Methods

5.2.1. Description of the study areas

This study was conducted inmost chickpea producing agro-ecological zones of Amhara, Oromiya, Tigray and SNNP regions in Ethiopia (Fig 5.1). These areas are geographically located in latitude 6.91'82.3''to14.90'66.1''(N) and longitude 36.92'37.1''to 42.99'03.8''(E) with altitude ranging from 1,484 to 2,913 meter above sea level (Appendix 1 and Table 5.1). In these areas, the average annual rainfall ranges from 800 mm to 2000 mm and chickpea is produced once per year rainfed condition. The dominant soil types in farmers' fields were heavy black soil (50.29 %) followed by light soil and light black soil with 27.56 % and 22.14 % respectively. Depending on the onset of rain and soil types, chickpea planting and harvesting time significantly varies from place to place. Generally, in these areas chickpea is mainly planted from middle of August to beginning of October, and harvested between November and end of January.

5.2.2. Sample collection

In 2015/16 cropping seasons, diseased chickpea plants with typical *Fusarium* wilt symptoms and other associated data including soil, seed samples and GPS coordinate were collected. Based on the extent of disease pressure in farmers field and the importance of chickpea crop in each zone and districts, a total of 217 samples were collected from 62 major chickpea growing districts located in 19 diverse agroecological zones of Ethiopia (Table 5.1 and Appendix.1).

Table 5.1. Summary of sample collection areas, altitudinal ranges and number of samples collected

Region	Ecological Zones	Number of Districts	Altitudinal range	Number of samples
Oromiya	East Shewa	5	1978-2556	27
	South West Shewa	7	1787-2421	28
	West Harerge	3	1714-2340	15
	East Harerge	4	1978-2556	13
	Arsi	2	2304-2595	12
	Bale	4	1996-2611	8
Amhara	North Shewa	6	2479-2913	24
	North Gonder	4	1810-2180	15
	South Gonder	2	1800-2766	5
	West Gojam	2	1802-2082	9
	East Gojam	2	1822-2457	6
	North Wollo	3	1784-1914	7
Tigray	Axum	3	1842-2202	11
	East Tigray	3	2233-2453	3
	West Tigray	4	1673-2626	17
SNNPR	Wolayita	2	1712-2071	7
	Gurage	2	1796-2142	6
	Hadiya	2	1892-1935	2
	Silte	2	2037-2076	2
Total	19	62	1673-2913	217

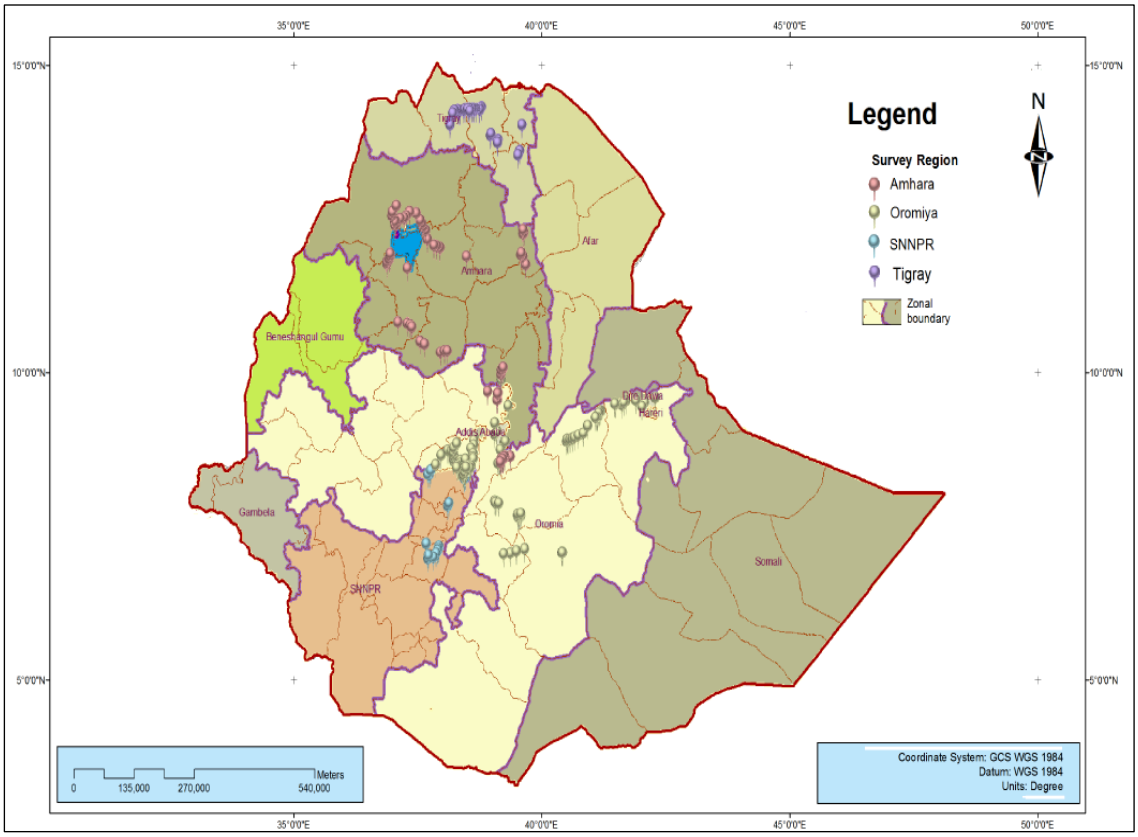


Fig.5.1. Sample collection regions and zones in Ethiopia

5.2.3. Foc strain isolation and purification

Ten pieces of diseased plant tissue were taken from the collar region of each sample and the remaining part was split lengthwise and checked for browning of vascular tissue (vascular discoloration) to confirm *Fusarium* wilting. Tissues of diseased plants taken from plants with clear vascular discoloration were surface disinfected with 10 % sodium hypochlorite solution for 2 to 3 minutes, rinsed extensively with sterile distilled water, and dried on filter paper and plated on sterilized potato dextrose agar (PDA) in 90 mm diameter Petri dishes. The plates were incubated at 25.8^{o}C for one week. After incubation, a single colony was cut from well separated colonies and transferred to a Petri dish containing solidified potato dextrose agar for further purification and Foc isolates identification.

5.2.4. Genomic DNA extraction

Well purified mycelium was grown on fresh PDA media for 7 to 8 days and conidia harvested for genomic DNA extraction. The harvested conidia passed through two layers of sterilized mira cloth and washed twice with sterilized distilled water. After the second wash, the conidial suspension was centrifuged at 8000 rpm for 15 min. By removing the supernatant, the pelleted spores were freezed at-80^{o}C overnight and dried by lyophilization.

From each sample, 250 mg to 500 mg lyophilized conidia were thoroughly grinded in liquid nitrogen 2 to 3 times for a total of 5 minutes. Genomic DNA was extracted using a modified phenol chloroform protocol. In this protocol, Sodium 4-aminosalicylate dehydrate, Triisopropyl naphthalene Sulfonic Acid Sodium and RNase were used to prepare the DNA extraction buffer, and proteinase K, Phenol Chloroform Isoamyl alcohol, Iso-Propyl Alcohol and NaCl were also used in the subsequent DNA extraction processes. The extracted genomic DNA cleaned under magnet by adding 0.45X AMpure bead to the DNA, and subsequently washing with 80 % ethanol twice. The final DNA was resuspended in 60μl EB buffer, and quantified with Nano drop, Pico green and Qubit. After repeated DNA extraction and quality checks, high quality gDNA samples were normalized to 6ng/μl for genomic library preparation.

5.2.5. Library preparation and whole genome sequencing

From the total of 217 samples collected in this study, 166 representative *Fusarium* isolates were prioritized and selected for whole genome sequencing. These isolates were selected mainly based on their regions of origin and good representativeness to

differentagro-ecological zones of the country (Appendix2). Genomic libraries were constructed for 166 isolates using QIAGEN, QIA Seq FX DNA library kit according to the manufactures protocol. First the gDNA fragmentation reaction time was optimized to 10 minutes with an input DNA of 100 ng to generate a fragment size between 400 to 600bpfor whole genome sequencing (Fig.5.2). Then, the genomic DNA was enzymatically sheared into smaller fragments, and fragmented DNA directly end-repaired and an 'A' was added to the 3' ends in the same tube following enzymatic shearing, making the DNA fragments ready for ligation. Following this step, specific adapters were ligated to both ends of the DNA fragments for sequencing.

The quality of the libraries were assessed using high sensitivity Agilent Bio Analyzer and the library DNA fragment distribution were between 400 to 600bp (Fig.5.3). Sequencing was performed on high quality genomic library using Illumina HiSeq 4000 platform at the University of California (UC) Davis, Genome Center.

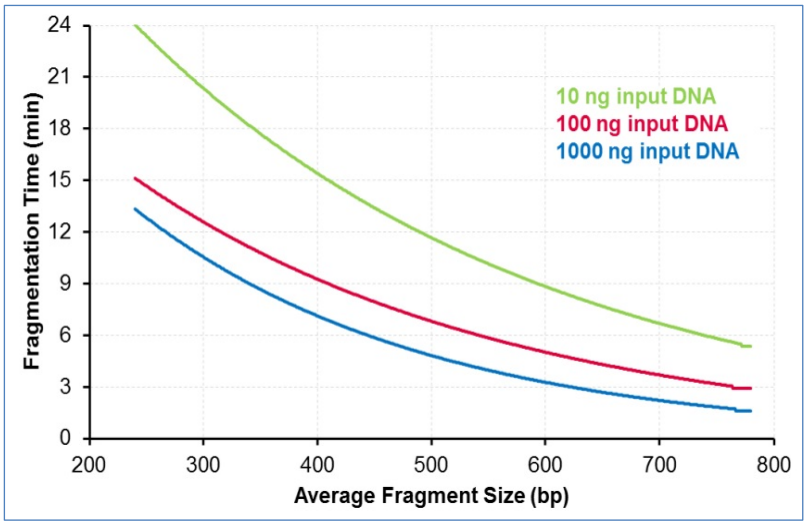


Fig.5.2. Fragmentation profile of different amounts of input DNA during genomic librarypreparation

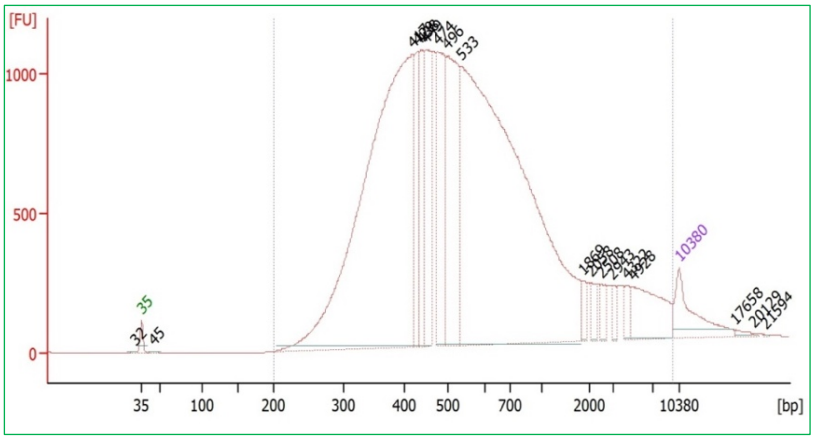


Fig 5.3. Pooled library fragmented DNA distribution of 166 Foc isolates prepared for whole genome sequencing

5.2.6. Pathogenicity test

Based on Nei's genetic diversity, geographical origin and sequence qualities (genome completeness and reasonable genome size), 27 representative *Fusarium* isolates were selected for the pathogenicity test (Table 5.2). Each Nei's cluster group was represented at least with one isolate. The wilt susceptible check (JG-62) and the resistant check (WR-315) were usedin the pathogenicity test to validate virulence level of each isolate. Two uninoculated checks with JG-62 and WR-315 genotypes were used as negative control. Atotal of 56 treatment combinations (27 test isolates combined with susceptible check JG-62 and resistant check WR-315, and two uninoculated negative controls) were used in this study.

This experiment was conducted from 2018 to 2019 at Debre Zeit Agricultural Research Center screen house using RCBD design with 4 replications. The standard *Fusarium* wilt pathogen sick pot screening techniques described by Pande et al. (2012) was used for virulence assay with some minor modifications asfollows. Sand maize meal medium was prepared by placing 100 g of riverbed sand, 200 g of maize meal and 100ml of distilled water in 500 ml Erlenmeyer flask. The medium was

autoclaved in the flasks at 121ºC for 20 minutes, and each flask was inoculated with small actively growing fungal culture and incubated at 25°C for 15 days. A fungus soil mixture was prepared by hand mixing contents of each flask with 5kg of autoclaved field soil under hygienic conditions.

A 25 cm diameter pots were filled with the inoculated 5kg soil, and each pot was watered and waited for 5 days. As per the experimental design,10 clean and healthy seeds of a highly susceptible or resistant genotypes were sown in different pots at 2-3 cm depth. Besides, the two uninoculated checks (negative controls) were filled with 5kg of autoclaved field soil and planted with JG-62 and WR-315 genotypes for comparison. Data on number of total plant emerged, healthy, diseased and dead plants were scored 10, 30, 45 and 60 days after planting.

5.2.7. Data analysis

Quality of the sequence data were assessed using Fast QC for per base and sequence quality score, per base GC content, per sequence GC content and sequence length distribution. Low quality reads and adapterwere trimmed using Trimmomatic program, anderror correction was performed using ALLPATHS-LG program. Draft genome assemblies were obtained using the A5 pipeline, initially using *Fusarium oxysporum* f. sp. *lycopersici* strain FOL4287 as a reference genome, and later detailed genomic analysis of each strain serve as a standalone entity, without an external reference. The genomic data has been deposited to the National Center for Biotechnology Information (NCBI) under Bio Project PRJNA412392. The quality of predicted gene calls from the genome assembly was assessed based on 3, 695 highly conserved Benchmarking Universal Single Copy Orthologs (BUSCO) genes with BUSCO-v3 software using sordariomyceta_odb9 database (Simao et al., 2015).

Nei's genetic distance matric generated using the SNP related package in R and Molecular Evolutionary Genetic Analysis (MEGA) version X (Kumar, Stecher, Li, Knyaz, and Tamura 2018). The phylogenetic relationship between different *Fusarium*isolates was generated based on genetic distance (SNP's) and 1,052 conserved BUSCO genes using Bayesian Evolutionary Analysis of Sample Tree (BEAST v1.10.4) software programs respectively.

The robustness of the tree topology was estimated from bootstrap value analysis with 1,000 replications. Average nucleotide identity (ANI) algorithm using BLAST (ANIb) and PYANI soft ware were used to generate ANI diversity groups and heatmap. A total of 3,695 highly conserved BUSCO genes that are common to *Fusarium* species were used to generate Average Nucleotide Identity (ANIb) between Foc isolates. In this analysis, the genome of each strain was aligned to the genome of every other strain, and the nucleotide identity for each pairwise comparison gives a measure of genomic distance between strains.

Principal coordinate analysis (PCoA) via distance matrix with data standardization was performed using GenAlEx 6.51b2.xlam software program. Mantel test was performed to estimate a correlation between the matrices of Nei's genetic distance and geographical distances using GenAlEx 6.51b2.xlam software program. Wilt incidence was computed using the standard formula suggested by Pande et al. (2012), and according to Sharma et al. (2014), the disease reactions was graded as resistant, moderately susceptible, susceptible and highly susceptible with wilt incidence of 0-20 %, 20-50 %, 50-80 % and 80-100 % respectively.

Based on the virulence data on susceptible and resistant checks, cluster analysis was performed using hierarchical clustering with the unweighted pair group average method (UPGMA) with DARwin (Dissimilarity Analysis Representation for window) computer program software Version 6 (http://darwin.cirad.fr).

Table 5.2. Lists of *Fusarium* isolates used in pathogenicity test and their estimated genome size, Nei's diversity group and regions of origins

Foc isolates	Genome size (Mbp)	Nei's diversity group	Geographical origin	
			Region	Sites
EtdFoc-55	54.84	Cluster -1	Oromiya	Doyokora
EthFoc-4	53.21	Cluster -3	Oromiya	Tilitigerbi
EtdFoc-13	53.33	Cluster -5	Oromiya	Ifabelem
EtdFoc-223	53.59	Cluster -7	Oromiya	Soyoma
EtdFoc-5	52.21	Cluster -13	Oromiya	Metokema
EtdFoc-160	54.36	Cluster -16	Oromiya	Gichie
EthFoc-22	53.43	Cluster -19	Oromiya	Ambelta
EthFoc-23	54.22	Cluster -19	Oromiya	Habrumiti
EthFoc-13	53.22	Cluster -3	Amhara	Bosoke
EtdFoc-88	52.78	Cluster -4	Amhara	Golashkan
EtdFoc-234	52.24	Cluster -6	Amhara	Norauda
EtdFoc-236	53.92	Cluster -6	Amhara	Campas
EthFoc-127	52.18	Cluster -10	Amhara	Wencher
EthFoc-118	53.41	Cluster -11	Amhara	Wejidewel
EthFoc-11	52.46	Cluster -12	Amhara	Samasenbet
EthFoc-12	52.87	Cluster -14	Amhara	Senbete
EtdFoc-186	59.30	Cluster -17	Amhara	Dingayqulqul
EthFoc-133	42.71	Cluster -20	Amhara	Zema
EtdFoc-258	51.40	Cluster -5	Tigray	Muhubal
EtdFoc-191	58.17	Cluster -8	Tigray	Kaka
EtdFoc-204	60.52	Cluster -15	Tigray	Madirash
EtdFoc-216	52.48	Cluster -16	Tigray	Maytsede
EtdFoc-208	57.04	Cluster -18	Tigray	Selam
EthFoc-078	51.62	Singletons	Tigray	Kushet
EtdFoc-220	58.20	Cluster -9	SNNPR	Badewchi
EtdFoc-218	53.03	Singleton	SNNPR	Ketefo
EtdFoc-72	53.95	Singleton	SNNPR	Adoch

Table.5.3. *Fusarium oxysporum* reference strains used in the present study and their genome size and BUSCO genes statistics

Strain	Hosts	Genome Size(Mb)	BUSCO genes summary			
			Duplicate	Single	Intact	% Present
USDA-Foc0	Chickpea	57.7	28	3647	3675	99.50
USDA-Foc1	Chickpea	58.0	34	3650	3684	99.7
USDA-Foc2	Chickpea	58.2	33	3624	3657	99.0
USDA-Foc3	Chickpea	58.1	31	3654	3685	99.7
USDA-Foc4	Chickpea	58.3	28	3653	3681	99.6
USDA-Foc5	Chickpea	58.3	31	3655	3686	99.8
Foc 381	Chickpea	54.8	23	3654	3677	99.51
Fo5176	Brassica	54.9	32	3621	3653	98.86
PHW808	Brassica	53.5	29	3653	3682	99.65
PHW815	Brassica	53.4	34	3653	3687	99.78
HDV247	Pisum	55.1	27	3652	3679	99.57
FOL 4287	Tomato	61.3	33	3560	3593	97.24
MN25	Tomato	48.6	22	3663	3685	99.81
CL57	Tomato	49.3	22	3661	3683	99.68
Melonis	Melon	54.0	28	3653	3681	99.62
NRRL32931	Human	47.9	20	3662	3682	99.65
Fo47	Soil	49.6	24	3660	3684	99.70
Cotton	Cotton	52.9	29	3658	3687	99.78
II5	Musa	46.5	13	3671	3684	99.70

5.3. Results

5.3.1. Whole genome sequencing of *Fusarium* isolates

Whole genome sequencing and genome assemblies of 166 *Fusarium* isolates resulted in 12,151,247 average raw reads and 9,372 scaffolds (Table 5.4A). This provides average draft genome size of 43.45Mbp. However, based on sequence qualities particularly based on genome completeness and reasonable genome size, a total of 128 *Fusarium* isolates, deposited to the NCBI data base under Bio Project PRJNA412392, were selected for further analyses. These isolates with average row reads of 27,459,890 were assembled in to 3,334 scaffolds with average draft genome size of 54.84 Mbp and 62.23X sequence coverage (Table 5.4A).

Benchmarking Universal Single Copy Orthologs (BUSCO) gene analysis showed that from a total of 3,695 highly conserved BUSCO genes that are common to *Fusarium species*, in average 3,641.11 genes representing 98.54 % were intact and present in 128 *Fusarium* isolates (Table 5.4B). This provides the high quality and integrity of the predicted BUSCO genes in the present study, and it is very comparable with genome statistics of selected reference strains (Table 5.3).

Table 5.4. Summaries of genome statistics and BUSCO genes for 166 and 128 *Fusarium* isolates

A. Genome assembly statistics

Number of isolates	Number of row reads	Draft genome size (Mbp)	N50	Scaffolds	Sequence coverage
166	12,151,247	43.45	162,004.4	9,372	33.25
128	27,459,890	54.84	162,719.1	3,334	62.23

B. BUSCO genes summaries

Isolates	Fragments	Duplicates	Singles	Intact	% Present
166	243.71	56.30	2,198.33	2,254.63	61.02
128	30.77	25.40	3,615.71	3,641.11	98.54

5.3.2. Genetic relationship of Foc isolates in Ethiopia

Nei's genetic distance analysis based on 196,495 SNPs markers generated from whole genome sequencing split 128 *Fusarium* isolates into 20 distinct clusters, and some isolates were separated alone as singletons (Fig.5.4 and Table 5.5). The number of isolates in each cluster ranged from 2 to 11. *Fusarium oxysporium* isolates in the first 16 clusters (fromcluster 1 to 16), most isolates that separated alone as singletonand all reference strainsused in the present study (*Fusarium oxysporium strains)* were closely related (Fig.5.4). However, *Fusarium* isolates in cluster 17,18, 19 and 20 were distantly out grouped from *Fusarium oxysporium* isolates. Phylogenetic and ANI

analyses assigned these isolates to different *Fusarium* species associated with the disease (Table 5.6).

Fusarium isolates collected from the same agroecological zones were assigned to different clusters irrespective of their regions of origin (Fig.5.4, Table 5.5 and Appendix x 1). Most reference strains from different forma speciales were clustered separately as singletons (Fig.5.4). For instance, Fo5176, PHW815, HDV247, NRRL32931, Cotton and II5 were clustered alone as singleton. Two reference strains from tomato (*Lycopersicum*) (CL57 and MN25) and one strain from soil (Fo47) grouped together. However, a single strain from Brassica (PHW808) was grouped in cluster-2 along with the Foc reference strains from USDA.

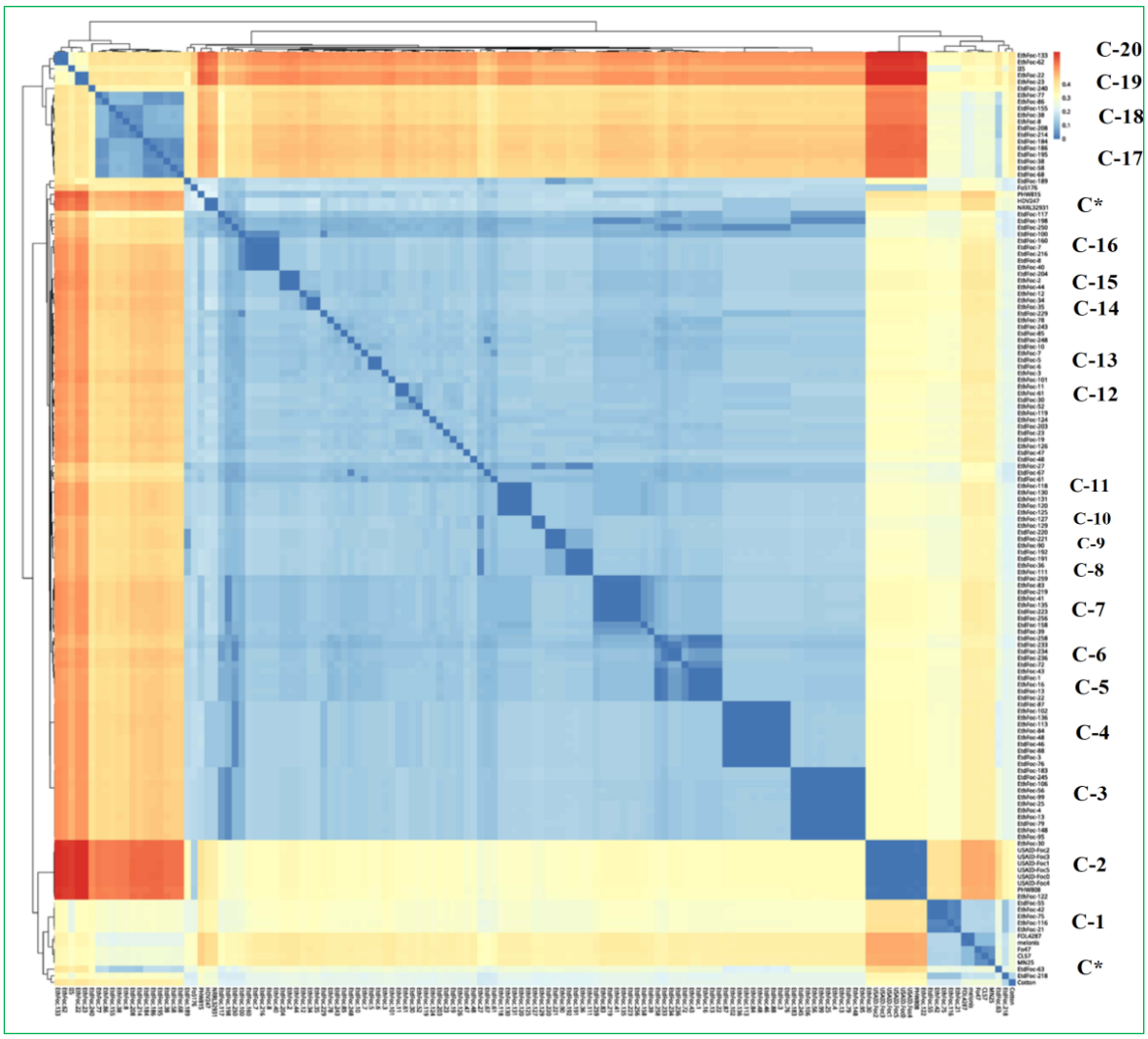


Fig. 5.4. Heatmap of Nei's genetic distance based on 196, 495 SNPs for 128 *Fusarium* isolates and 12 reference strains.

C- represent cluster;and C indicates clusters ofreference strains*

In the heatmap,isolates with zero(0)genetic distance are shown with blue cells, and color intensity fades as the genetic distance between isolates approach 0.1. Red cells correspond to genetic distanceabove 0.4.

Table 5.5. Lists of 128 Ethiopian *Fusarium* isolates in Nei's clusters generated based on whole genome SNPs

Nei's clusters	Number of isolates ID	*Fusarium* isolates
Cluster-1	5	EtdFoc-55,EthFoc-21,EthFoc-42,EthFoc-75 and EthFoc-116
Cluster-2	8	USDA-Foc 0, USDA-Foc 1, USDA-Foc 2, USDA-Foc 3,USDA-Foc 4, USDA-Foc 5, EthFoc-122 and EthFoc-30
Cluster-3	11	EthFoc-4, EthFoc-25, EthFoc-13, EthFoc-56, EtdFoc-79, EtdFoc-245,EthFoc-95, EthFoc-99, EthFoc106, EthFoc-148 and EtdFoc-183
Cluster-4	10	EtdFoc-3, EtdFoc-46, EtdFoc-76, EtdFoc-87, EtdFoc-88,EthFoc-102, EthFoc-113, EthFoc-136, EthFoc-48 and EthFoc-84
Cluster-5	6	EtdFoc-1, EtdFoc-13, EthFoc-16, EtdFoc-22, EthFoc-43and EtdFoc-258
Cluster-6	2	EtdFoc-234 and EtdFoc-236
Cluster-7	7	EthFoc-41, EthFoc-83, EthFoc-135, EtdFoc-219, EtdFoc-223, EtdFoc-256 and EtdFoc-259
Cluster-8	4	EthFoc-36, EthFoc-111, EtdFoc-191 and EtdFoc-192
Cluster-9	3	EthFoc-90, EtdFoc-220 and EtdFoc-221
Cluster-10	2	EthFoc-127 and EthFoc-129
Cluster-11	5	EthFoc-118, EthFoc-120, EthFoc-125,EthFoc-130 and EthFoc-131
Cluster-12	4	EthFoc-11,EthFoc-61, EtdFoc-30 and EthFoc-52
Cluster-13	2	EtdFoc-5 and EtdFoc-6
Cluster-14	3	EthFoc-12, EthFoc-34 and EthFoc-35
Cluster-15	3	EthFoc-2, EthFoc-44 and EtdFoc-204
Cluster-16	5	EtdFoc-7, EtdFoc-8, EthFoc-40, EtdFoc-160 and EtdFoc-216
Singletons	29	EthFoc-3,EthFoc-7,EtdFoc-10,EtdFoc-19,EtdFoc-23, EthFoc-27, EtdFoc-39,EtdFoc-47,EtdFoc-48,EtdFoc-61,EtdFoc72, EthFoc-78, EtdFoc-85,EtdFoc-100, EthFoc-101, EtdFoc-117, EthFoc-119, EthFoc-124, EthFoc-126, EtdFoc-158, EtdFoc-189, EtdFoc-198, EtdFoc-218, EtdFoc-203, EtdFoc-229, EtdFoc-233, EtdFoc-243,EtdFoc-248 and EtdFoc-250
Cluster-17	8	EtdFoc-38, EtdFoc-58,EtdFoc-68, EtdFoc-184, EtdFoc-186, EtdFoc-195, EthFoc-86 &EthFoc-77
Cluster-18	5	EthFoc-8,EthFoc-38,EtdFoc-63,EtdFoc-155,EtdFoc-208and EtdFoc-214
Singleton	1	EtdFoc-240
Cluster-19	2	EthFoc-22 and EthFoc-23
Cluster-20	2	EthFoc-62 and EthFoc-133
Total	**128**	

5.3.3. Phylogenetic relationship of *Fusarium* isolates

The taxonomic positions of *Fusarium* isolates were investigated using highly conserved BUSCO genes that are common to *Fusarium species*. Phylogenetic tree built based on 1,052 highly conserved BUSCO genes grouped *Fusarium* isolates into six distinct clades/species (Fig5.5). Consistent with Nei's diversity analysis, *Fusarium oxysporium* isolates in clade-I were further subdivided into 16 sub-groups with high bootstrap values at each nodes (Fig 5.5). These sub-groups correspond to Focisolates in Nei's diversity analysis from cluster 1 to 16 (Fig 5.6). Similar with Nei's diversity analysis, *Fusarium* isolates from clade/species II to V were distantly related from Focisolates in clade I (Fig 5.5). These isolates correspond to *Fusarium* isolates in Nei's clusters 17, 18, 19 and 20.

All Foc reference strains from the same formae speciales (USDA and ICRISAT Foc isolates) were clustered along with *Fusarium oxysporum* isolates in clade-I (Fig 5.5). However, reference strains from different formae speciales were clustered separately within clade-I. Three strains from tomato/*Lycopersicum* (FOL 4287, CL57 and MN25) and three strains from soil (Fo47), human (NRRL32931) and Melon (Melonis) were grouped together. Two strains from Brassica (PHW808 and Fo5176) were grouped in clade I together with USDA reference strains. Two strains from cotton and Musa families (cotton and II5) were consistently separated alone as singleton in different analyses.

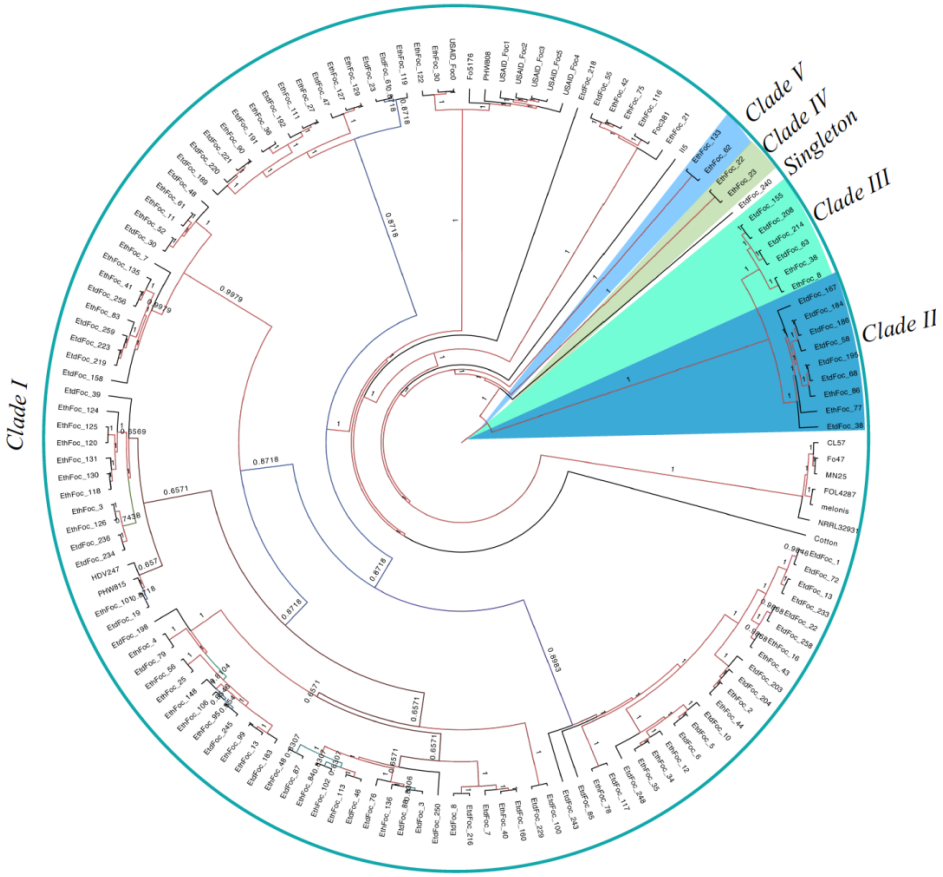


Fig.5.5. Phylogenetic relationship of 142 *Fusarium* isolates based on 1,052 conserved BUSCO genes using BEAST (Bayesian Evolutionary Analysis of Sample Tree)

5.3.4. Average Nucleotide Identity (ANI) Analysis

Pairwise average nucleotide identity (ANI) analysis based on 3,695 highly conserved BUSCO genes divided *Fusarium* isolates into 6 distinctspecies using 95% ANI (ANI_{95}) as the lower species boundary (Fig 5.6). ANI groups werevery consistent both with phylogenetics clades and Nei's genetic diversity group's. There are no cases of a single Nei's group split among multiple ANI groups and phylogenetic clades (Table 5.6). *Fusarium* isolates in ANI group I, II, III, IV and V correspond to the phylogenetic clade I, II, III, IV and V, respectively. Similar to the phylogenetic analysis, *Fusarium* isolates in ANI group II, III,IV and V were out grouped from Foc isolates in ANI group-I (Fig 5.6 and Table 5.6).

Both ANI and phylogenetic analyses confirmed that all *Fusarium* isolates in ANI group-I belong to *Fusarium oxysporium ciceris* (Foc). However, *Fusarium* isolates in ANI group-II, III, IV, V and one singleton were assigned to *Fusarium falciforme, Fusarium solani, Fusarium hostae, Fusarium verticillioides and Fusarium aywerte,* respectively (Table 5.6).

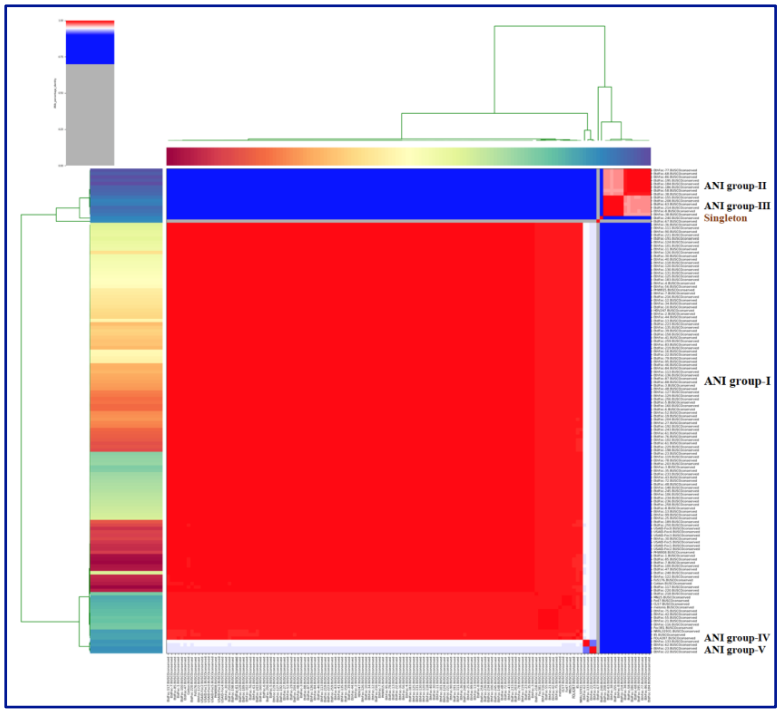


Fig. 5.6. Heat map of Average nucleotide identityfor 3,695 single copyBUSCO genes present in Foc isolates.

Foc isolates with above 95% ANIb sequence identity are shown with red cells in the heatmap and color intensity fades as the comparisons approach to 95 % ANIb sequence identity.Blue cells correspond to ANIb comparisons indicating isolates do not belong to the same species. Color bars above and to the left of the heatmap correspond to Foc isolate used in analysis.

Table 5.6. Nei's diversity clusters, corresponding ANI groups and phylogenetic clades and *Fusarium* species

Nei's diversity groups	Phylogenetic clades	ANI groups	Number of isolates	Speciesname
Cluster1 to 16	I	I	80	*Fusarium oxysporium*
Singletons	I	I	29	*Fusarium oxysporium*
Cluster-17	II	II	8	*Fusarium falciforme*
Cluster-18	III	III	6	*Fusarium solani*
Cluster-19	IV	IV	2	*Fusarium hostae*
Cluster-20	V	V	2	*Fusarium verticillioides*
Singleton	-	-	1	*Fusarium aywerte*

The percentage of the genome of each isolate alignment coverage to the genome of every other isolates is also essential to confirm ANI groups based genome relatedness between isolates. For instance, two isolates with 95 % ANI and alignment of 95 % of their genomes are more closely related than two isolates with 99.5 % ANI but that align with only 75 % of their genome. In the present study, there are several isolates with very high alignment coverage/high percentages of their genomes aligning, in other cases very little of the genomes aligns in some pairs (Fig. 5.7). The genome of *Fusarium* isolates in ANI group I, IV and V align with very high alignment coverage (> 95 %) of their genomes. Similarly, isolates in ANI group-II had high alignment coverage (> 95 %) with isolates in ANI group III. However, isolates in ANI group II and III poorly aligned (< 25 %) with isolates in ANI group I, IV and V (Fig 5.7).

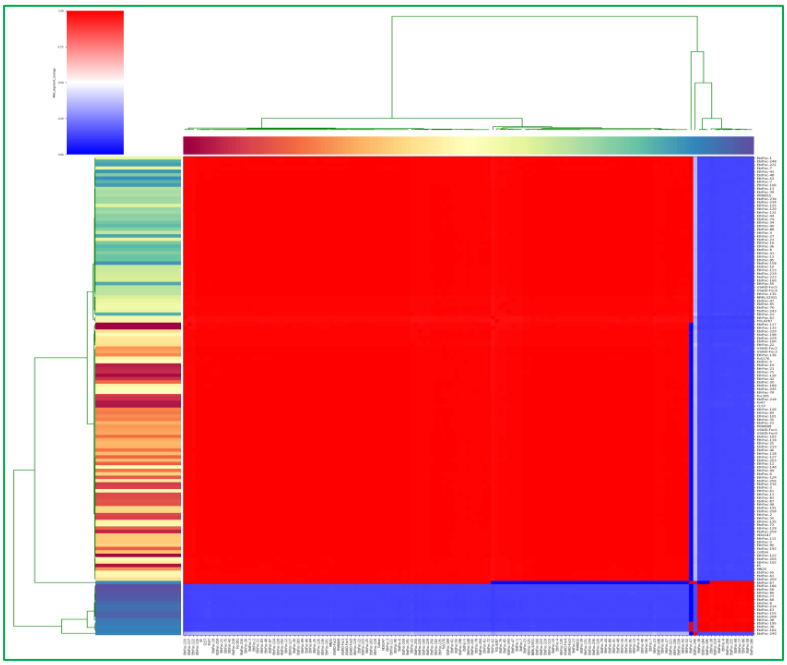


Fig.5.7. Heat map of Average nucleotide Identity alignment coverage for single copy BUSCO genes present in *Fusarium* test isolates

Foc isolates with above 50 % alignment coverage are shown with red cells in the heatmap and color intensity fades as the comparisons approach 50 % alignment coverage. Blue cells correspond to < 50 % alignment coverage. Color bars above and to the left of the heatmap correspond to each Foc isolate used in analysis.

5.3.5. Principal coordinates analysis

To better visualize the clustering patterns of the test isolates, principal coordinate analysis (PCoA) was performed using genetic distance matrix generated from whole genome SNPs. The result showed that the first three principal coordinate accounted for 81.66 % of the total variation with PC-1, PC-2, and PC-3 contributing 58.65 %, 15.00 % and 8.01 %, respectively (Table 5.7). PCoA further confirmed the six distinct species identified by average nucleotide identity analysis (ANI) based on highly conserved BUSCO genes (Fig 5.8).

The two distinct groups in the lower left hand quadrat corresponds to *Fusarium* isolates in ANI group II and III. The two groups in the upper left hand quadrate corresponds to *Fusarium* isolates in ANI group IV and V. *Fusarium* isolates in the upper and the lower right hand quadrates corresponded to Foc isolates in ANI group-Iincluding the reference strains from USDA.This group corresponded to the Foc isolates from cluster 1 to 16 in Nei's diversity analysis.

Table 5.7. Percentage of variation explained by the first three principal coordinate

	PC-1	PC-2	PC-3
Egen Value	3.65	0.94	0.50
Percent of variation explained	58.65	15.00	8.01
Cumulative (%)	58.65	73.65	81.66

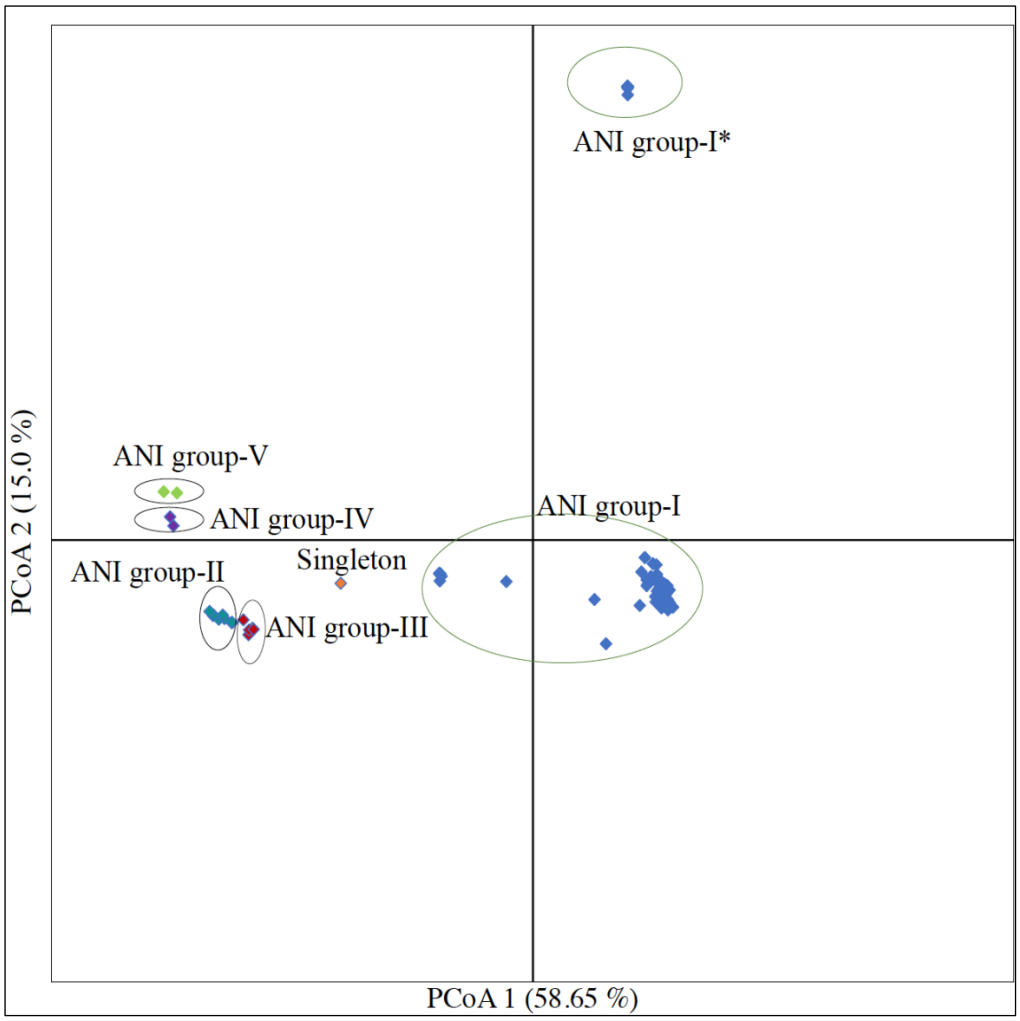


Fig.5.8. Principal coordinate analysis (PCoA) of 128 *Fusarium* isolates
**The six Foc reference strains from USDAalong with two Ethiopia Foc isolates*

5.3.6. Distribution of *Fusarium*isolates in Ethiopia

For all *Fusarium* isolates considered in the present study, mantel correlation estimate showed weak correlation between geographical distance and genetic distance (*P = 0.280 and* R^2 *= 0.0006)* (Fig 5.9). The geographic distance between *Fusarium* isolates ranged from 0.226 to 807.751 kilometer, with a mean of 316.97 km. The Nei's genetic distances between *Fusarium* isolates ranged from 0.00 to 0.64 with a mean genetic distance of 0.225.

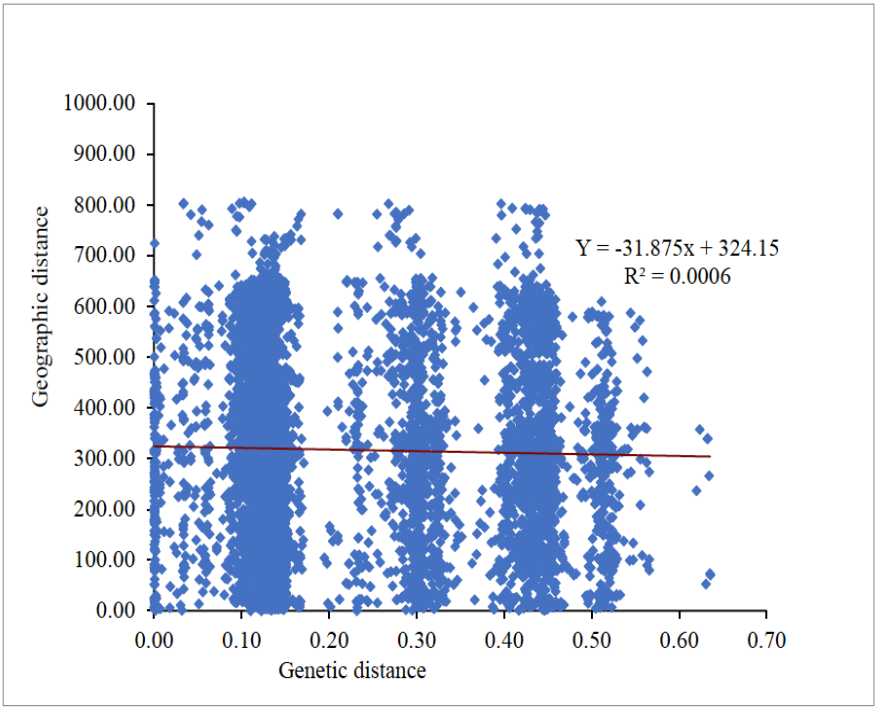


Fig.5.9. Genetic distance Vs Geographic distance

5.3.7. Pathogenic variability of *Fusarium* isolates

Fusarium isolates used in pathogenicity test induced various degree of disease incidence both in resistant and susceptible checks.The disease incidence in susceptible check (JG-62) ranged from 8.1 % to 97.4 %, however, in the resistant check (WR 315), it rangedfrom 0 % to 11.8 % (Table 5.8). As expected, no disease symptoms was observed in all uninoculated checks evaluated using the susceptible check, JG-62 and the resistant check, WR315.

Dendrogram built based on virulence data grouped test Foc isolates into four distinct virulence groups irrespective of their geographical origin(Fig 5.10). Most isolates from the same Nei's clusters were grouped together in the same virulence group indicating good relationship between the virulence level of the pathogens and their respective genetic diversity groups. For instance, EtdFoc-236 and EtdFoc-234 from

cluster-6, and EtdFoc-013 and EtdFoc-258 from cluster-5 were clustered in virulence group-I. EtdFoc-160 and EtdFoc-216 from cluster-16, and EthFoc-4 and EthFoc-13 from cluster-3 were clustered in group-II and group-III, respectively.

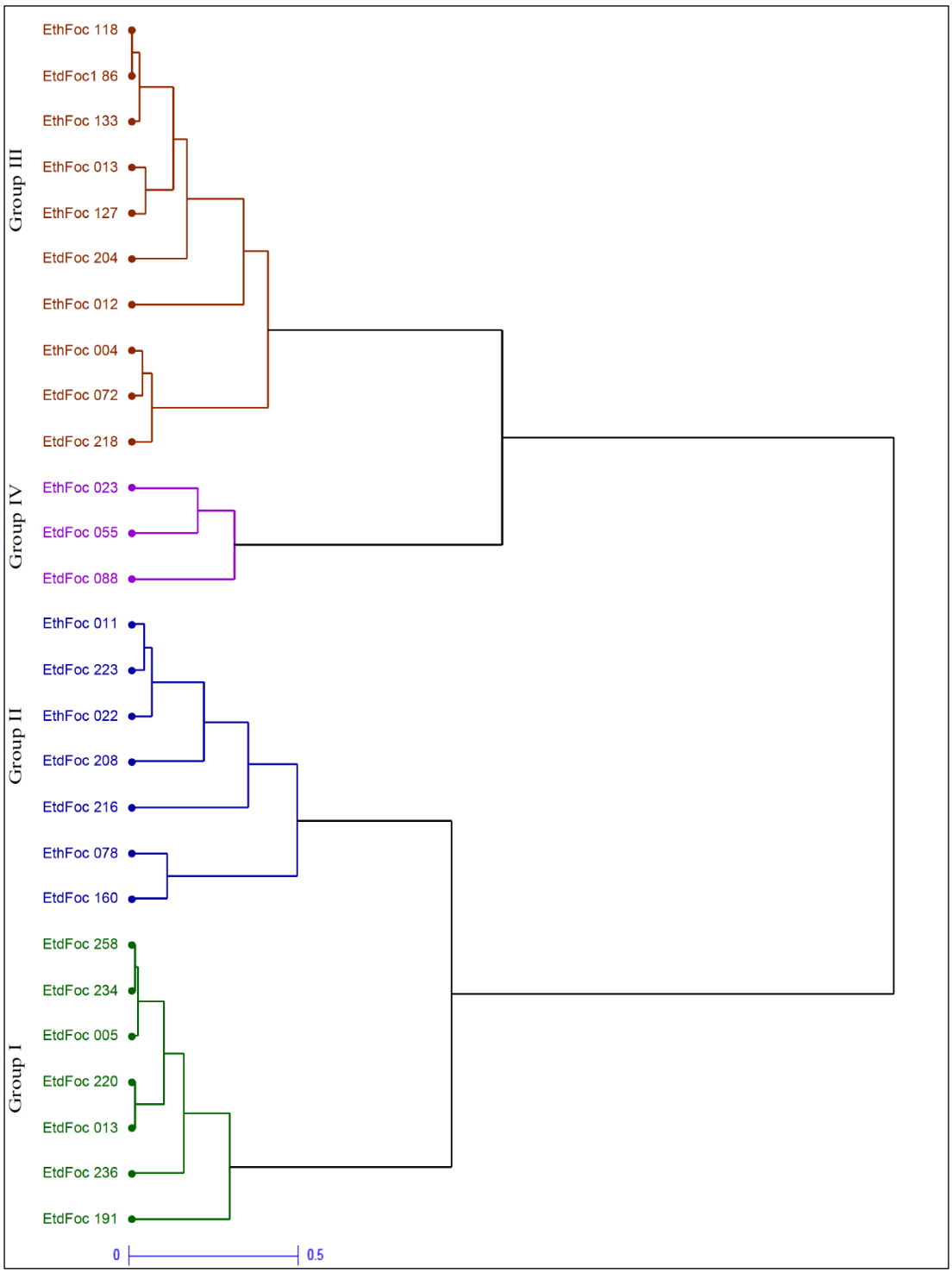


Fig 5.10. Dendrogram built using UPGMA based on virulence data of Foc isolates on susceptible and resistant checks

Table 5.8. Disease incidence induced with each *Fusarium oxysporium* isolateused in pathogenicity test using the resistant and susceptible checks and their respective Nei'sdiversitygroups

Virulence group	*Fusarium* isolates	Disease incidence %		Nei's Cluster
		WR315	JG-62	
Group-I	EtdFoc-236	5.7	97.4	6
	EtdFoc-234	0.0	94.9	6
	EtdFoc-258	0.0	94.6	5
	EtdFoc-5	0.0	94.3	13
	EtdFoc-013	10.5	92.3	5
	EtdFoc-220	0.0	92.1	9
	EtdFoc-191	0.0	87.2	8
Group-II	EthFoc-78	5.4	79.5	Singleton
	EtdFoc-160	5.1	76.9	16
	EtdFoc-208	11.8	71.8	18
	EtdFoc-223	7.9	67.6	7
	EthFoc-11	2.6	66.7	12
	EthFoc-22	0.0	65.6	19
	EtdFoc-216	2.7	59.5	16
Group-III	EthFoc-12	2.6	48.6	14
	EtdFoc-204	0.0	43.6	15
	EthFoc-118	10.8	40.5	11
	EtdFoc-186	2.6	40.5	17
	EthFoc-133	2.9	40.0	20
	EthFoc-13	0.0	37.8	3
	EthFoc-127	2.7	36.8	10
	EtdFoc-72	8.6	33.3	Singleton
	EthFoc-4	2.8	32.5	3
	EtdFoc-218	6.1	31.4	Singleton
Group-IV	EtdFoc-55	0.0	17.9	1
	EthFoc -23	0.0	13.2	19
	EtdFoc-088	0.0	8.1	4

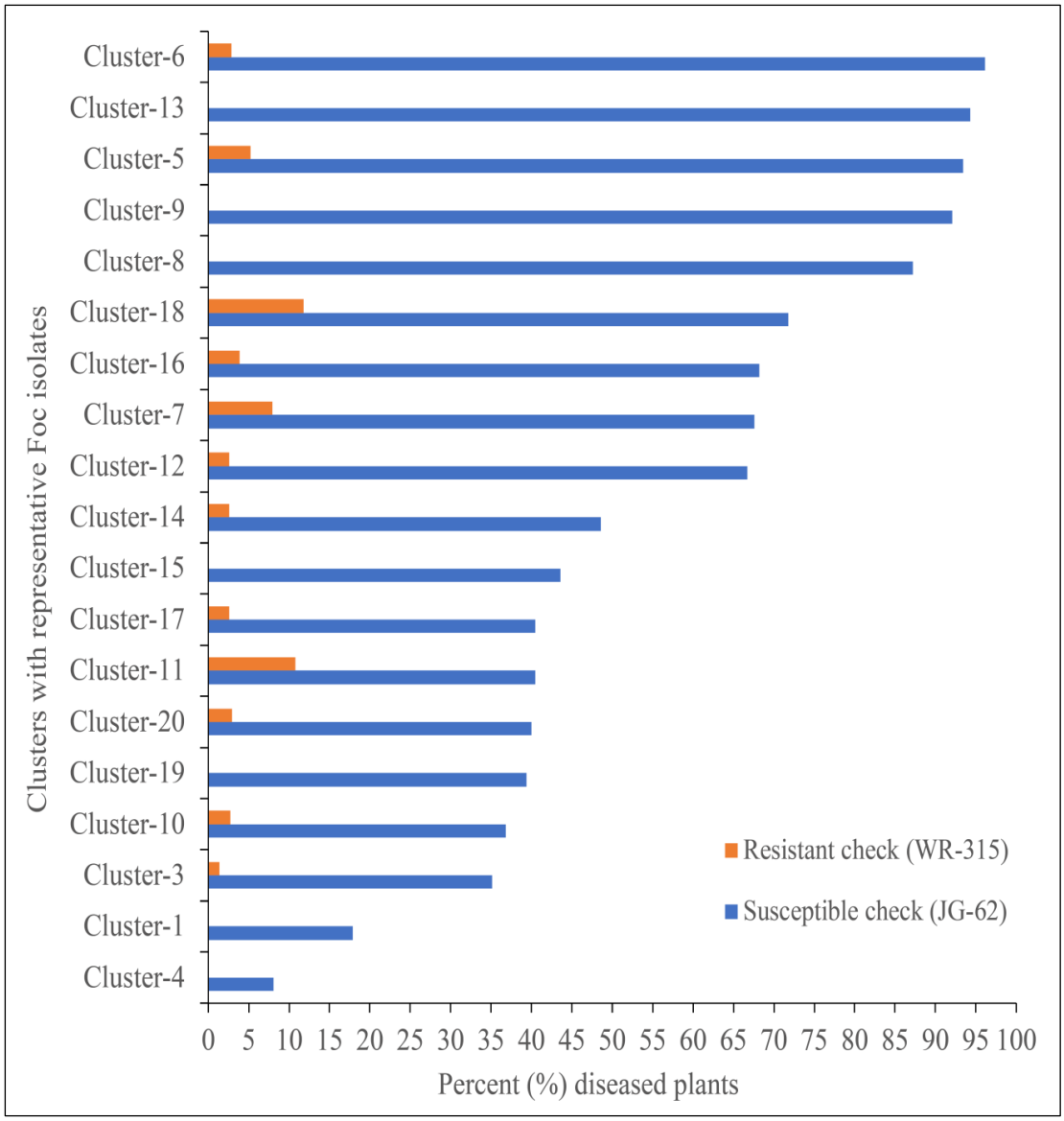


Fig 5.11. Mean virulence level of *Fusarium* isolates representing each Nei'sclusters in virulence assay (ordered with virulence level)

5.4. Discussion

Due to the soil borne nature of Foc pathogen, chemical control is not effective and practical (Sharma et al.,2019). Use of resistant varieties is the most economical and practical solution to control *Fusarium* wiltin chickpea (Sharma et al., 2014, 2019). However, chickpea breeding for *Fusarium* wilt resistance is often challenged with high pathogen variability and the existence of different physiological races (Jimenez Gasco et al., 2004a; Sharma et al., 2014; Jimenez-Diaz et al., 2015). Therefore, understanding the genetic diversity, geographical distributions and pathogenic variability of Foc pathogen in Ethiopia is prerequisitefor developing effective breeding strategy andintegrated disease management measures.

The results of the present study indicated the presenceof significant genetic diversity and pathogenic variability between Foc isolates in Ethiopia. Nei's genetic diversity analysis based on whole genome SNPs divided Ethiopian Foc isolates into 16 distinct clusters. Similarly, different previous studies based on different DNA molecular markers showed the existence of significant genetic diversity and pathogenic variability between Foc isolatesin different countries (Kelly et al., 1994, Jimenez-Gasco et al., 2001; Barve et al., 2001; Honnareddy and Dubey; 2006, Dubey and Singh, 2008; Gurjar et al., 2009; Sharma et al., 2009, 2014).

Consistent with Nei's genetic diversity analysis, phylogenetic analysis based on 1,052 highly conserved BUSCO genes split Ethiopian *Fusarium* isolates into 6 distinct species and 16 sub-groups. Similarly, pairwise average nucleotide identity (ANI) analysis based on highly conserved BUSCO genes split *Fusarium* isolates into 6 distinct species using 95 % ANI (ANI_{95}) as the lower boundary. There are no cases

of a single Nei's and phylogenetic groups split among multiple ANI groups. Consistent with phylogenetic clades and Nei's diversity analysis, *Fusarium* isolates in ANI group II, III,IV and V were out grouped from *Fusarium oxysporium* isolates in the main ANI group-I, and belong to closely related *Fusarium* species. As a result, *Fusarium* isolates in ANI group-II, III, IV and V were assigned to *Fusarium falciforme, Fusarium solani, Fusarium hostae and Fusarium verticillioides,* respectively. A single isolate (EtdFoc 240) was also assigned to *Fusarium aywerte*. Similar previous studies also reported that two strains showing pairwise ANI values below 95 or 96 % belong to different species and otherwise to the same species (Goris et al., 2007; Richter and Rossello Mora, 2009).

All *Fusarium oxysporum* isolates included in the present study share a common ancestor supporting the monophyletic origin. Previous studies also reported that *Fusarium oxysporum* isolates from different formae speciales may share a common ancestor (monophyletic origin) (Kistler,1997) and different isolates from a single forma speciales may have independent evolutionary (polyphyletic origins) (Baayen et al., 2000; Skovgaard et al. 2001; Cramer et al., 2003). Similar earlier studies also proved that, except few formae specialis like Foc which are monophyletic orgin (Jimenez-Gasco et al., 2002; Wunsch et al., 2009), most *Fusarium* formae speciales are polyphyletic in nature where isolates may have adapted to the same host by convergent evolution (Ma et al., 2013).

Previous studies reported that, the morphological discriptions of *Fusarium* species have been rigorously challenged with different advanced molecular tools used to delineate phylogenetic species (Leslie and Summerell, 2006). The most common error

resulting from a morphological species description in *Fusarium* is the grouping of isolates that should be separated into distinct species into a common species. Similary the most common error with phylogenetic species description in *Fusarium* is the splitting of isolates into more groups than is biologically meaningful (Leslie and Summerell, 2006).

In recent years, the increasing use of advanced genomic tools and techniques in fungal diagnostics has emerged as a possible answer to resolve the problems associated with existing phenotypic identification and classification systems. Different studies reported that ANI is one of the best approach to determine genetic relatedness between two genomes as it evaluates a large number of genes in its calculation, including slowly and quickly evolving genes (Konstantinidis and Tiedje, 2005; Pritchard et al., 2016).

The most reliable method to validate the pathogenicity of Foc isolatesis through pathogenicity assay in which host plants are inoculated with the pathogens to observe disease development. The present study successfully demonstrated the pathogenic variability of Foc isolates in Ethiopia. Virulence data split test isolates into four distinct virulence groups irrespective of their geographical origin. Similar previous study in India also demonestrated that there was no apparent correlation between geographical origin and virulence of the Foc isolates (Sharma et al., 2009).

Fusarium isolates from the same Nei's clusters were grouped together in the same virulence groups indicating the presence of significant correlation between the virulence level of the Foc isolates and its respective genetic diversity groups.

Similarly, recent work in India revealed that DArT markers were able to group the Foc isolates consistent with its virulence group (Sharma et al., 2014). Earlier studies confirmed that highly polymorphic DNA markers clearly delineated pathogenic and non-pathogenic Foc isolates (Kelly et al.,1994; Jimenez-Gasco et al., 2001; Jimnez-Gasco et al., 2004a;et al., 2004b; Sharma et al., 2009).

In different parts of the world, several physiological races of *Foc* have been proposed based on differential responses of a standard set of chickpea cultivars (Jimenez-Gasco and Jimenez-Diaz, 2003). In light of the increasing appreciation that *Fusarium oxysporum* is a multi-species assemblage (Zhang and Ma, 2017) and that host range is a polyphyletic character (Epstein et al., 2017), the assumption that the previously assigned races are phylogenetically coherent remains untested. Moreover, *Foc* race assignments lack certainty, because the genetic assignment of chickpea host differentials lacks precision.

Besides, the experimental and greenhouse conditions such as temperature, humidity, spore concentration, etc. also can have a significant effect on the level of pathogen virulence leading to an incorrect result. Therefore, races are difficult to determine correctly based on phenotypic observations, and further study is underway to assign the 16 distinct diversity groups identified in the present study into specific races using molecular markers.

Similarly, previous studies showed that the two pathotypes and all pathogenic races were clearly descriminated using highly polymorphic molecular markers (Jimnez-Gasco et al.2004; Sharma et al., 2009). Recent studies also reported change in the race scenario of Foc isolates, and many new pathogenic races were suscpeted in different

parts of the world (Dubey et al., 2012, Sharma et al., 2013, 2014). Mantel correlation analysis showed very poor correlation between geographic and genetic distances of Ethiopia Foc isolates. This suggests that few closely related Foc isolates are widely distributed throughout chickpea growing areas of the country. Therefore, all Foc races in the country possibly found in different chickpea growing areas of the country. Similarly, Foc isolates collected from the same agroecological zones were distributed to different Nei's genetic diversity groups irrespectives of their geographical origin. In agreement with the present findings, several previous studies reported the existence of significant genetic diversity between Foc isolates irrespective of their geographic origin (Jimenez-Gasco et al., 2002; Gurjar et al., 2009, Dubey et al., 2012).

In conclusion, the results of the present study suggest the presence of high genetic diversity and pathogenic variability among Foc isolates in Ethiopia irrespectives of their regions of geographical origin. Therefore, designing effective country wide breeding strategies is indispensable to break the recurrent disease cycle in the country.

CHAPTER SIX

PCR Based Diagnostic Assayand 18S Amplicon Sequencing for Identification of *Fusarium* Wilt Pathogen of Chickpea (*Fusarium oxysporum* f. sp. *ciceris)*

Abstract

Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) can significantly reduce through appropriate integrated disease management measures. However, this requires rapid, accurate and reliable disease diagnosis methods. Symptom based diagnosis alone often leads to disease misdiagnosis. To develop simple, cost effective and reliable disease diagnostic assay for rapid and accurate detection of chickpea wilt pathogen directly from symptomatic plants, broad specific PCR primers were designed using a set of 66 high quality draft genomes spanning 16 diversity groups of chickpea-associated *Fusaria oxysporium* sampled in Ethiopia. On test with *Fusarium* EOG09331-PTT primers (PTT-F1R2 primer), 97.5 % of diseased plants with typical symptoms (39 of 40 plants) from five farmers' fields gave consistently strong amplification with the identity of amplicons confirmed by Sanger sequencing. However, PCR based diagnostic assay is unable to assess the presence of all other microbes that might cause the disease symptoms. To address these issues, microbial community composition were surveyed using 18S amplicon sequencing. The result nominated *Phytophthora medicaginis* (that cause *Phytophthora* root rot in chickpea) as alternative pathogen in farmers' fields where *Fusarium* wilt was suspected. The resulting information about the prevalence of active pathogens could inform agronomic practices in subsequent seasons, and it could be used retrospectively to evaluate the impact of subclinical disease on crop yields. Community analyses represent a potentially powerful alternative to traditional plant disease diagnostics. Without the constraints of culturability and the bias of endpoint PCR, amplicon sequencing can provide powerful insights into disease dynamics.

Keywords/phrases: Amplicon sequencing, Disease diagnosis, *Fusarium*, *Phytophthora,* symptomatic plant

6.1. Background and Justification

Recent surveys of chickpea diseases in Ethiopia rank wilt, caused by *Fusarium oxysporum* f.sp. *ciceris* (Foc), as among the most important diseases of chickpea (Yimer et al.,2018). Accurate disease diagnosis is essential to the effective control measure of plant diseases (Navas Cortes et al., 2012). Importantly, misdiagnosis can lead to inappropriate and ineffective management responses. In most agricultural situations diagnosis of *Fusarium* wilt is indirect. Agriculturalists often extrapolate from published descriptions and observations made under controlled conditions, that may or not be relevant to local circumstances.

Moreover, because *Fusarium oxysporum* is an effective soil inhabitant and plant colonizer, isolation of the organism from symptomatic plants is an insufficient criterion for disease diagnosis. Rarely there are steps taken to confidently identify the causal agent of observed symptoms. In different parts of the world, due to lack of good experiences and resource, the most prevalent techniques used to identify plant pathogens relied upon culture-based morphological approaches. This has certainly been the history of *Fusarium* wilt diagnosis in Ethiopia (Beniwal et al.,1992), a situation that is exemplary of most regions of chickpea cultivation, and indeed of many crops worldwide.

These classical culture-based plant pathogen detection methods are now being increasingly replaced by culture independent molecular detection techniques which are much faster, highly sensitive, more specific and reliable (Jimenez-Fernandez et al., 2011a). The polymerase chain reaction (PCR) is one of the most important and sensitive technique for the detection of different plant pathogens. PCR-based methods

can detect and identify a wide range of plant pathogens in several plant species (Jimenez Gasco and Jimenez Diaz, 2003; Jimenez-Fernandez et al., 2010, 2011a; Mirmajlessi et al., 2015). Depending on the specificity of the primers, identification of fungal pathogens based on PCR assays can be achieved at different taxonomic levels, genus, species or strain level (Jimenez-Gasco et al., 2001; Jimenez Gasco and Jimenez Diaz, 2003; Jimenez-Fernandez et al., 2010, 2011a).

Moreover, with the advent of low cost, high throughput amplicon sequencing it is increasingly feasible to take a non-biased approach to characterize microbes that associate with diseased and healthy individuals, essentially revisiting the first of Koch's postulates (Fredericks and Relman 1996; Adams et al., 2018). Prevailing methods treat the resulting data as community surveys and apply a variety of population genetic and statistical methods that together comprise the rapidly expanding field of microbiome research.

In this study, the full genome sequence of 66 *Fusarium oxysporum* isolates collected from diverse Ethiopian chickpea geographies were used to develop broad-specificity oligonucleotide primers for Foc pathogen detection by means of the PCR. Moreover, a culture-independent broad-range18S amplicon survey was conducted to characterize chickpea-associated eukaryotic communities and test for the enrichment of specific taxa by field site and according to disease symptoms.

6.2. Materials and Methods

6.2.1. Field sample collection

From October 2017 to December 2018, a total of 179 chickpea plants were sampled from fifteen farmers' fields and the sick plot of the Debre Zeit Agricultural Research Center (Table 6.1). Eleven fields were sampled for symptomatic plants only, four fields were sampled for a combination of symptomatic and asymptomatic plants, and a single field contained only asymptomatic plants (Fig 6.1). Individual plant phenotypes were recoded as either asymptomatic (no visible disease) or symptomatic, and fields were given a score of given a score of "low", "medium", "high" or "asymptomatic". Root tissues of plants at Lume, Welenso, Bolo, and Debre Zeit were collected in October 2017 and at Abasamuel, Geradogemeda, Jewe, Foka, Kaliti, Gasho, Bole, and Tuludimitu in December 2017. Dissected stem tissue was collected in December 2018 at Denkaka and Algie.

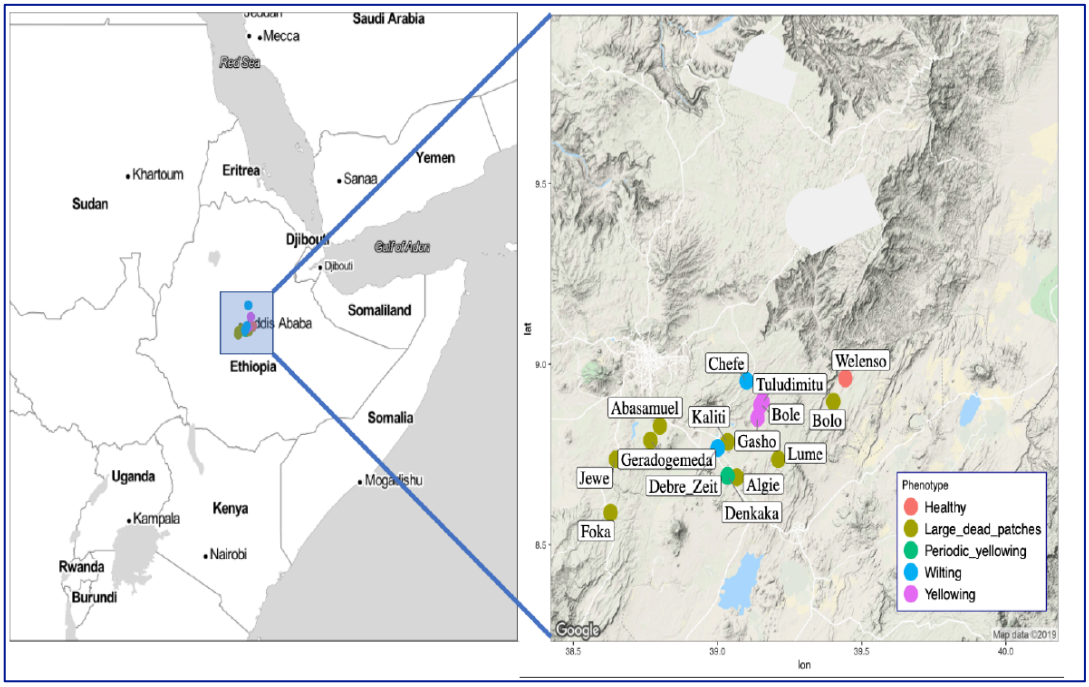


Fig 6.1. Sample collection areas map

Table 6.1. Sample collection areas, disease level, and samples collected per field

Location	Collection Date	Altitude (masl)	Disease Instance	Symptomatic plants	Total plants
Debre Zeit-1	Oct 2017	1887	Moderate	5	5
Bolo	Oct 2017	1807	High	3	3
	Oct 2017	1807	Asymptomatic	0	5*
Welenso	Oct 2017	2496	Asymptomatic	0	5*
Lume	Oct 2017	2835	High	3	3
	Oct 2017	2835	Asymptomatic	0	5*
Debre Zeit-2	Dec2017	1887	Moderate	10	10
Bole	Dec2017	2200	Moderate	10	10
Gasho	Dec 2017	2093	Moderate	10	10
Tuludimitu	Dec 2017	2178	Moderate	10	10
Abasamuel	Dec 2017	2073	High	10	10
G/Gemeda	Dec 2017	2115	High	10	10
Chefe	Dec 2017	2436	Moderate	10	10
Kaliti	Dec 2017	1886	High	10	10
Foka	Dec 2017	2112	High	10	10
Jewe	Dec 2017	2137	High	10	10
Algie	Oct 2018	1832	High	18	18
	Oct 2018	1832	Asymptomatic	0	10*
Denkaka	Oct 2018	1885	Low	15	15
	Oct 2018	1885	Asymptomatic	0	10*

*Oct = October; Dec = December; * Asymptomatic collected to use as a PCR negative control*

6.2.2. DNA extraction

DNA was extracted from samples within 24 hours of collection using laboratory facilities at Addis Ababa University and Holetta Agricultural Research Center of the Ethiopian Institute of Agricultural Research. Root DNA extraction was performed using the Omega Plant (Omega) DNA extraction kit, and stem DNA extractions were performed with the DNeasy Plant Mini kit (Qiagen) according to manufacturers' instructions.

6.2.3. Design and testing of Foc PCR primers

PCR primers were designed based on the alignment of selected single copy BUSCO (Simao et al., 2015) genes present and highly conserved in the genomes of a set of 66 *Fusarium* isolates (BioSample SAMN07711041; PRJNA412392). Briefly, nucleotide sequences were aligned using ClustalW (Larkins et al., 2007) and PCR primers were designed complementary to invariant sites separated by 1-2k bp using Primer3 (Untergasser et al., 2012).

Using a small subset of three BUSCO genes (PTT, TPO and 4TJ), four forward and reverse primer sets were tested in PCR assays using an annealing temperature of 56^{o}C (Table6.2) and 20 ng purified Foc gDNA isolated from strain EtdFoc-2 (PRJNA412392, BioSample SAMN07711041). From tested primer pairs, PTT-F_1R_2, TPO-F_1R_2 and 4TJ-F_1R_1 were better amplified Foc culture gDNA (Fig. 6.2 & 6.3).

Among these primer pairs, BUSCO gene EOG09331-PTT was selected as a target based on its strong and uniform amplification of Foc strain gDNA using primersPTT_F_1 and PTT_R2. The broad specificity and sensitivity of PTT-F_1R_2 was confirmed using 12 ng of EtdFoc-2genomic DNA, and 12 ng of healthy plant DNA as negative controls (Fig.6.2).PTT-F_1R_2 primer selectively amplified only Foc culture gDNA in lanes 82, 84, 86, 88 and 90. However, TPO-F_1R_2 and 4JI-F_1R_2 primers lacks specificity to Foc pathogen (Fig. 6.3 A and B).

Table 6.2. Selected BUSCO genes, a sequence of forward (F) and reverse (R) primers, primers length (bp), melting temperature (T_m) and percent GC content.

Primer Name	Sequence 5' to 3'	Length (bp)	T_m	% GC
1.**EOG09331-PTT**				
PTT_F_1	TGTTCGTGACCGTAATTGGA	20	59.96	45
PTT_R_1	AACCTTGCCCTGTGTTTGAC	20	60.01	50
PTT_ F_2	CCCAAAGGACGACATCATCT	20	59.93	50
PTT_ R_2	CACCTTCAACAACACCAACG	20	60.04	**50**
2. **EOG09331-TPO**				
TPO_ F_1	CAAGGTCTACGACTGCACCA	20	59.90	55
TPO_ R_1	CAATGTAGGCGATGAAACCA	20	59.54	45
TPO_ F_2	ACGAGCACCCGTACGTTATC	20	60.02	55
TPO_ R_2	CGCTGCGATCCTTATCTTTC	20	59.94	50
3. **EOG09331-4TJ**				
4TJ_ F_1	CTCATAGACACCGCTCGTGA	20	60.01	55
4TJ_ R_1	CGGATCAAAAGGACGAACAT	20	59.93	45
4TJ_ F_2	TGCCCAGGTCTTTACCATTC	20	59.93	50
4TJ_ R_2	CCTTAGACATGGCACCCAGT	20	59.99	55

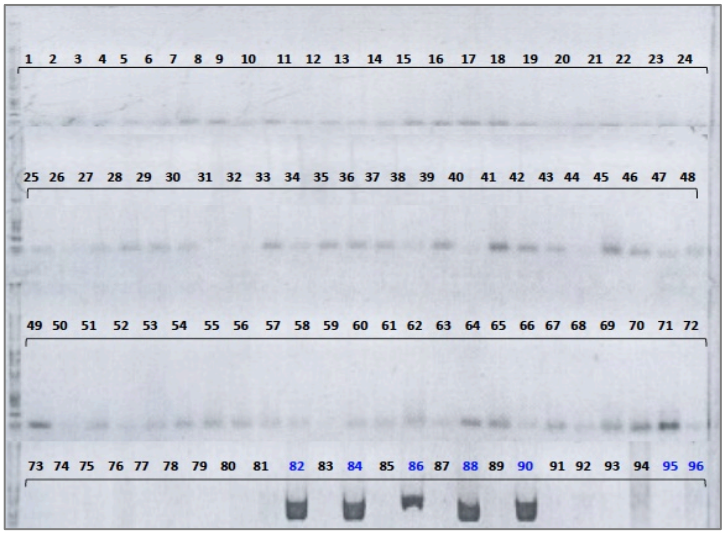
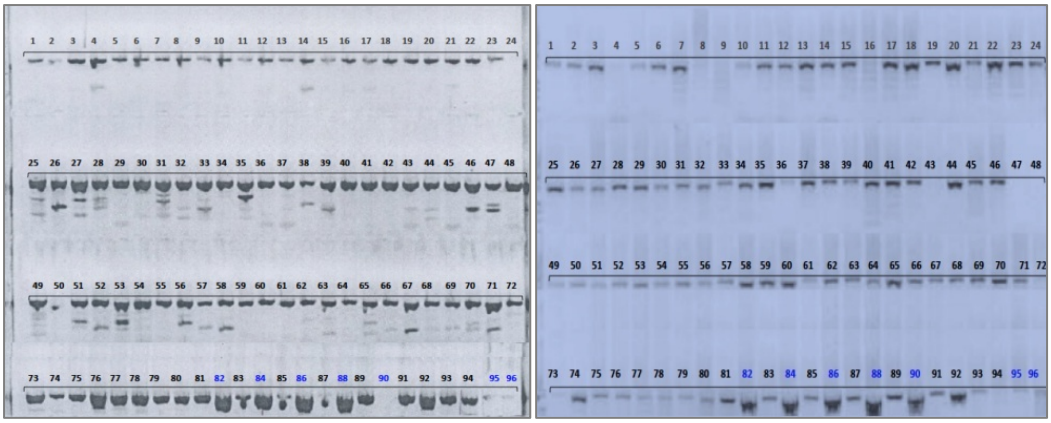


Fig.6.2. Agarose gel electrophoresis showing PCR amplification of 12 ng Foc and plant DNAs using PTT-F_1R_2 primer.

*Lanes labelled with number **82,84, 86, 88** and **90** were corresponded to Foc culture genomic DNA. Lane 95 and 96 corresponds to negative controls with DNA from healthy chickpea plants. All other lanes from 1 to 94 were corresponding to healthy plant DNA extracts used as negative controls.*



A. TPO-F_1R_2 primer B. 4TJ-F_1R_1 primer

Fig.6.3. Agarose gel electrophoresis showing PCR amplification of Foc and plant DNAs(12ng) using TPO-F_1R_2 (A) and 4TJ-F_1R_1 (B) primers.

*Lanes labelled with **82,84, 86, 88** and **90** were corresponds to Foc culture genomic DNA(12ng), and lanes with 95 and 96 corresponds to negative controls with DNA(12ng) from healthy chickpea plants. All other lanes from 1 to 94 were corresponding to healthy plant DNA extracts used as negative controls.*

6.2.4. PCR assays and library preparation

For assays of field-collected plant samples, PCR was conducted in 25μl final volumes containing 20 ng template genomic DNA, 0.4 μM each of primers PTT_F_1 and PTT_R_2 and 2X GoTaq buffer. Thermal cycling conditions consisted of a single cycle of 95^{o}C for 3 minutes, followed by 40 cycles of 94^{o}C for 30 seconds, 56^{o}C for 30 seconds, and 72^{o}C for 1 minute, with a final extension cycle at 72^{o}C for 10 minute and 25^{o}C for 10 seconds with lid constant temperature of 85^{o}C.

Amplicon libraries were constructed based on the Illumina Amplicon Library Preparation protocol (Illumina, 2013) using the Earth Microbiome Project primers for 18S (Ammaral-Zetter 2009; Stoek, 2010). Amplicons were barcoded with the NexteraXT index kit v2 (Illumina, 2013). Individual libraries were assessed for size and quantified using the Agilent Bioanalyzer DNA HS kit (Agilent Technologies). Equal quantities of each library were pooled to create mixed samples for sequencing

at the University of California-Davis DNA Technologies and Expression Analysis Core facility.

6.2.5. Amplicon data preparation and analysis

Demultiplexed sequences were preprocessed using the DADA2 R library (Callahan, 2016). Each sequencing run was processed separately to allow the software to more accurately predict the error from different sequencing runs. Samples with less than 100 total reads were removed. Reads were re-oriented using the conserved region of the Eukaryota _1391f primer and low quality regions were trimmed to remove bases that did not meet a Q2 threshold, allowing two expected errors. Reads containing Ns were removed and primer sequences were trimmed. All sequence were merged to generate a single amplicon sequence variant (ASV). Taxonomy was assigned to ASVs using the DADA2 “assign Taxonomy” function with the Silva 16/18S v 32 database (Callahan, 2018). Data was filteredto remove reads not assigned to the kingdom Eukaryota. Following filtering for taxa, samples with less than 1,000 reads remaining were removed from the dataset. Representative sequences were aligned with Multiple Alignment using Fast Fourier Transform (MAFFT).

6.3. Results

6.3.1. Pathogen association with symptoms using PCR assay

PCR primers were designed for broad specificity, using a set of 66 high quality draft genomes of chickpea-associated *Fusaria* samples. PCR assay confirmed the presence of *Fusarium oxysporum* f. sp. *ciceris* (Foc) in symptomatic plants. On test with *Fusarium* EOG09331-PTT primers (PTT-F_1R_2 primer), 97.5 % of diseased plants with typical symptoms (39 out of 40 plants) from five farmers' fields sampled in December 2017 gave uniformly strong amplification (Fig 6.4) with the identity of amplicons confirmed by Sanger sequencing of one amplicon from each of the five fields. However, some plants from additional farmers' fields yielded either rare or absent amplification with positives exhibiting only weak amplification (Fig 6.4; Table 6.3).

To further test the correspondence between whole plant symptoms and *Fusarium* DNA amplification, in 2018 fifty-three plants (20 asymptomatic and 33 heavily symptomatic plants) were sampled at two farmers' fields, Algie and Denkaka. The two field sites had distinct symtpomatologies: the Algie field contained large dead patches with dying yellowed plants at the border, while the Denkaka field contained isolated yellowed plants mixed among asymptomatic individuals (Table 6.3). With the intent of enriching infected tissue and reducing the chance of false negative outcomes, vascular tissue was excised and DNA was extracted from surface sterilized stem sections.

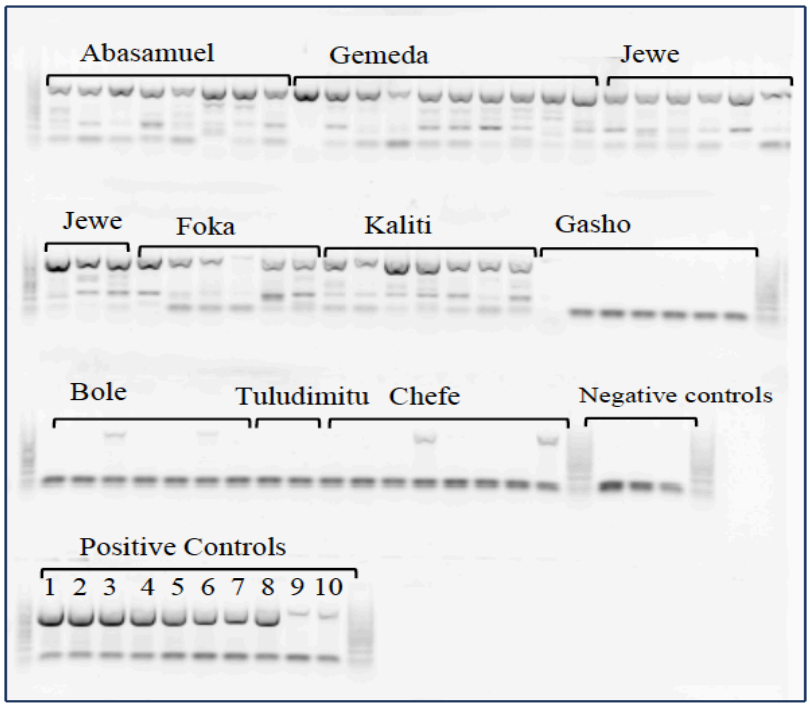


Fig. 6.4. Agarose gel electrophoresis showing PCR amplification of PTT-F_1R_2 primers with samples of gDNA from farmers' fields.

Abasamuel, Gemeda, Jewe, Foka, Gasho, Bole, Tuludimitu and Chefe were farmers' fields with symptomatic diseased plants. All lanes with negative controls correspond to 12ng healthy plant gDNA. For positive controls; Lanes labeled with number 1,3,5,7 & 9 corresponds to serial dilution of Foc culture genomic DNA with 6ng, 2ng, 0.7ng, 0.23ng & 0.08ng respectively. Whereas lanes labeled with number 2, 4, 6, 8,10 corresponds to an artificial mixture of 12ng healthy plant gDNA to each serial dilution.

Besides, on amplification with broad specificity *Fusarium* primers (PTT-F_1R_2 primer), plants from the Algie site yielded a mixture of positive and negative outcomes among both symptomatic and asymptomatic individuals (Table 6.3). From 18 symptomatic plants, 13 (72.2 %) were positive indicating Foc pathogen in diseased plants (Table 6.3). Among theten asymptomatic plants, 5 (50 %) were positive (Table 6.3), suggesting asymptomatic infection with Foc pathogen. By contrast, at the Denkaka site only a single PCR-positive plant, with faint amplification was observed among 15 symptomatic plants. All asymptomatic plants were 100 % PCR-negative indicating the absence of Foc pathogen in healthy plants (Table 6.3).

Table 6.3.PTT PCR results for symptomatic and asymptomatic samples collected from 16 different sites

Location	Disease Instance	Samples in PCR	Amplified with PTT	% PCR positive
Geradogemeda	High	9	9	100
Jewe	High	9	9	100
Abasamuel	High	8	8	100
Kaliti	High	7	7	100
Foka	High	6	6	100
Bolo	High	3	3	100
	Asymptomatic	5	2	40.0
Algie	High	18	13	72.20
	Asymptomatic	10*	5#	50.0
Lume	High	3	2	66.6
	Asymptomatic	5	0	0.00
Welenso	Asymptomatic	5	1	20.0
Denkaka	Moderate	15	2#	13.3
	Asymptomatic	10	0	0.00
Chefe	Moderate	3	1	33.3
Bole	Moderate	7	2	28.50
Debre Zeit-1	Moderate	4	1	25.0
Gasho	Moderate	6	0	0.00
Debre Zeit-2	Moderate	5	0	0.00
Tuludimitu	Moderate	2	0	0.00
PCR control	Positive control**	10	10	100
	Negative control***	3	0	0.00

Among the 10 asymptomatic plants collected from Algie field, one sample showed necrotic vascular tissue during DNA extraction. **Fusarium culture gDNA DNA extract from greenhouse grown chickpea, # Sum total consists of some weak bands*

6.3.2. Microbial community analysis

To explore the microbial community landscape on chickpea without the bias of targeted PCR, we used 18S amplicon sequencing. DNAs were amplified using oligonucleotide primers that anneal to highly conserved regions of eukaryotic 18S rDNA and sequence variation in the intervening variable regions was used for taxonomic assignment. Analysis of community diversity focused on four fields (Bolo, Lume, Algie and Denkaka) in which both symptomatic and asymptomatic plants included were indicated that Ascomycota and Basidiomycota as well as Peronosporomycota (*Oomycetes*) were predominant in all samples (Fig 6.5).

Amplicon sequence variants (ASVs) enriched in symptomatic samples at more than one field site belonged to Orders Hypocreales, Peronosporomycetes, Arachnida, and Glomerales (Fig.6.6). The most consistent, significantly enriched ASV in diseased samples at three sites (Algie, Lume and Bolo) was ASV3, an Oomycete taxonomically assigned to the genus *Phytophthora*.

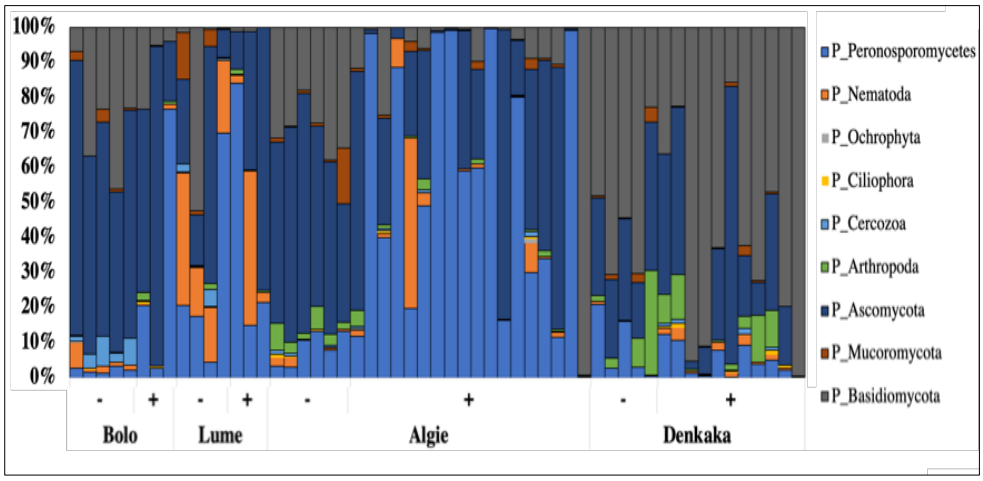


Fig 6.5. Identity and diversity of 18S reads in field sites with paired asymptomatic(-) and symptomatic (+) specimen collection.

Host removed relative abundance phyla level taxon summary plot. Asymptomatic samples represented with a minus(-) and symptomatic samples represented with a plus (+)

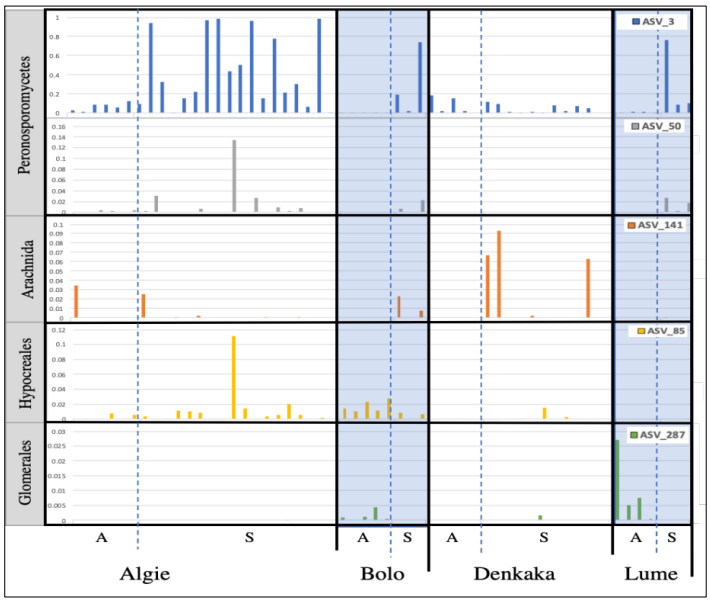


Fig 6.6. Host removed 18S relative abundance of ASVs identified as differentially abundant between asymptomatic (**A**) and symptomatic (**S**) plants.

In Algie, Bolo, Lume, and Denkaka symptomatic samples, the sites with only symptomatic plants were dominated by a small number of highly abundant taxa. Filtering each sample to remove ASVs with less than 1% abundance reduced the number of ASVs in the dataset from 1,067 to 177. The same filter reduced the number of ASVs per sample to between 1 and 29 ASVs, while retaining 64 % to 99 % total abundance for each sample (Fig 6.7 A).

Plots of ASV abundances for each sample by rank shows that the top ranked ASV typically exceeded 50% of total reads in symptomatic, but not typically in asymptomatic plants (Fig 6.7B). The presence of such high abundance first rank ASVs correspond to the identity of the ASV in the top rank. Algie, Bole, Bolo, Foka, Lume, and Kaliti contained ASVs associated with *Phytophthora* in the top rank (Fig 6.7**)**.

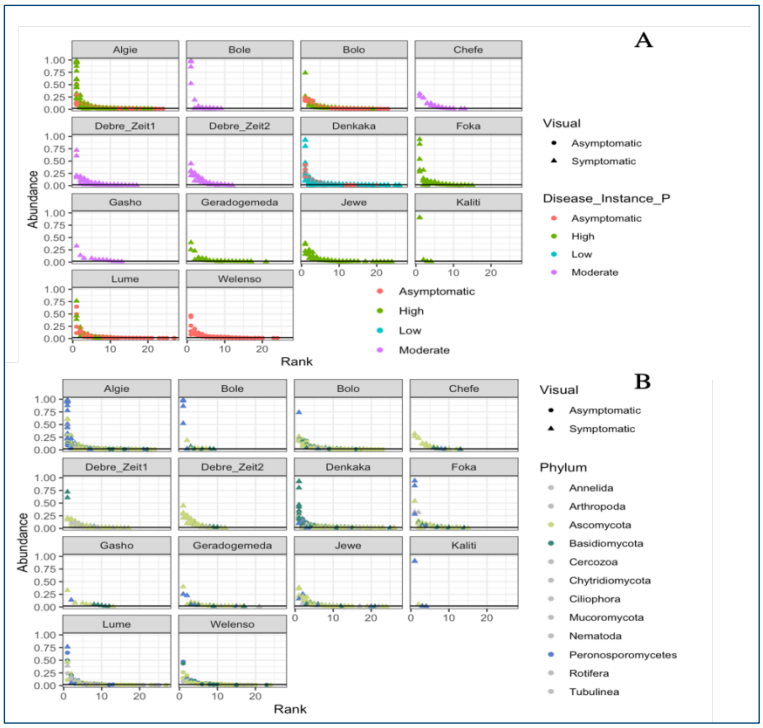


Fig 6.7. Abundance rank plot of all 18S samples.
A. ASVs for each sample colored by field appearance with 1% threshold line.
B. ASVs colored by phyla membership

6.4. Discussion

In chickpea, symptoms based diagnosis of *Fusarium* wilt and root rot complex diseases often leads to misdiagnosis. Developing simple, cost effective, rapid and highly sensitive pathogen detection method is one of the most important steps to properly apply integrated disease management measures (Jimenez-Fernandez et al., 2010; Jimenez-Fernandez et al., 2011a; Chilvers, 2012; Chitrampalam, 2018). In the present study, a survey of farmers' fields in Ethiopia using endpoint PCR was successfully detected Foc pathogen, particularly in high disease incidence fields with typical disease symptoms. Several previous studies on plant diseases confirmed the significance of culture independent PCR-based disease diagnostic assays in determining the prevalence of the disease in the field (Chitrampalam, 2018).

To date an increasing number of diagnostic laboratories is adapting PCR-based disease diagnostic assays for routine detection of plant pathogens (Fang and Ramasamy, 2015). According to Chilvers (2012*)*, the advantages of PCR-based disease diagnostic assaysinclude ability to detect the pathogen rapidly without prior culturing, accurate, sensitive and ability to identify pathogens specific strains. However, in somefields inconsistent association of *Foc* pathogen with symptomatic plants was observedindicatingthe presence of other chickpea associated pathogens causing the disease symptoms.

Disease diagnosis using organism-specific DNA amplification is unable to assess the presence of other microbes, or features of community dynamics, that might better inform diagnosis. To address these issues, microbial community composition were surveyed using 18S amplicon sequencing and the result suggested that *Phytophthora*

amedicaginis (that cause *Phytophthora* root rot in chickpeas) as the causal agent at some fields where *Fusarium* wilt was suspected. Community genomics analyses represent a potentially powerful alternative to traditional plant disease diagnostics. Without the constraints of culturability and the bias of endpoint PCR, amplicon sequencing can provide powerful insights into disease dynamics. Plant-associated microbial communities have been studied extensively in microbial ecology (Hartmann, 2014; Banerjee, 2019; Thiergart, 2020), including in relation to plant disease (Lamelas, 2020), while the use of community analyses as a tool in plant disease diagnosis is comparatively limited (Hu, 2019).

Disease diagnosis is most effective if it leads to effective changes in integrated disease management options. These include effective crop rotation cycles, appropriate planting times and field management practices and use of disease-resistant varieties that significantly reduce recurrent yield loss and disease inoculum build up in farmers. Therefore, the need for easy, cost effective, rapid and accurate disease diagnosis of chickpea wilt disease for farmers has several advantages to apply appropriate integrated disease management measures on time and to break the disease cycle over years. In conclusion, *Fusarium* wilt is among the most prominent factors impacting chickpea production in Ethiopia and is a primary cause of declining acreage devoted to the crop. Therefore, accurate pathogen identification is vital to reversing this trend by informing effective breeding, management and extension recommendations to growers. This study suggested *Phytophthora* as alternative pathogens that cause the disease symptom at some fields where *Fusarium* wilt was suspected. I acknowledge Ms. Betsy Alford of UC, Davis for 18S amplicon data analysis.

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATIONS

Chickpea (*Cicer arietinum*L.) is one of the most economically important nutritious food legume cultivated in different parts of the world. Ethiopia is the largest producer, consumer and exporterof chickpea in Africa, every year producing around half million tons. However, several biotic and abiotic stresses restrict its potential productivity. In order to enhance chickpea production and productivity in Ethiopia, the major challenges posed by various biotic and abiotic stresses must be addressed.

Fusarium wilt, caused by the asexual soil borne and seed borne fungus (Foc), is the most destructive root infecting pathogen affecting chickpea production and productivity in Ethiopia. Foc is pathogenic to multiple species with the genus *Cicer*, of which chickpea is the only cultivated member causing highly catastrophic vascular wilt disease. It is seed borne but is also a persistent soil fungus, capable of surviving in the soil for more than six years. High humidity, moderate temperature, accessibility of plant nutrients in soil are the prime conditions for fast growth of the pathogen and greater chance of disease development.

In the present study both morphological and genomic data clearly showed that in Ethiopia Foc is the most predominant and widely distributed pathogen in chickpea. The high disease pressure on farmers field was significantly associated with heavy black soil, Desi type local cultivars, early planting, flowering and plant maturity. The highest rates of disease incidence were recorded in regions with the higher usage of desi type local cultivars. Crop rotation and late planting were also correlated with

decreased disease incidence. In the absence of rotation or when chickpea was grown after other pulse crops, disease was more severe. Foc pathogen can colonize the roots of other pulse crops without developing external symptoms, thus increasing inoculum density within the soil (Jendoubi et al., 2017). Therefore, traditional cropping practices in the country, particularly cultivation of local cultivars, poor crop rotation and agronomic practices and sowing by broadcast method favor the inoculum build up in the soil and thereby the occurrence of the disease every year.

This study suggested the prevalence of wilt and root rot complex throughout chickpea growing regions of Ethiopia likely owes to the persistence of the pathogens in infested soils, and the long history of chickpea cultivation which derives from the great importance of chickpea as a cash and nutritional crop in affected areas. Special attention and strong integrated approach that can address all these issuesis required to break the recurrent wilt and root rot diseasescycle in the country.

Breeding for host plant resistance is the most cost efficient and ecofriendly strategy to control the disease. Although traditional breeding efforts have contributed to reducing the effect of wilt disease, introduction of effective resistance into farmers preferred varieties and against geographically-disperse pathogen genotypes remains elusive. The deployment of wilt resistant varieties has not been extensively used in Ethiopia due to lack of stable resistance source for the pathogen with desirable yield and yield-related morphological traits.

In the presentstudy, *Fusarium* wilt highly resistant wild chickpea introgression linesand advanced recombinant inbred lines (RILs) were identified. These novel wilt

resistant source can be utilizedin any chickpea breeding program either as a crossing parent or for direct release as wilt resistant varieties after rigorous evaluation for high yield and yield-related morphological traits. Moreover, the identified newwilt resistance source can be used to identify and map novel *Fusarium* wilt resistant QTL in chickpea to facilitate new gene discovery and trait transfer. Similarly, extensive screening work is required to identify more new wilt resistant sources from above 10,000 advanced wild introgression lines developed at UC, Davis by crossing wild chickpea species with Ethiopian and Indian elite varieties.

Genomics assisted breeding has great potential to accelerate the introgression and combination of resistance traits into farmerpreferred varieties, but this depends on the identification of an effective QTL and tightly linked molecular markers for trait transfer. Interestingly, in the present study, a novel QTL for *Fusarium* wilt resistance is identified.This major effect QTL and the associated genetic markers arean important target to facilitate the discovery of new wilt resistance genes in chickpea to develop wilt resistant variety through marker assisted selection. This QTL can be transferred to differentgenetic backgrounds of farmer-preferred and widely cultivated wilt susceptible chickpea varieties through marker assisted backcrossing (MABC) or marker assisted recurrent selection (MARS) in chickpea breeding programs.

However, to further delineate the locus and fully understand the gene responsible for *Fusarium* wilt resistance, it is highly recommended to get sufficient recombination with more mapping population. In the present study, the small population size limited to get sufficient recombination to delineate the locus. Therefore, further investigations

and fine mapping with a larger population and high density markers is recommended to effectively apply the present finding in different chickpea breeding programs.

Chickpea breeding for *Fusarium* wilt resistance is regularly challenged with narrow genetic base in cultivated chickpea, high pathogenic variability and the existence of different pathogenic races in the country. Understanding the genetic diversity, geographical distribution and pathogenic variability of Foc pathogen in Ethiopia will allow us to design effective breeding and integrated disease managementstrategies to reduce or eliminate the emerging threats in our chickpea production system.The results of the present study clearly showed the presence of significant genetic diversity and pathogenic variability among Foc isolates in Ethiopia. Both, the Nei's genetic diversityanalysis based on whole genome SNPs and the phylogenetic analysis based on highly conserved BUSCO genes divided Ethiopian Foc isolates in to 16 distinct groups irrespective of their geographical origin. Further study is underway to assign the 16 distinct diversity groups into specific races using highly polymorphic molecular markers.

This study indicated weak correlation between geographical distance and genetic distance of Foc isolates in Ethiopia and the presence of high genetic diversity and pathogenic variability among Foc isolates irrespective of their region of origin. Therefore, effective breeding and integrated disease management strategies should be developed to break the recurrent disease cycle in the country. To overcome the current disease condition by informing effective breeding and integrated disease management measures to researchers and growers, simple, cost effective and reliable disease diagnosis assay isalso required. In the present study Foc specific PCR primer

that can specifically detect Foc pathogen from infected plants was successfully developed. However, some symptomatic plants yielded inconsistent results with Foc specific PCR primer, that calls for wider scope and magnitude to get at conclusive point.

To address these issues, microbial community composition were surveyed using 18S amplicon sequencing and the result nominated *Phytophthora medicaginis* as alternative pathogens in farmers' fields where *Fusarium* wilt was suspected. The resulting information about the prevalence of active pathogens could inform agronomic practices in subsequent seasons, and it could be used retrospectively to evaluate the impact of subclinical disease on crop yields. In Ethiopia, *Phytophthora* root rot of chickpeasis reported for the first time in this study. Therefore, detailed study on *Phytophthora* root rot of chickpeasis recommendedto understand the basic biology, and the genetic and geographic diversityof *Phytophthora medicaginis*in Ethiopia to better manage and assess risks that associated with this new pathogen in the current and future chickpea production.

In conclusion, the results of the present study provide detailed information and appropriate frame work to develop effective country wide *Fusarium* wilt disease resistance breeding strategies, integrated disease management measures and rapid and accurate disease diagnostic technique to combat wilt disease of chickpea in Ethiopia.

8. Lists of Publications/manuscripts

I. **Bekele, D**.,Tesfaye, K., Fikre, A. Cook, D.R. (2021).The extent and association of chickpea Fusarium wilt and root rot disease pressure with major biophysical factors in Ethiopia. *J. Plant Pathol*. https://doi.org/10.1007/s42161-021-00779-4

II. **Bekele, D**., Tesfaye, K., Fikre, A. (2020).Genetic diversity analysis of advanced chickpea (*Cicer arietinum* L.) genotypes in Ethiopia for identification of high-yielding and novel *Fusarium* wilt resistance sources. *Journal of Crop Sci. Biotechnol.* **24**:191–201.https://doi.org/10.1007/s12892-020-00071-4

III. **Bekele, D**., Tesfaye, K., Fikre, A. Cook, D.R.(2021). Identification of a Novel Quantitative Trait Loci (QTL) for *Fusarium* Wilt Resistance in Chickpea (*Cicer arietinum* L.). (*In preparation)*

IV. **Bekele, D**., Tesfaye, K., Fikre, A. Cook, D.R.(2021). Genomic diversity and geographic distribution of *Fusarium*wilt pathogen of chickpea (*Fusarium oxysporum f. sp. ciceris*) in Ethiopia (*In preparation*)

V. Alford, B., **Bekele, D**., Fayyaz, A.,Garcia, N.C., Tesfaye, K., Fikre, A., and Cook, D.R. (2021).Utilization of microbial community ecology analysis to identify and characterize wilt diseases of chickpea in Ethiopia. (*In preparation*)

9. References

Abera, M., Sakha, P.K. Fininsa, C., Ahmed, S. (2011). Status of Chickpea *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *ciceris*) in North Western Ethiopia. *Arch. Phytopathol. Plant Prot.* **44**:1261-1272. doi:10.1080/03235408.2010.490410

Abi-Ghanem, R., Carpenter-Boggs, L., Smith, J.L., 3 and Valdemar, G.J. (2012). Nitrogen Fixation by US and Middle Eastern Chickpeas with Commercial and Wild Middle Eastern Inocula. International Scholarly Research Network ISRN Soil Science, Volume **2012**: 1-5. ID 981842, doi:10.5402/2012/981842

Abraham, A.D., Menzel, W., Varrelmann, M., Vetten H.J. (2009). Molecular, serological and biological variation among chickpea chlorotic stunt virus isolates from five countries of North Africa and West Asia. *Arch. Virol.* **154**:791–799.

Adams, I.P., Fox, A., Boonham, N. (2018).The impact of high throughput sequencing on plant health diagnostics. *Eur.J.Plant Pathol.* **152:** 909–919. https://doi.org/10.1007/s10658-018-1570-0

Agarwal, G., Garg, V., Kudapa, H., Doddamani, D., Pazhamala, L.T., Khan, A.W., Thudi, M., Lee S.H., Varshney, R.K.(2016). Genome-wide dissection of AP2/ERF and HSP90 gene families in five legumes and expression profiles in chickpea and pigeonpea. *Plant Biotechnol. J.* **14**:1563–1577

Agrios, G.N. (2005). Plant Pathology, 5th ed. *Elsevier Academic Press*, *San Diego,* pp.725-750.

Ahmad, F., Gaur, P.M., Croser, J. (2005). Chickpea (*Cicer arietinum* L.). In Grain Legumes, Genetic Resources, Chromosome Engineering, and Crop Improvement; Singh, R.J., Jauhar, P.P., Eds.; CRC Press: Boca Raton, FL, USA, Volume 1, pp. 187–217.

Ahmed, S., Melkamu, A. (2006). Chickpea, lentil, grass pea, fenugreek and lupine disease researchin Ethiopia. In: Ali, K., Kenneni, G., Ahmed, S., Malhorta, R., Beniwal, S., Makkouk, K., Halila, M.H., (Eds.). Food and forage legumes of Ethiopia: progress and prospects. Proceedings of the Workshop on Food and Forage Legume, pp. 215–220.

Asrat, Z. (2017). Significance and Managment of Chickpea Wilt/root rot and Future Prospects in Ethiopia. *A Review, Int.J.life Sci.***5**:117-136

Al-taae, A.K., Hadwan, H.A., Al-jobory, S.A.E. (2013). Physiological Races of *Fusarium oxysporum* f. sp. *Ciceris* in Iraq. *J. Life Sci.* **7**: 1070–1075.

Ali, M.Y., Johansen, C., Krishnamurthy, L. Hamid, A. (2005). Genotypic variation in root systems of chickpea (*Cicer arietinum* L.) across environments. *J. Agron. Crop Sci.* **191**: 464–472.

Allard, R.W.(1960). Principles of Plant Breeding. *John Willey and Sons. Inc*. New York, pp: 36.

Amaral Zettler, L. A., Mc Climent, E. A., Ducklow, H. W., Huse, S. M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA Genes. *PLoS ONE* 4:e 6372. http://doi.org/10.1371/journal.pone.0006372.

Appel, D.J., Gordon,T.R.(1995).Intraspecific variation within populations of Fusarium oxysporum based on RFLP analysis of the intergenic spacer region of the rDNA. *Exper. Mycol.* **19:** 120-128.

Asnake Fikre**,** Haile Desmae, and Seid Ahmed (2020).Tapping the Economic Potential of Chickpea in Sub-Saharan Africa. *Agron*. **10(**11**):**1707; doi:10.3390/agronomy10111707

Baayen, R.P., ODonnel, K., Bonants, P.J.M., Cigelnik, E., Kroon, L.P.N.M., Roebroeck, E.J.A., and Waalwijk, C. (2000). Gene genealogies and AFLP analyses in

the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. *Phytopathol.* **90**:891-900. doi: 10.1094/phyto.2000.90.8.891

Barve, M.P., Haware, M.P., Sainani, M.N., Ranjekar, P.K., Gupta, V.S (2001). Potential of microsatellites to distinguish four races of *Fusarium oxysporum* f. sp. *ciceri* prevalent in India. *Theor. Appl. Genet.* **102**:138–147.

Banerjee, S., Walder, F., Buchi, L., Meyer, M., Held, A., Gattinger, A., Keller, T., Charles, R., Heijden, M.G.(2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J.* **13**: 1722–1736. https://doi.org/10.1038/s41396-019-0383-2.

Bayraktar, H., Dolar, F.S. (2012). Pathogenic variability of Fusarium oxysporum f. sp. ciceris isolates from chickpea in Turkey. *Pakistan J. Bot*. **44**: 821–823.

Bejiga, G., Tulu A., Seifu, T. (1994). Effect of sowing date and seeding rate on the yield and other characters of chickpea (*Cicer arietinum* L). *Eth. J. Agr. Sci.* **14**:7–14.

Bejiga, G., Eshete, M., Anbessa, Y. (1996). Improved cultivars and production technology of Chickpea in Ethiopia. Debre Zeit Agricultural Research Center (DZARC), Debre Zeit, Ethiopia, 60pp.

Beniwal, S.P.S., Ahmed, S., Gorfu, D.(1992). Wilt/root rot diseases of chickpea in Ethiopia. *Trop. Pest Manag.* **38**:48–51.

Berhanu, B., Kumari, S.G., Kemal, A., Yusuf, A., Makkouk, K.M., Mandefro, A., Melkamu, A., Gelelbelu, G., Dereje, H. (2005). Survey of viruses affecting legume crops in the Amhara and Oromia regions of Ethiopia. *Phytopathol. Mediterr.* **44**:235–246.

Bevan, M.W., Flavell, R.B., Chilton, M.D. (1983). A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. *Nat***. 304**: 184–187

Buddenhagen, I.W., Richards, R.A.(1988). Breeding cool season food legumes for improved performance in stress environments. In: World Crops: Cool Season Food Legumes, pp. 81-95, (Summer field, R.J., ed). Kluwer Academic Publishers, The Netherlands.

Callahan, B.J, McMurde, P.J., Rosen, M.J., Han, A.W., Johnson A.J.A., Holmes S.P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods*. 13: 581–583.

Callahan, B.J. (2018). Silva taxonomic training data formatted for DADA2 (Silva version 132) [Data set]. Zenodo. http://doi.org/10.5281/zenodo.1172783

Central Statistical Agency (2016). Agricultural sample survey 2015/2016. Report on area and production of major crops. Statistical Bulletin no. 583. Addis Ababa: The Federal Democratic Republic of Ethiopia.

Chitrampalam P., Abraham N., Nelson, B.D. (2018). Culture Independent PCR Based Assay to Detect the Root Rot Pathogen *Fusarium* solani Species Complex 11 from Soybean Roots and Soil. Plant Disease, 102327-333, https://doi.org/10.1094/PDIS-03-17-0447-RE

Chand, H., Khirbat, S.K.(2009). Chickpea Wilt and Its Management: *Agri. Rev.* 30:1–12.

Chilvers, M.I. (2012). Molecular Diagnostics in Plant Disease Diagnostic Clinics…What's the Status? *Fungal Genom Biol.* **2**:1 doi:10.4172/2165-8056.1000e102

Cho, S., Muehlbauer, F. J. (2004). Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (*Cicer arietinum* L.). *Physiol. Mol. Plant Pathol.* **64**: 57–66.

Cho, S., Kumar, J., Shultz, J.L., Anupama, K., Tefera, F., Muehlbauer, F.J.(2002). Mapping genes for double podding and other morphological traits in chickpea. *Euphytica*. **128**:285–292.

Chobe, D.R, Gupta, O, Pawar, M. (2016). Radiation induced mutation for resistance against races/ pathotypes of *Fusarium oxysporum* f.sp *ciceris* in chickpea (*Cicer arietinum* L.). *Indian Phytopath*. **69**:260–265. Available, online at: http://epubs.icar.org.in/ejournal/index.php/IPPJ/article/view/71426.

Chongo, G., Gossen, B. D., Buchwaldt, L., Adhikari, T., Rimmer, S. R. (2004). Genetic diversity of *Ascochyta* rabiei in Canada. *Plant Dis.* **88**: 4–10.

Choudhary, P., Khanna, S.M., Jain, P.K., Bharadwaj, C., Kumar, J., Lakhera, P.C., Srinivasan, R. (2012a). Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genet.Mol. Res.***11(2)**:891-905. http://dx.doi.org/10.4238

Chu, Y., Chee, P., Culbreath, A., Isleib, T.G., Holbrook, C.C., Ozias, A. P. (2019). Major QTLs for Resistance to Early and Late Leaf Spot Diseases Are Identified on Chromosomes 3 and 5 in Peanut (*Arachis hypogaea*). *Front. Plant Sci.* 10883. Doi: 10.3389/fpls.2019.00883

Cobos, M.J., Winter, P., Kharrat, M., Cubero, J.I., Gil, J., Millan, T., Rubio, J.(2009). Genetic analysis of agronomic traits in a wide cross of chickpea. *Field Crop Res*. **111**:130–136

Cobos, M.J., Fernandez, M.J., Rubio, J., Kharrat, M., Moreno, M.T., Gil, J., Millan, T. (2005). A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli x Desi crosses: Location of genes for resistance to Fusarium wilt race 0. *Theor. Appl. Genet.* **110**:1347–1353.

Cobos, M.J., Rubio, J., Fernandez-Romero, M.D., Garza, R., Moreno, M.T., Millan, T., Gil, J. (2007). Genetic analysis of seed size, yield and days to flowering in a

chickpea recombinent inbred line population derived from a kabuli x desi cross. *Ann. Appl. Biol.* **151**: 33–42.

Collard, B.C.Y., Mackill, D.J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B*. **363**:557–572.

Cooper, M., Gho, C., Leafgren, R., Tang, T., Messina, C.(2014). Breeding drought-tolerant maize hybrids for the us corn-belt: discovery to product. *J. Exp. Bot*. **65**: 6191–6204.doi:10.1093/jxb/eru064.

Cramer, R.A., Byrne, P.F., Brick, M.A., Panella, L., Wickliffe, E., Schwartz, H.F.(2003). Characterization of Fusarium oxysporum isolates from common bean and sugar beet using pathogenicity assays and random amplified polymorphic DNA markers. *J. Phytopathol.* **151**:352–360

Cunnington, J., Lindbeck, K., Jones, R.H. (2007). National Diagnostic Protocol for the Detection of Fusarium Wilt of Chickpea (*Fusarium Oxysporum* f. sp. *ciceris*). Plant Health Australia, Camberra, Australia.

Dakora, F.D., Keya, S.O.(2000). Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biol. Bioch*. **29**:809-817.

Damte T, Ojiewo C.O.(2016). Current status of wilt/root rot diseases in major chickpea growing areas of Ethiopia. *Arch. Phytopathol. and Plant Prot.*. ISSN: 1477-2906. Available at http://dx.doi.org/10.1080/03235408.2016.1180925

DARwin (Dissimilarity Analysis Representation for window) computer program software Version 6. Available at (http://darwin.cirad.fr). Accessed on May 20, 2020.

Dean, R. (2012).The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **1**:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Delp, B.R., Stowell, L.J., Msrois, J.J. (1986). Evaluation of field sampling techniques for estimation of disease incidence. *Phytopathol.* **76**:1299-1305.

Demers, J.E, Garzon, C.D., Jimenez-Gasco M.M. (2014). Striking genetic similarity between races of Fusarium oxysporum f. sp. ciceris confirms a monophyletic origin and clonal evolution of the chickpea vascular wilt pathogen. *Eur. J. Plant. Pathol.* **139**:309–324. DOI 10.1007/s10658-014-0387-8

Deokar, A., Sagi, M., Tar'an, B. (2019). Genome-wide SNP discovery for development of high-density genetic map and QTL mapping of ascochyta blight resistance in chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet.* **132**:1861–1872

Dikkar, M. and Deshmukh, V.V. (2003). Nutrional requirement of *Fusarium* wilt of chickpea. *Crop Res.* **25(1)**: 197-199.

Dita, M.A., Rispail, N., Prats, E., Rubiales, D. and Singh, K.B. (2006). Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. *Euphytica* **147**:1–24

Dubey, S.C., Priyanka, K., Singh, V., Singh, B. (2012). Race profiling and molecular diversity analysis of *Fusarium oxysporum f. sp. ciceris* causing wilt in Chickpea. *J Phytopathol*. **160**:576–587.

Dubey, S.C., Singh, S.R.(2008).Virulence analysis and oligonucleotide fingerprinting to detect diversity among Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Mycopathol.***165**(6):389–406.

Dubey, S.C., Suresh, M., Singh, B. (2007). Evaluation of Trichoderma species against *Fusarium oxysporum* f.sp *ciceris* for integrated management of chickpea wilt. *Biol. Cont.* **40**: 118-127.

Dubey, S.C, Priyanka, K., Singh, V. (2014). Phylogenetic relationship between different race representative populations of *Fusarium oxysporum* f. sp. *ciceris* in

respect of translation elongation factor-1α, β-tubulin, and internal transcribed spacer region genes. *Arch. Microbiol.* **196**:445–452, DOI 10.1007/s00203-014-0976-0

Ebbole, D. Sachs, M. S. (1990). A rapid and simple method for isolation of Neurospora crassa homokaryons using microconidia. *Fungal Genet.* **37**:17-18.

Edel, V., C. Steinberg, N. Gautheron, C. Alabouvette (2000). Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. *Mycol. Res.***104**: 518-526.

Edwards, M.D., Stuber, C.W., Wendel, J.F. (1987). Molecular marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genet.* **116**: 113–125

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS ONE* **6**:e19379.

Epstein, L., Kaur, S., Chang, P.L., Carrasquilla-Garcia, N., Lyu, G., Cook, D.R., Subbarao, K.V. and O'Donnell, K.(2017). Races of the celery pathogen *Fusarium oxysporum* f. sp. *apii* are polyphyletic. *Phytopathol*. **107**:463-473. https://doi.org/10.1094/PHYTO-04-16-0174-R.

Eyasu, E.(2016). Soils of the Ethiopian Highlands: Geomorphology and Properties. CASCAPE Project, ALTERA, Wageningen University and Research Centre (Wageningen UR). The Netherlands. 385pp

Fageria, N.K., Baligar, V.C., Li, Y.C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *J. Plant Nutr.* **31**:1121–1157.

FAO (1986). The Ethiopian Highlands Reclamation Study (EHRS). Volume 1 Main Report. Food and Agriculture Organization, FAO, Rome

FAOSTAT (Food and Agriculture Organization of the United Nations Statistical Database) (2018). FAOSTAT Production Statistics of Crops. Available at http://www.fao.org/faostat/en/#data/QC. Accessed on 16, August, 2020.

FAOSTAT (Food and Agriculture Organization of the United Nations Statistical Database) (2019). FAOSTAT Production Statistics of Crops. Available at http://www.fao.org/faostat/en/#data/QC. Accessed on 29, May, 2021.

Fang Y., Ramasamy R.P.(2015). Current and Prospective Methods for Plant Disease Detection. *Biosensors.* **4:** 537-56, ISSN 2079-6374 http:// doi:10.3390/bios5030537.

Farr, D. F., Bills, G, F., Chamuris, G. P. and Rossman, A.Y.(1989). Fungi on plant and plant product in the United States. *American Phytophythological Society*, St. Paul.

Fikre, A. (2014). An overview of chickpea improvement research program in Ethiopia. *J. Inter. Legume Soci.* 3: Available at https://www.researchgate.net/publication/313852048. Accessed on 11, August 2019.

Fikre, A., Korbu, L., Eshete, M., Bekele, D., Girma,N., Mohamed, R. (2018). A decade of research progress in chickpea and lentil breeding and genetics. *Ethiop. J. Crop Sci.* **6:**101–113. Available online at: http://oar.icrisat.org/11056/1/110-122.pdf

Fininsa C., Yuen, J. (2001).Association of maize rust and leaf blight epidemics with cropping systems in Hararghe Highlands, Eastern Ethiopia. *Crop Prot*. **20**:669–678. https://doi.org/10.1016/S0261-2194(01)00033-3.

Fischer, R.A., Byerlee, D., Edmeades, G.O. (2009). Can Technology Deliver on the Yield Challenge to 2050. Rome: Food and Agriculture Organization of the United Nations.

Fraley, R.T., Rogers, S.G., Horsch, R.B., Sanders, P.R., Flick, J.S., Adams, S.P., Bittner, M.L., Brand, L.A., Fink, C.L., Fry, J.S. (1983). Expression of bacterial genes in plant cells. *Proc. Natl. Acad. Sci. USA*. **80**: 4803–4807.

Fredericks, D.N., Relman, D. A.(1996). Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. *Clin. Microbiol. Revi*, **9:**18–33.

Frias, J., Vidal-Valverde, C., Sotomayor, S., Diaz-Pollan, C., Urbano, G. (2000). Influence of processing on available carbohydrate content and ant nutritional factors of chickpeas. *Eur. Food Res. Technol.*, **210 (5),** 340–345.

Garg, T., Mallikarjuna, BP, Thudi, M. Samineni, S., Singh, S., Sandhu, JS, Kaur, L. Singh, I. Sirari, A. Basandrai, A.K. Basandrai, D., Varshney, R.K.. Gaur, P.M. (2018). Identification of QTLs for resistance to Fusarium wilt and Ascochyta blight in a recombinant inbred population of chickpea (Cicer arietinum L.). *Euphytica*. **214**:45. https://doi.org/10.1007/s10681-018-2125-3

Gaur, P.M., Jukanti, A.K., Srinivasan, S., Gowda, C.L.L.(2012a). Chickpea (*Cicer arietinum* L.), In: Bharadwaj DN(ed) Breeding of field crops. Agrobios (India), Jodhpur pp 165–194

Gaur, P.M., Jukanti, A.K., Varshney, R.K. (2012b). Impact of Genomic Technologies on Chickpea Breeding Strategies. *Agron*. **2(3)**:199-221. http://doi:10.3390/ agronomy 2030199

Gaur, P.M., Kumar, J., Gowda, C.L.L., Pande, S., Siddique, K.H.M., Khan, T.N., Warkentin, T.O., Chaturvedi, S.K., Than, A.M., Ketema, D. (2005). Breeding Chickpea for Early Phenology: Perspectives, Progress and Prospects. In Food Legumes for Nutritional Security and Sustainable Agriculture, Proceedings of Fourth International Food legumes Research Conference; Kharkwal, M.C., Ed.; IARI, New Delhi, India, 18–22 October.

Gaur, P.M., Mallikarjuna, N., Knights, T., Beebe, S., Debouck, D., Mejia, A., Malhotra, R.S., Imtiaz, M., Sarker, A., Tripathi, S. (2010). Gene Introgression in Grain Legumes. In Grain Legumes: Genetic Improvement, Management and Trade; Gupta, S., Ali, M., Singh, B.B., Eds.; Indian Society of Pulses Research and Development: IIPR, Kanpur, India,; pp. 1–17.

Gaur, P.M., Thudi, M., Samineni, S., Varshney, R.K.(2014). Advances in Chickpea Genomics, in Legumes in the Omic Era. Gupta S, Nadarajan N, Gupta DS(eds). ISBN 978-1-4614-8369-4, DOI 10.1007/978-1-4614-8370-0. Springer, New York, Heidelberg Dordrecht, London, pp. 73-94. https://doi 10.1007/978-1-4614-8370-0

Getachew Agegnehu and Woldeyesus Sinebo (2012). Drainage, sowing date and variety effects on chickpea grown on a Vertisol in Ethiopia. *Arch. Agron. Soil Sci.* **58**:1,101-113

Gomez, K.A., Gomez A.(1984). Statistical Procedures for Agricultural Research. John Wiley & Sons. New York

Gordon, T. R., Martyn, R.D.(1997). The evolutionary biology of Fusarium oxysporum. *Ann. Rev. Phytopathol.* **35**:111-128. doi: 10.1146/annurev.phyto.35.1.111

Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007). DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int. J. Syst. Evol. Microbiol.* **57(1):** 81–91. doi: 10.1099/ijs.0.64483-0

Gowda, S.J.M., Radhika, P., Kadoo, N.Y., Mhase, L.B., Gupta, V.S. (2009). Molecular mapping of wilt resistance genes in chickpea. *Mol. Breed.*, **24**:177–183.

Groenewald, S., Berg, N. V. D., Marasas, W. F. O. and Viljoen A. (2006). Biological, physiological and pathogenic variation in a genetically homogenous population of *Fusarium oxysporum f. sp. cubense*. *Austr. Plant Pathol.*. **35**: 401-409.

Gumber, R.K., Kumar, J., Hanaware, M.P (1995). Inheritance of resistance to *Fusarium* wilt in chickpea. *Plant Breed.* **114**: 277–279.

Gupta, O., Kotasthane, S.R., Khare, M.N.(1987). Factors influencing epidemiology of vascular wilt of chickpea. *Proc. Natl. Acad. Sci.* **57**: 86-91.

Gupta P K, Langridge P, Mir R R. (2010). Marker-assisted wheat breeding: Present status and future possibilities. *Mol. Breed.*, **26**:145–161.

Gupta, O, Khare, M. N. and Kotasthane, S. R. (1986). Variability among six isolates of *Fusarium oxysporum* f.sp. *ciceris* causing wilt of chickpea. *Indian Phytopathol.* **39**:279-281.

Gupta, S., Nadarajan, N., Gupta, D.S. (2014). *Legumes in Omic Era: Retrospects and Prospects in* S. Gupta et al. (eds.), Legumes in the Omic Era, DOI 10.1007/978-1-4614-8370-0_1

Gur, A., Zamir, D. (2004). Unused natural variation can lift yield barriers in plant breeding. *PLoS Biol.* **2(10)**:245.

Gurjar, G., Brave, M., Giri, A., Gupta, V. (2009). Identification of Indian pathogenic races of *Fusarium oxysporum* f. sp. *ciceris* with gene specific, ITS and random markers. *Mycol.*. **101**:484–495

Hajibarat, Z., Saidi, A., Hajibarat, Z., Talebi, R. (2014). Genetic diversity and population structure analysis of landrace and improved chickpea (*Cicer arietinum* L.) genotypes using morphological and microsatellite markers. *Environ. Exper.* **12**:161-166. ISSN 2255-9582

Halila, H.M., Strange, R.(1996). Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f. sp. *ciceris race* 0. *Phytopathol.Mediter.* **35**:67-74

Halila, I., Cobos, M.J., Rubio, J., Millan, T., Kharrat, M., Marrakchi, M., Gil, J. (2009).Tagging and mapping a second resistance gene for *Fusarium* wilt race 0 in chickpea. *Eur. J. Plant Pathol1*. **24:**87–92

Halila, I., Rubio, J., Millan, T., Gil, J., Kharrat, M., Marrakchi, M. (2010). Resistance in chickpea (*Cicer arietinum*) to *Fusarium* wilt race "0". *Plant Breed.***129**:563–566.

Hammer, K. (1984). Das Domestikations syndrom. *Kulturp.* **32**: 11–34.

Han, S., Yuan, M., Clevenger, J.P., Li, C., Hagan, A., Zhang, X., Chen, C. He, G. (2018). A SNP-Based Linkage Map Revealed QTLs for Resistance to Early and Late Leaf Spot Diseases in Peanut (*Arachis hypogaea* L.). *Front. Plant Sci.* **9** :10-12. Doi: 10.3389/fpls.2018.01012

Hartmann, M., Frey, B., Mayer(2015). Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J*. **9:**1177–1194. https://doi.org/10.1038/ismej.2014.210

Haque, I., Jutzi, S. (1984). Nitrogen fixation by forage legumes in sub-Saharan Africa: Potential and limitations. *ILCA Bulletin.* **20**: 2-13.

Haware, M.P.(1990a). Fusarium wilt and other important diseases chickpea in the Mediterranean area. In Present Status and Future Prospects of Chickpea Crop Production and Improvement in the Mediterranean Countries; Saxena, M.C., Cubero, J.I.,Wery, J., Eds.; CIHEAM: Zaragoza, Spain; pp. 61–64.

Haware, M.P., Jim_enez-Diaz, R.M., Amin, K.S., Phillips, J.C., Halila, M.H.(1990b). Integrated management of wilt and root rots of chickpea. In: Chickpea in the Nineties (Ed.), ICRISAT (International Crops Research Institute for the Semi Arid Tropics). ICRISAT Center, Patancheru, India, pp. 129-133.

Haware, M.P., Nene, Y.L (1980). Influence of wilt at different stages on the yield loss in chickpea.Tropical Grain Legume Bulletin **19**:8–40.

Haware, M.P., Nene, Y.L., Natarajan, M. (1996). Survival of Fusarium oxysporum f.sp. ciceri in soil in the absence of chickpea. *Phytopathol. Mediter*. 35:9-12, ISSN 0031-9465.

Haware, M.P., Nene, Y.L., Rajeshwari, R. (1978). Eradication of *Fusarium oxysporum* f.sp. *ciceris* transmitted in chickpea seed. *Phytopathol.* **68**:1364–1367.

Haware, M. P., Nene, Y. L. (1982). Races of *Fusarium oxysporum* f. sp. *ciceris. Plant Dis.* **66**: 809–810.

Herrera-Estrella, L., Depicker, A., van Montagu, M., Schell, J. (1983) Expression of chimaeric genes transferred into plant cells using a Ti-plasmidderived vector. *Nat.* **303:** 209–213

Hiremath, P.J., Kumar, A., Penmetsa, R.V., Farmer, A., Schlueter, J.A., Chamarthi, S.K., Whaley, A.M., Garcia, N.C., Gaur, P.M., Upadhyaya, H.D., Kavi, K.P.B., Shah, T.M., Cook, D.R., Varshney, R.K. (2012). Large-scale development of cost effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes. *Plant Biotechnol. J.* **10**: 716–732. https:// doi: 10.1111/j.1467-7652.2012.00710.x

Honnareddy, N., Dubey, S.C. (2006). Pathogenic and molecular characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Curr. Sci.* **91**:661–666.

Hossain, M. M., Nilufar, H., Farjana, S., Shah, M., Naimul, I., Shaikul, M.d., and Khurshed, M.d., Bhuiyan, A. (2013). Integrated management of Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris* with microbial antagonist, botanical extract and fungicide. *Afr.J. Biotechnol.* **12 (29)**:4699-4706.

Hossain, S., Ford, R., McNeil, D., Pittock, C., Panozzo, J.F. (2010). Inheritance of seed size in chickpea (*Cicer arietinum* L.) and identification of QTL based on 100-seed weight and seed size index. *Aust.J.Crop Sci.* **4:**126–135.https://doi.org/10.1007/s10681-018-2125-3

Hu, Y., Green, G.S., Milgate, A.W., Stone, E.A., Rathjen, J.P.,Schwessinger., B. (2019). Pathogen Detection and Microbiome Analysis of Infected Wheat Using a Portable DNA Sequencer. *Phytobio. J.* **3:**92-101.

Huynh, B.L., Ehlers, J.D., Huang, B.E., Amatriaın, M.M., Lonardi, L., Santos, J.R.P., Ndeve, A., Batieno, B.J., Boukar, Q., Cisse, N., Drabo, I., Fatokun, C., Kusi, C., Agyare, R.Y., Guo, Y.N., Herniter, I., Lo, S., Wanamaker, S.I., Xu, S., Close, T.G., Roberts, P.A.(2018). A multi-parent advanced generation inter-cross (MAGIC) population for genetic analysis and improvement of cowpea (Vigna unguiculata L. Walp.). *The Plant J.* **93**:1129–1142. https://doi:10.1111/tpj.13827

Iruela, M., Rubio, J., Cubero, J.I., Gil, J., Milan, T. (2002). Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. *Theor. Appl. Genet*. **104**:643–651

Jabran, K., A. Ali, Z. Ali, A. Sat tar, A. Aulakh, M., Iqbal J., Nadeem. M.A. (2012). Higher seed rates increase grain yield in chickpea (*Cicer arietinum* L.) strains. *Crop Environ*. 3:19-22.

Jacobson, D. J., Gordon. T. R. (1990). Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. *Mycolog. Res.*. **94:** 734-744.

Jaganathan, D., Thudi, M., Kale, S., Azam, S., Roorkiwal, M., Gaur, P.M., Kishor, P.B., Nguyen, H.T., Sutton, T., Varshney, R.K.(2015). Genotyping- by-sequencing based intra-specific genetic map refines a ‘‘QTL-hotspot” region for drought tolerance in chickpea. *Mol. Genet Genomics*. **290**:559–571

Jain, M., Misra, G., Patel, R.K., Priya, P., Jhanwar, S., Khan, A.W., Shah, N., Singh, V.K., Garg, R., Jeena, G., Yadav, M., Kant, C., Sharma, P., Yadav, G., Bhatia, S., Tyagi, A.K., Chattopadhyay, D. (2013). A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). *Plant J.* **74**:715–29.

Jalali, B.L., Chand, H.(1992). Chickpea wilt. In: Singh, U.S., Mukhopadhayay, A.N., Kumar, J., Chaube, H.S. (Eds.), Plant Diseases of International Importance, Diseases of Cereals and Pulses, vol. I. Prentice Hall, Englewood Cliffs, NJ, USA, pp. 429-444

Jamil, F.F., Sarwar, N., Sarwar, M., Khan J.A., Geinstlinger, J.,Kahal, G.(2000). Genetic and pathogenic diversity with in Ascochyta rabiei (Pass.) Lab. Populations in Pakistan causing blight of chickpea (Cicer arientinum L.). *Physiol. Mol. Plant Pathol.* **57**: 243-254.

Jannatabadi, A.A., Talebi, R., Armin, M., Jamalabadi, J., Baghebani, N. (2014). Genetic diversity of Iranian landrace chickpea (*Cicer arietinum* L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers. *J. Plant. Biochem. Biotechnol*.**23**:225-229

Jendoubi, W., Bouhadida, M., Boukteb, A., Beji, M., Kharrat, M .(2017). Fusarium Wilt Affecting Chickpea Crop. *J. Agri.* **7**:23. https://doi:10.3390/agriculture,7030023.

Jimenez-Diaz, R.M, Castillo P, Jimenez Gasco, M.M, Landa, B.B., Navas Cortes, J.A. (2015).*Fusarium* wilt ofchickpeas: biology, ecology and management. *Crop Prot.* **73:**16–27. https://doi.org/10.1016/j.cropro.2015.02.023.

Jimenez-Diaz, R.M., JimEnez-Gasco, M.M.(2011). Integrated Management of *Fusarium* Wilt Diseases. In Control of Fusarium Diseases; Transworld Research Network: Kerala, India, pp. 177–215.

Jimenez-Diaz, R. M., Trapero-Casas, A., and de la Colina, J. C.(1989). Races of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea in Southern Spain, in Vascular Wilt Diseases of Plants, NATO ASI Series, eds E. C. Tjamos and C. H. Beckman (Berlin: Springer Verlag), 515–520. doi: 10.1007/978-3-642-73166-2_39

Jimenez-Fernandez, D., Landa, B.B., Kang, S., Jimenez-Diaz, R.M., Navas-Cortes, J.A.(2013). Quantitative and microscopic assessment of compatible and incompatible interactions between chickpea cultivars and *Fusarium oxysporum* f. sp. *ciceris* races. *PLoS One* 8 (4), e61360. http://dx.doi.org/10.1371/journal.pone.0061360.

Jimenez-Fernandez, D., Montes-Borrego, M., Navas-Cortes, J.A., Jimenez Diaz, R.M., Landa, B.B., (2010). Identification and quantification of Fusarium oxysporum

in planta and soil by means of an improved specific and quantitative PCR assay. *Appl. Soil. Ecol*. **46**: 372-382.

Jimenez-Fernandez, D., Montes-Borrego, M., Jimenez-Diaz, R.M., Navas-Cortes, J.A., Landa, B.B. (2011a). In Planta and Soil Quantification of *Fusarium oxysporum f. sp. ciceris* and Evaluation of FusariumWilt Resistance in Chickpea with a Newly Developed Quantitative Polymerase Chain Reaction Assay. *Phytopathol.* **101**:250–262

Jimenez-Fernandez, D., Navas-Cortes, J.A., Montes-Borrego, M., Jimenez-Diaz, R.M., Landa, B.B., (2011b). Molecular and pathogenic characterization of Fusarium redolens, a new causal agent of Fusarium yellows in chickpea. *Plant Dis.* **95**: 860-870.

Jimenez Gasco, M.M., Jimenez Diaz, R.M (2003). Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 5, and 6. *Phytopathol.* **93**: 200-209.

Jimenez Gasco, M., Perez-Artes, E., Jimenez-Diaz, R.M. (2001). Identification of Pathogenic Races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f. sp. *ciceris* with Random Amplified Polymorphic DNA (RAPD). *Eur. J. Plant Pathol*.**107**: 237–248.

Jimenez-Gasco MM, Milgroom MG, Jimenez-Diaz RM (2002). Gene genealogies support *Fusarium oxysporum* f. sp. *ciceris* as a monophyletic group. *Plant Pathol*. **51**:72–77.

Jimenez-Gasco MM, Milgroom MG, Jimenez-Diaz R M (2004a). Stepwise evolution of races in *Fusarium oxysporum f. sp. ciceris* inferred from fingerprinting with repetitive DNA sequences. *Phytopathol*. **94**:228–235.

Jimenez-Gasco M, Navas-Cortes JA, Jimenez-Diaz RM (2004b). The *Fusarium oxysporum* f.sp *ciceris*/*Cicer arietinum* pathosystem: a case study of the evolution of plant-pathogenic fungi into races and pathotypes. *Int. Microbiol.*. 7, 95–104.

Joshi, P.K., Rao, P., Gowda, C.L.L., Jones, R.B., Silim, S.N., Saxena, K.B., Kumar, J. (2001). The World Chickpea and Pigeonpea Economies: Facts, Trends and Outlook. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pardesh, India.

Kaiser, W.J., Klein, R.E., Larsen, R.C., Wyatt, S.D.(1993). Chickpea wilt incited by Pea streak carlavirus. *Plant Dis*. 77, 922-926.

Kale, S.M., Jaganathan, D., Ruperao, P., Chen, C., Punna, R., Kudapa, H., Thudi, M., Roorkiwal M., Katta, M.A., Doddamani, D., Garg, V., Kishor, P.B.K., Gaur, P.M., Nguyen, H.T., Batley, J., Edwards, D., Sutton, T., Varshney, R.K. (2015). Prioritization of candidate genes in "QTL-hotspot" region for drought tolerance in chickpea (*Cicer arietinum* L.). *Sci. Rep*. **19**:15296

Kashiwagi, J., Krishnamurthy, L., Crouch, J.H., Serraj, R. (2006). Variability of root length density and its contribution to seed yield in chickpea (*Cicer arietinum* L) under terminal drought stress. *Field Crops Res*. **95**:171–181.

Kassie, M., Shiferaw, B., Asfaw, S., Abate, T., Muricho, G., Ferede, S., Eshete, M. and Assefa, K. (2009). Current situation and future outlooks of the chickpea sub-sector in Ethiopia. ICRISAT and EIAR. (http://www.icrisat.org/tropicallegumesII/pdfs/Current_Situation.pdf).

Katan, T. (1999). Current status of vegetative compatibility groups in Fusarium oxysporum. *Phytoparas.***27**: 51-64.

Kelly, A.G., Alcala-Jimenez, A.R., Bainbridge, B.W., Heale, J.B., Perez-Artes, E., Jimenez-Diaz, R.M.(1994). Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea. *Phytopathol.* 4:1293–1298.

Kelly, A. G., Bainbridge, B. W, Heale, J. B., Perez-Artes. E. and Jimenez-Diaz, R. M. (1998). In planta PCR detection of the wilt-inducing pathotype of *Fusarium*

oxysporum f.sp. *ciceris* in chickpea (*Cicer arietinum* L). *Physiol. Mol. Plant Pathol.* **52**: 397–409

Keneni, G., Bekele, E., Imtiaz, M., Dagne, K., Getu, E., Assefa, F. (2012). Genetic Diversity and Population Structure of Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Accessions from Different Geographical Origins as Revealed by Microsatellite Markers. *Plant Mol. Biol. Rep*. **30**:654–665. https://doi 10.1007/s11105-011-0374-6.

Kirkegaard, J., Christen, O., Krupinsky, J. and Layzell, D. (2008). Break crop benefits in temperate wheat production. *Field Crops Res.*. **107**:185–195.

Kistler, H.C. (1997). Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathol*. **87**:474-479.

Kole, C., Muthamilarasan, M., Henry, R., Edwards, D., Sharma, R., Abberton, M., Batley, J., Bentley, A., Blakeney, M., Bryant, J., Cai, H., Cakir, M., Cseke, L.J., Cockram, J., de Oliveira A.C., De Pace, C., Dempewolf, H., Ellison, S., Gepts, P., Greenland, A., Hall, A., Hori, K., Hughes, S., Humphreys, M.W., Iorizzo, M., Ismail, A.M., Marshall, A., Mayes, S., Nguyen, H.T., Ogbonnaya, F.C., Ortiz, R., Paterson, A.H., Simon, P.W., Tohme, J., Tuberosa, R., Valliyodan, B., Varshney, R.K., Wullschleger, S.D., Yano, M. and Prasad, M. (2015). Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. *Front. Plant Sci.* **6**:563. doi: 10.3389/fpls.2015.00563

Konstantinidis, K. T., Tiedje, J. M. (2005). Genomic insights that advance the species definition for prokaryotes. *Proc. Natl. Acad. Sci. US A*. **102**:2567–2572.

Krishnamurthy, L., Gaur, P.M., Basu, P.S., Chaturvedi, S.K., Tripathi, S., Vadez, V., Rathore, A., Varshney, R.K., Gowda, C.L.L. (2011). Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. *Plant Genet. Res*. **9**:59–69.

Krishnamurthy, L., Ito, O., Johansen, C. (1996). Genotypic Differences in Root Growth Dynamics and its Implications for Drought Resistance in Chickpea. In Dynamics of Roots and Nitrogen in Cropping Systems of the Semi-Arid Tropics; JIRCAS Agriculture Series No. 3; Ito, O., Johansen, C., Adu Gyamfi, J.J., Katayama, K., Kumar Rao, J.V.D.K., Rego, T.J., Eds.; Japan International Research Center for Agriculture Sciences: Tsukuba, Japan,; pp. 235–250.

Kudapa, H., Azam, S., Sharpe, A.G., Taran, B., Li, R., Deonovic, B., Cameron, C., Farmer, A.D., Cannon, S.B., Varshney, R.K.(2014). Comprehensive transcriptome assembly of chickpea (*Cicer arietinum* L.) using Sanger and next generation sequencing platforms: development and applications. *PLoS ONE* 9:e86039

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol.Evol.* 35:1547-1549.

Kumar, V., Tripathi, S., Gowda, C.L.L., Sharma, S., Singh, S., Tyagi, A.K., Parida, S.K (2015). A genome-wide SNP scan accelerates trait-regulatory genomic loci identification in chickpea. *Scientific Reports* | 5 11166 | DOI: 10.1038/srep11166. www.nature.com/scientificreports/

Kumar, J., Vijayalakshmi, V.S., Rao, T.S. (2000). Inheritance of flower color in chickpea. *J. Hered.* **91**: 416–417.

Kumar, S.(1998). Inheritance of resistance to Fusarium wilt (race 2) in chickpea. *Plant Breed*. **117**:139–142.

Ladizinsky, G, Adler, A.(1976). The origin of chickpea, *Cicer arietinum* L. *Euphytica*. 25:211-217.

Lamelas, A., Desgarennes, D., Lopez-Lima, D., Villain, L., Alonso-Sanchez, A., Artacho, A., Latorre, A., Moya, A., Carrion, G. (2020) The Bacterial Microbiome of Meloidogne- Based disease Complex in Tabacco and Tomato. *Front.Plant Sci*. **11**:136. doi: 10.3389/fpls.2020.00136

Landa, B. B., Navas-Cortes, J. A. and Jimenez-Diaz, R. M. (2004). Integrated management of Fusarium wilt of chickpea with sowing date, host resistance and biological control. *Phytopathol.* **94**:946-960.

Landau, S., Everitt, B.S.(2004). A handbook of Statistical Analyses using SPSS. Chapman and Hall/CRC Press LLC, USA, pp 339.

Leport, L., Turner, N.C., French, R.J., Barr, M.D., Duda, R., Davies, S.L., Tennant, D. and Siddique, K.H.M.(1999). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Eur. J. Agron*. **11**:279–291.

Leslie, J.F., Summerell, B.A. (2006). The Fusarium Laboratory Manual. Blackwell Professional, 2121 State Avenue, Ames, Iowa 50014, USA, pp. 81–250.

Li, H, Rodda, M., Gnanasambandam, A., Aftab, M., Redden, R., Hobson, K., Rosewarne, G., Materne, M.K.S., Slater, A.T.(2015). Breeding for biotic stress resistance in chickpea: progress and prospects. *Euphytica.* **204**:257–288. https://doi: 10.1007/s10681-015-1462-8

Lichtenzveig, J., Bonfil, D.J., Zhang, H.B., Shtienberg, D., Abbo, S. (2006). Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to Didymella rabiei the causal agent of Ascochyta blight. *Theor. Appl. Genet*. **113**:1357–1369.

Lievens, B., Rep, M., and Thomma, B.P. (2008). Recent developments in the molecular discrimination of *formae speciales* of *Fusarium oxysporum*. *Pest. Manag. Sci.* **64**:781–788.

Lincoln, M., Kumar, V.S., Saugata S, Rani EA, Lal J, Anil, K. (2018). Multi-Parent Advanced Generation Intercross (MAGIC) Population for Genome Mapping in Plant. *Int.. J. Genet. 10(2)*: 343-345. http://dx.doi.org/10.9735/0975-2862.10.2.343-345

Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trail, F., Gardiner, D.M., Manners, J.M., and Kazan, K.(2013). *Fusarium* pathogenomics. *Annu. Rev. Microbiol.* **67**:399-416.

Malcolm, G.M., Kuldau, G.A., Gugino, B.K., Jimenez-Gasco, M.M.(2013). Hidden host plant associations of soil borne fungal pathogens: an ecological perspective. *Phytopathol.* **103**:538-544

Malik, S. R., Iqbal, S. M., Haqqani, A. M. (2006). Resistance screening to Ascochyta blight disease of chickpea in Pakistan. International Chickpea and Pigeonpea Newsletter.**13:**30–31.

Maliro, M.F.A., McNeil, D., Kollmorgen, J., Pittock, C., Redden, R.(2004). Screening Chickpea (*Cicer arietinum* L.) and Wild Relatives Germplasm from Diverse Country Sources for Salt Tolerance. In Proceedings of the International Crop Science Congress; Brisbane, Australia, 26 September –1 October 2004.

Mannetje, L., O'Connor, K.F., Burt, R. L.(1980).The use and adaptation of pasture and fodder legumes. In: Summer field R J and Bunting A. H (Eds). *Advances in legume science*. Royal Botanical Gardens, Kew. pp. 537-551.

Mannur, D.M., Babbar, A., Thudi, M., Sabbavarapu, M.M., Roorkiwal, M., Sharanabasappa, Y.B., Bansal, V.P., Jayalakshmi, S.K., Yadav, S.S., Rathore, A., Chamarthi, S.K., Mallikarjuna, B.P., Gaur, P.M., Varshney, R.K.(2019). Super Annigeri 1 and improved JG 74: two Fusarium wilt-resistant introgression lines developed using marker-assisted backcrossing approach in chickpea (*Cicer arietinum* L.). *Mol. Breed*. **39**:2. https://doi.org/10.1007/s11032-018-0908-9

Mashaki, K.M, Garg, V., Ghomi, A.A.N., Kudapa, H., Chitikineni, A., Nezhad, K.Z., Yamchi, A., Soltanloo, H., Varshney, R.K., Thudi, M.(2018). RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). *PLoS ONE*. **13**:0199774

Mc Couch, S. (2004). Diversifying selection in plant breeding. *PLoS Biol*. 2 (10): e347.

Menale ,K., Bekele S., Solomon A., Tsedeke A., Geoffrey M., SetotawF., Million E., and Kebebew A.(2009). Current Situation and Future Outlooks of the Chickpea sub-sector in Ethiopia; Ethiopian Institute of Agricultural Research (EIAR), Debre Zeit Agricultural Research Center, Debre Zeit , Ethiopia

Mesfin Abebe (1998). Nature and Management of Ethiopian Soils. Intuitional Livestock Research Institute (ILRI). 272 pp.

Michielse, C.B., and Rep, M. (2009). Pathogen profile update: *Fusarium oxysporum. Mol. Plant Pathol*. **10**:311–324.

Millan T, Clarke HJ, Siddique KHM, Buhariwalla HK, Gaur PM et al (2006) Chickpea molecular breeding: new tools and concepts. *Euphytica.* **147**:81–103

Millan, T., Madrid, E., Imtiaz, M., Kharrat, M., Chen, W. (2013). Disease Resistance in Chickpea, in Translational Genomics for Crop Breeding, Volume I: Biotic Stress. Varshney, R. K. and Tuberosa, R. (eds). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118728475.ch11

Millan, T., Madrid, E., Cubero, J.I., Amri, M., Castro, P., Rubio, J. (2015). Chickpea. In Grain Legumes; De Ron Antonio,M., Ed.; Springer: New York, NY, USA.

Mirzapour, S., Darvishnia, M., Bazgir, E., and Goodarzi, D. (2014). Identification of resistant sources in chickpea against Fusarium wilt under greenhouse condition. *Int. J. Farm. Alli. Sci.* **3**: 772–776. Available online at: http://ijfas. com/wp content/uploads/2014/08/772-776.pdf

Mirmajlessi, S.M., Destefanis, M., Gottsberger, R.A., Mand, M., Loit, E. (2015). PCR-based specific techniques used for detecting the most important pathogens on strawberry. *System. Rev.* **4:**9. doi:10.1186/2046-4053-4-9

Moose, S. P., Mumm R. H.(2008). Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol*. **147(3):** 969-977.

Muehlbauer, F.J., Sarker, A. (2017). Economic importance of chickpea: Production, value, and world trade. In: Varshney RK, Thudi M, Muehlbauer F (eds) The Chickpea Genome. Springer, Cham, pp 5–12. doi:10.1007/978-3-319-66117-9_2

Munoz, N., Liu, A., Kan, L., Li, M.W., Lam, H.M. (2017). Potential Uses of Wild Germplasms of Grain Legumes for Crop Improvement. *Int. J. Mol. Sci.* **18**:328. http://doi:10.3390/ijms18020328.

Murumkar, C.V. and Chavan, I.D. (1985). Physiological changes in chickpea level infected by *Fusarium* wilt. *Biovegy.***11(1)**:118-120.

Navas Cortes J.A., M.P. Ana, A. Ana, S.T. Palom (2012). Molecular Tools for Detection of Plant Pathogenic Fungi and Fungicide Resistance, Plant Pathology, Christian Joseph Cumagun (Ed.), ISBN: 978-953-51-0489-6, In Tech, Available from: http://www.intechopen.com/books/plantpathology/molecular-tools-for-detection-of-plant-pathogenic-fungi-and-fungicide-resistance

Navas Cortes, J.A., Hau, B., Jimenez Diaz, R.M.(2000). Yield loss in chickpeas in relation to development of *Fusarium* wilt epidemics. *Phytopathol.* **90**:1269–1278.

Navas Cortes JA, Landa BB, Rodriguez Lopez J, Jimenez Diaz RM, Castillo P (2008). Infection by *Meloidogyne eartiellia* does not break down resistance to races 0, 1A, and 2 of *Fusarium oxysporum* f. sp. *ciceris* in chickpea genotypes. *Phytopathol*. 98:709-718. doi:10.1094 / PHYTO-98-6-0709.

Navas-Cortés, J.A., Hau, B., Jimenez-Diaz, R.M.(1998). Effect of Sowing Date, Host Cultivar, and Race of Fusarium oxysporum f. sp. ciceris on Development of Fusarium Wilt of Chickpea. *Phytopathol.* **88**:1338–1346.

Naveed M, Shafiq M, Rafiq CM, Zahid MA (2015). Genetic diversity in new chickpea accessions for *Fusarium* wilt resistance, canopy temperature and yield components under drought milieus. *Afr.J.C.S.* **9**(6):538-544. ISSN:1835-2707

Nayak SN, Zhu H, Varghese N, Datta S, Choi HK, Horres R, Jungling R, Singh J, Kishor PB, Sivaramakrishnan S, Hoisington DA, Kahl G, Winter P, Cook DR, Varshney RK (2010). Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. *Theor. Appl. Genet*. 120:1415–1441. https://doi: 10.1007/s00122-010-1265-1

Nelson, P. E., Toussoun, T. A. and Marassas W. F. O.(1983). Fusarium species. An illustrated manual for identification.' The Pennsylvania State University Press, USA

Nene, Y.L., Haware, M.P., Reddy, M.V.(1978). Diagnosis of some wilt-like disorders of chickpea (*Cicer arietinum* L.). In: Information Bull., vol. 3. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India

Nene, Y.L., Reddy, M.V. (1987). Chickpea diseases and their control. In: Saxena MC, Singh KB (eds.), The Chickpea. CAB International, Oxon, UK, pp. 233-270

Nene, Y.L., Sheila, V.K. and Sharma, S.B., (1996). A world list of Chickpea and Pigeon pea pathogens . 5th ed., ICRISAT, Patancheru India, p 27.

Nikam, P. S., Jagtap, G.P. and Sontakke, P.L.(2007). Management of chickpea wilt caused by *Fusarium oxysporum* f. sp. ciceris. *Afr.J.agri.. Res.* **2(2):** 692-697.

Ohlson, E.W., H. Ashrafi, and M.R (2018). Identification and mapping of late blight resistance quantitative trait loci in tomato accession PI 163245. *Plant Geno.* 11180007. Doi:10.3835/plantgenome2018.01.0007

Pande, S., Kishore, G.K., Upadhyaya, H.D., Rao, J.N. (2006). Identification of Sources of Multiple Disease Resistance in Mini-core Collection of Chickpea. *Plant Dis.* **90**:1214-1218. https://doi:10.1094/PD-90-1214.

Pande, S., Narayana, Rao, J., Sharma, M.(2007). Establishment of the chickpea wilt pathogen Fusarium oxysporum f.sp. ciceris in the soil through seed transmission. *Plant Pathol. J*. **23(1)**: 3-6

Pande, S., Sharma, M., Nagavardhini, A., Rameshwar, T.(2012). High Throughput Phenotyping of Chickpea Diseases: Stepwise identification of host plant resistance. Information Bulletin No.92. Patancheru 502 324, Andhra Pradesh, India: ICRISAT 56 pp. ISBN 978-92-9066-552-6.

Pande, S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W., Crouch, J.H.(2005). Ascochyta blight of chickpea (*Cicer arietinum* L.): A review of biology pathogenicity and disease management. *Aust.J.Agric. Res.***56**:317–332.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., Tanksley, S.D.(1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nat.* **335:** 721–726

Patil, B.S., Ravikumar, R.L., Bhat, J.S., Soregeon, C.D (2014). Molecular mapping of QTLs for resistance to early and late Fusarium wilt in chickpea. *Czech J. Genet. Plant Breed.* **50**:171-176

Patil, M., Om, G., Pawar, M., and Chobe, D. R.(2015). Effect of culture media, pH and temperature on the mycelial growth and sporulation of *Fusarium oxysporum f. sp. ciceris* isolates of chickpea from Central Zone of India. Res. J. Appl. Sci. **49**:54–60.

Paul, P.J., Samineni, S., Thudi, M., Sajja, S.B., Rathore, A., Das, R.R., Khan, A.W., Chaturvedi, S.K., Lavanya, G.R., Varshney, R.K., Gaur, P.M.(2018). Molecular mapping of QTLs for heat tolerance in chickpea. *Int. J. Mol Sci*. **19**:2166

Pearson, C.J., Norman, D.W. and Dixon, J.(1995). Sustainable dryland cropping in relation to soil productivity - FAO soils bulletin 72. Food and Agriculture Organization of the United Nations (FAO). Rome.

Penmetsa, R.V., Carrasquilla-Garcia, N., Bergmann, E.M., Vance, L., Castro, B., Kassa, M.T., Sarma, B.K., Datta, S., Dubey, A., Gujaria, N., Baek, J.M., Woodward, J.E., Farmer, A.D., Coyne, C.J., von Wettberg, E.J.B., Varshney, R.K., Cook, D.R. (2016). Multiple post-domestication origins of *Kabuli* chickpea through allelic variation in a diversification-associated transcription factor. *New Phytolog*. **211**:1440-1451. https://doi.org/10.1111/nph.14010.

Pingali, P. L. (2012). Green revolution: impact, limits and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* **109**:12302–11230. doi:10.1073/pnas.09129. 53109.

Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., Toth, I.K.(2016). Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Anal. Methods.* 8.12

Puhalla, J. E. (1985). Classification of Fusarium oxysporum on the basis of vegetative compatibility. *Canad. J. Bot.* **63:** 179-183.

Pundir, R.P.S., Rao, N.K., van der Maesen, L.J.G. (1985). Distribution of qualitative traits in the world germplasm of chickpea (*Cicer arietinum* L). *Euphytica.* 34: 697–703.

Rachana, S.(2002). Annual plant protection science. In: Gireesh Chand, Santosh Kumar (eds.), Crop Disease and Their Management: Integrated Approaches. CRC Press, pp.43–46.

Rajesh, P.N., Tullu, A., Gil, J., Gupta, V.S., Ranjekar, P.K., Muehlbauer, F.J. (2002). Identification of an STMS marker for the double-podding gene in chickpea. *Theor. Appl. Genet.* **105**:604–607.

Ranalli, P. and Cubero, J.I.(1997). Bases for genetic improvement of grain legumes. *Field Crop Res*. **53:**69-82

Rao, L., Usha Rani, P., Deshmukh, P., Kumar, P., Panguluri, S.(2006). RAPD and ISSR finger printing in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor Cicer reticulatum Ladizinsky. *Genet Resour. Crop Evol*. **54**:1235–1244

Rasheed, A., Hao, Y., Xia, X., Khan, A., Xu, Y., Varshney, R.K., He, Z.(2017). Crop breeding chips and genotyping platforms: progress, challenges, and perspectives. *Mol. Plant*. **10:**1047–1064

Rasu, T., Sevugapperumal, N., Thiruvengadam, R., Ramasamy, S.(2013). Morphological and genomic variability among *Sclerotium rolfsii* populations. *The Bioscan.* **8 (4)**: 1425-1430.

Redden, R.J., Berger, J.(2007). History and Origin of Chickpea. In: Chickpea Breeding and Management, eds Yadav SS, Redden RJ, Chen W, Sharma B (CABI, Oxfordshire, United Kingdom), pp 1–13. 1st Ed.

Richter M, Rossello M.R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences of the United States of America*, **106**. 19126–19131.

Roorkiwal, M., Jain A, Kale, S.M., Doddamani, D., Chitikineni, A., Thudi, M., Varshney, R.K. (2018). Development and evaluation of high density Axiom .*Cicer* SNP Array for high-resolution genetic mapping and breeding applications in chickpea. *Plant. Biotechnol. J.* **16**:890–901

Roorkiwal, M., von Wettberg, E.J., Upadhyaya, H.D., Warschefsky, E.J., Rathore, A., Varshney, R.V. (2014). Exploring germplasm diversity to understand the domestication process in chickpea (*Cicer*) using SNP and DArT markers. *PLoS One* **9:** e102016.

Roorkiwal, M., Bharadwaj, C., Barmukh, R., Dixit, GP., Thudi M., Gaur, PM., Chaturvedi, SK., Fikre, A., Hamwieh, A., Kumar S., Sachdeva, S., Ojiewo, C.O., Taran, B., Girma, N., Singh, N.P., Siddique, K.H.M.,Varshney, R.K. (2020).

Integrating genomics for chickpea improvement: achievements and opportunities. *Theor. Appl. Genet.*. https://doi.org/10.1007/s00122-020-03584-2

Rubio, J., Hajj Moussa, E., Kharrat, M., Moreno, M.T., Millan, T., Gill, J. (2003). Two genes and linked RAPD markers involved in resistance to *Fusarium oxysporum* f. sp. *ciceris* race 0 in chickpea. *Plant Breed*. **122:**188–191

Sabbavarapu, M., Sharma, M., Chamarthi, S.K., Swapna, N., Rathore, A., Thudi, M., Gaur, P.M., Pande, S., Singh, S., Kaur, L. Varshney, R.K. (2013). Molecular mapping of QTLs for resistance to Fusarium wilt (race 1) and Ascochyta blight in chickpea (Cicer arietinum L.). *Euphytica*. **193**:121–133. https://doi 10.1007/s10681-013-0959-2

Salimath, P.M., Toker, C., Sandhu, J.S., Kumar, J., Suma, B., Yadav, S.S., Bahl, P.N. (2007). Conventional breeding methods. In Chickpea Breeding and Management; Yadav, S.S., Redden, R.J., Chen, W., Sharma, B., Eds.; CABI:Wallingford, UK.

SAS Institute Inc (2008). SAS/STATA User Guide for Personal Computers Version 9.2 Edition. SAS Institute, Carry, NC, USA.

Saxena, M. C., Singh, K.B. (1987). The chickpea published by CAB. International. ICARDA. 250-252.

Saxena, N.P. (2003). Management of drought in chickpea A holistic approach. *In*: N.P. Saxena (ed.), Management of agricultural drought-agronomic and genetic options, pp. 103-122. Oxford & IBH Publishing Co.Pvt.Ltd. New Delhi, India

Saxena, N.P., Krishnamurthy, L., Johansen, C. (1993). Registration of a drought-resistant chickpea germplasm. *Crop Sci.* **33**:14-24.

Saxena, K.B., Kishor, P.B.K., Varshney, R.K.(2016). Construction of genotyping-by sequencing based high-density genetic maps and QTL mapping for fusarium wilt resistance in pigeonpea. *Sci. Rep.* | 7 (2016) 1911 | DOI:10.1038/s41598-017-01537-2. www.nature.com/scientificreports/

Scheben, A., Batley, J. Edwards, D., (2017). Genotyping-by-sequencing approaches to Characterize crop genomes: choosing the right tool for the right application. *Plant Biotechnol. J.* **15**:149-161.

Sharma, K.D., Chen, W., Muehlbauer FJ (2005). Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for Fusarium oxysporum f. sp ciceris. *Plant. Dis.* **89**:385–390. https://doi: 10.1094/PD-89-0385

Sharma, K.D., Muehlbauer, F.J. (2007). *Fusarium* wilt of chickpea: Physiological specialization, genetics of resistance and resistance gene tagging. *Euphytica.* **157**:1–14. https://doi.org/10.1007/s10681-007-9401-y

Sharma, K.D., Winter, P., Kahl, G., Muehlbauer, F.J (2004). Molecular mapping of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea. *Theor. Appl. Genet.* **108**:1243–1248

Sharma, M., Ghosh, R., Ramesh, R.K., Upala, N.M., Chamarthi, S., Varshney, R., Pande. S. (2012a). Molecularand morphological diversity in *Rhizoctonia bataticola* isolates causing dry root rot inchickpea (*Cicer arietinum* L.) in India. *Afr. J. Biotechnol*. **11**:8948–8959.

Sharma, M., Kiran, B,T,, Gaur, P.M., Ghosh, R., Rameshwar, T., Chaudhary, R.G., Upadhyay, J.P., Gupta, O., Saxena, D.R., Kaur, D.S.C., Anandani, V.P., Harer, P.N., Rathore, A., Pande, S. (2012b). Identification and multi-environment validation of resistance to *Fusarium oxysporum* f. sp *ciceri*s in chickpea. *Field Crop Res*. **135**:82–88. https://doi.org/10.1016/j.fcr.2012.07.004.

Sharma, M., Nagavardhini, A., Thudi, M., Ghosh, R., Pande, S., Varshney, R.(2014). Development of DArT markers and assessment of diversity in *Fusarium oxysporum* f. sp. *ciceris*, wilt pathogen of chickpea (*Cicer arietinum* L.). *BMC Genomics*, **15**:454.

Sharma, M., Ghosh, R. (2016). An update on genetic resistance of chickpea to Ascochyta blight. *Agron*. **6**:18

Sharma, M., Ghosh, R., Pande, S. (2015). Dry root rot (*Rhizoctonia bataticola* (Taub.) Butler): an emerging disease of chickpea – where do we stand. *Arch. Phytopathol. and Plant Prot.,* **48**:13-16, 797-812, https://doi: 10.1080/03235408.2016.1140564

Sharma, M., Ghosh, R., Tarafdar, A., Rathore, A., Chobe, D.R., Kumar, A.V., Gaur, P.M., Samineni, S., Gupta, O., Singh, N.P., Saxena, D.R., Saifulla, M., Pithia, M.S., Ghante, P.H., Mahalinga, D.M., Upadhyay, J.B. and Harer, P.N.(2019). Exploring the Genetic Cipher of Chickpea (*Cicer arietinum* L.) Through Identification and Multi-environment Validation of Resistant Sources Against *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *ciceris*). *Front. Sustain. Food Syst.* **3**:78. doi: 10.3389/fsufs.2019.00078

Sharma, M., Nagavardhini, A., Pande, S.(2013). Race scenario of *Fusarium oxysporum f.sp. ciceris*, wilt pathogen of chickpea (*Cicer arietinum* L.). Bordeaux, France: 12th Europian *Fusarium* Seminar; 2013:58.

Sharma, M., Sengupta, A., Ghosh, R., Agarwal, G., Tarafdar, A., Nagavardhini, A., Pande, S., Varshney, R.K. (2016). Genome wide transcriptome profiling of *Fusarium oxysporum* f sp. *ciceris* conidial germination reveals new insights into infection related genes. Scientific Reports **6**:37353. https://doi.org/10.1038/srep37353.

Sharma, M., Varshney, R.K., Rao, J.N., Kannan, S., Hoisington, D., Pande, S. (2009). Genetic diversity in Indian isolates of *Fusarium oxysporum* f. sp. *ciceris*, chickpea wilt pathogen. *Afr.J. Biotechnol*. **8**:1016–1023.

Shehabu, M., Ahmed, S., Sakhuja, P.K.(2008). Pathogenic variability in Ethiopian isolates of *Fusarium oxysporum* f. sp. *ciceris* and reaction of chickpea improved varieties to the isolates. *Int. Pest Manag.,* **54(2)**:143-149, *DOI: 10.1080/09670870701780153*

Singh, R.K., Chaudhary, B.D. (1985). Biometrical Method in Quantitative Genetics Analysis. Kalyani Publishers, New Delhi, pp 56.

Singh, F., Diwakar, B. (1995). Chickpea botany and production practices. *Skill Devel. Seri.* **16:** 8–9.

Singh, H., Kumar, J., Haware, M.P.(1987). Genetics of resistance to *Fusarium* wilt in chickpeas. In: Day PR, Jellis GJ (eds). Genetics and plant pathogenesis. Blackwell Scientific Publications, Oxford, pp 339–342

Singh, R., Sharma, P., Varshney, R., Sharma, S.K., Singh, N.K.(2008). Chickpea Improvement: Role of Wild Species and Genetic Markers, Biotechnology and Genetic Engineering.*Rev.*, **25(1)**:267-314. https://doi.org/10.5661/bger-25-267.

Singh, G., Chen, W., Rubiales, D., Moore, K., Sharma, Y. R. and Gan, Y. (2007). Diseases and their management. pp. 497-519. In: Chickpea Breeding and Management Yadav, Redden., Chen. and Sharma (eds.). CAB International

Singh, K.B. (1987). Chickpea Breeding. In The Chickpea; Centre for Agriculture and Bioscience International (CABI): Wallingford, UK. Singh, K.B. (Eds.), The Chickpea. CAB Int., Oxon, UK, pp. 233-270.

Sivashakthi, S., Thudi, M., Tharanya, M., Kale, S.M., Kholova, J., Halime, M.H., Jaganathan, D., Baddam, R., Thirunalasundari, T., Gaur, P.M., Varshney, R.K., Vadez, V.(2018). Plant vigour QTLs co-map withan earlier reported QTL-hotspot for drought tolerance while water saving QTLs map in other regions of the chickpea genome. *BMC Plant Biol.* **18**:29.

Skovgaard, K., H. I. Nirenberg, K. O'Donnell, and S. Rosendahl. (2001). Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. *Phytopathol.* **91**: 1231-1237.

Sneh, B., Burpee, L., Ogoshi, A. (1991). Identification of *Rhizoctonia* species. Am Phytopath Soc Press, St. Paul, MN, USA.

Stevenson, P.C., Turner, H.C., Haware, M.P., (1997). Phytoalexin accumulation in the roots of chickpea (*Cicer arietinum* L.) seedlings associated with resistance to

Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*). *Physiol. Mol. Plant Pathol.* **50:** 167-178.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H.-W., & Richards, T. A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology, 19 Suppl 1, 21–31. http://doi.org/10.1111/j.1365-294X.2009.04480.x

Sudupak, M.A., Akkaya, M.S., Kence, A. (2002). Analysis of genetic relationships among perennial and annual Cicer species growing in Turkey using RAPD markers. *Theor. Appl. Genet*. **105:**1220–1228.

Tadesse, N. (1996). Survey for race situation and causal organisms of wilt/root rot diseases of Chickpea and lentil in Ethiopia. In: *Nile Valley and Red Sea Regional Program on cool-season food legumesand cereals*. Annual report (1994/95) Addis Ababa pp. 59–61.

Tadesse, N., Melkamu, A., Bejiga, G. (1998a). Survey and identification of the major organisms causing wilt and root-rots and their relative importance in chickpea and lentil in Ethiopia. In: *Nile Valley and RedSea Regional Program on cool-season food legumes and cereals*. ICARDA/NVRSRP/NW-DOC-006, pp.89–90.

Tadesse, N., Bejiga, G., Ayalew, M.(1998b). *Fusarium* wilt of chickpea and lentil in Ethiopia: Race identification and pathogen variability.pp35. In: Nile valley and Red Sea Regional Program on Cool season Food Legumes and Cereals. Regional Networks. Anual Report (1997/98). Cairo, Egypt.

Tarafdar, A., Rani, T. S., Chandran, U. S. S., Ghosh, R., and Sharma, M. (2017). "Impact of moisture stress on collar rot (Sclerotium rolfsii Sacc.) development in chickpea (*Cicer arietinum* L.)," in Proceedings of ISMPP International Conference on "Plant Health for Human Welfare" (Jaipur: Indian Society of Mycology and Plant Pathology), 119.

Tarafdar, A., Rani, T. S., Chandran, U. S. S., Ghosh, R., Chobe, D. R., and Sharma, M. (2018). Exploring combined effect of abiotic (soil moisture) and biotic (Sclerotium rolfsii Sacc.) stress on collar rot development in chickpea. *Front. Plant Sci.* **9**:1154. doi: 10.3389/fpls.2018.01154

Tekeoglu, M., Tullu, A., Kaiser, W.J., Muehlbauer, F.J.(2000). Inheritance and linkage of two genes that confer resistance to Fusarium wilt in chickpea. *Crop Sci.* **40**:1247–1251.

Tester, M., Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Sci*. **327**(5967): 818-822.

Thiergart, T., Duran, P., Ellis, T., Vannier, N., Garrido Oter, R., Roux, F., Alonso-Blanco, C., Agren, J., Schulze Lefert, P., Hacquard, S. (2020) Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. *Nature Ecol. and Evol.* **4**:122-131, doi:10.1038/s41559-019-1063-3.

Thudi, M., Bohra, A., Nayak, S.N., Varghese, N., Shah, T.M., Penmetsa, R.V., Thirunavukkarasu, N., Gudipati, S., Gaur, P.M., Kulwal, P.L., Upadhyaya, H.D., Kavikishor, P.B., Winter, P., Kahl, G., Town, C.D., Kilian, A., Cook, D.R., Varshney, R.K. (2011). Novel SSR markers from BAC end sequences, DArT arrays and a comprehensive genetic map with 1291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS ONE* **6**:e27275

Thudi, M., Chitikineni, A., Liu, X., He, W., Roorkiwal, M., Yang, W., Jian, J., Doddamani, D., Gaur, P.M., Rathore, A., Samineni, S., Saxena, R.K., Xu, D., Singh, N.P., Chaturvedi, S.K., Zhang, G., Wang, J., Datta, S.K., Xu, X., Varshney, R.K.(2016a). Recent breeding programs enhanced genetic diversity in both desi and kabuli varieties of chickpea (*Cicer arietinum* L.). *Sci. Rep.* **6**:38636. https://doi:10.1038/srep38636.www.nature.com/scientific reports.

Thudi, M., Khan, A.W., Kumar, V., Gaur, P.M., Katta, A.V.S.K., Garg, V., Roorkiwal, M., Samineni, S., Varshney, R.K.(2016b). Whole genome re-sequencing reveals genome wide variations among parental lines of mapping populations in chickpea (Cicer arietinum). *BMC Plant Biol.* **16:**10. https://doi: 10.1186/s12870-015-0690-3

Thudi, M., Upadhyaya, H.D., Rathore, A., Gaur, P.M., Krishnamurthy, L., Roorkiwal, M., Nayak, S.N., Chaturvedi, S.K., Basu, P.S., Gangarao, N.V.P.R., Fikre, A., Kimurto, P., Sharma, P.C., Sheshashayee, M.S., Tobita, S., Kashiwagi, J., Ito, O., Killian, A., Varshney, R.K.(2014). Genetic dissection of drought and heat tolerance in chickpea through genomewide and candidate gene-based association mapping approaches. *PLoS ONE 9*: 96758

Trapero-Casas, A., Jimenez-Diaz, R.M.(1985). Fungal wilt and root rot diseases of chickpea in southern Spain. *Phytopathol.* **75**:1146 -1151.

Tullu, A., Kaiser, W.J., Kraft ,J.M. (1999). A second gene for resistance to race 4 of Fusarium wilt in chickpea and linkage with a RAPD marker. *Euphytica.* **109**:43–50. Doi: 10.1023/A:1003604209476.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G. (2012). Primer 3 - new capabilities and interfaces. *Nucleic Acids Res.* **40**(15):e115.

Upadhyaya, H.D., Bajaj, D., Das, S., Kumar, V., Gowda, C.L.L., Sharma, S., Tyagi, A.K., Parida, S.K.(2016). Genetic dissection of seed-iron and zinc concentrations in chickpea. *Sci. Rep.* **6**:24050

Upadhyaya, H.D., Dwivedi, S.L., Baum, M., Varshney, R.K., Udupa, S.M., Gowda, C.L., Hoisington, D., Singh, S. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biol.* **8**: 106–117.

Upadhyaya, H.D., Thudi, M., Dronavalli, N., Gujaria, N., Singh, S., Sharma, S., Varshney, R.K.(2011). Genomic tools and germplasm diversity for chickpea improvement. *Plant Gen. Res. Char. Util.* **9**:45–48.

Upasani, M.L., Limaye, B.M., Gurjar, G.S., Kasibhatla, S.M., Joshi, R.R., Kadoo, N.Y., Vidya, S., Gupta, V.S. (2017). Chickpea *Fusarium oxysporum* interaction

transcriptome reveals differential modulation of plant defense strategies. *Sci. Rep*. https://doi:10.1038/s41598-017- 07114-x www.nature.com.

Van der Maesen, L.J.G. (1987). Origin, history and taxonomy of chickpea. In: The Chickpea, PP. 11 34, (Saxena, M.C., and Singh, K.B., eds). CAB international,Wallingfold, UK.

Varshney, R.K.(2016). Exciting journey of 10 years from genomes to fields and markets: Some success stories of genomics-assisted breeding in chickpea, pigeonpea and groundnut. *Plant Sci.* **242**:98–107

Varshney, R.K., Mohan, S.M., Gaur, P.M., Chamarthi, S.K., Singh, V.K., Srinivasan, S., Swapna, N., Sharma, M, Singh, S., Kaur, L., Pande, S. (2014c). Marker-assisted backcrossing to introgress resistance to *Fusarium* wilt race 1 and Ascochyta blight in C 214, an elite cultivar of chickpea. Plant Genome 7, pp. 1-11. ISSN 1940-3372

Varshney, R.K., Kudapa, H., Roorkiwal, M., Thudi, M., Pandey, M.K., Saxena, R.K., Chamarthi, S.K., Mohan, S.M., Mallikarjuna, N., Upadhyaya, H., Gaur, P.M., Krishnamurthy, L., Saxena, K.B., Nigam, S.N. , Pande, S. (2012). Advances in genetics and molecular breeding of three legume crops of semi-arid tropics using next-generation sequencing and high-throughput genotyping technologies. *J. Biosci.* **37**:811–820] DOI 10.1007/s12038-012-9228-0

Varshney, R.K., Mir, R.R., Bhatia, S., Thudi, M., Hu, Y., Azam, S., Zhang, Y., Jaganathan, D., You, F.M., Gao, J., Riera, L.O., Luo, M.C. (2014b). Integrated physical, genetic and genome map of chickpea (*Cicer arietinum* L.). *Funct. Integr. Genom.* **14**:59–73. https://doi:10.1007/s10142-014-0363-6

Varshney, R.K., Pandey, M.K., Bohra, A., Singh, V.K., Thudi, M., Saxena, R.K. (2019b). Toward the sequence-based breeding in legumes in the post-genome sequencing era. *Theor. Appl. Genet.* **132**:797–816

Varshney, R.K., Saxena, R.K., Upadhyaya, H.D., Khan, A.W., Yu, Y., Kim, C., Rathore, A., Kim, D., Kim, J., An, S., Kumar, V., Anuradha, G., Yamini, K.N., Zhang, W., Muniswamy, S., Kim, J.S., Penmetsa, R.V., von Wettberg, E., Datta, S.K. (2017). Whole-genome resequencing of 292 pigeonpea accessions identifies genomic regions associated with domestication and agronomic traits. *Nat. Genet.* **49**:1082–1088

Varshney, R.K, Song, C., Saxena, R.K., Azam, S., Yu, S., Sharpe, A., Cannon, S., Baek, J., Rosen, B.D., Taran, B., Millan, T., Zhang, X., Ramsay, L.D., Iwata, A., Wang, Y., Nelson, W., Farmer, A.D., Gaur, P.M., Soderlund, C., Penmetsa, R.V., Xu, C., Bharti, A.K., He, W., Winter, P., Zhao, S., Hane, J.K., Carrasquilla-Garcia, N., Condie, J.A., Upadhyaya, H.D., Luo, M.C., Thudi, M., Gowda, C.L.L., Singh, N.P., Lichtenzveig, J, Gali, K.K., Rubio, J., Nadarajan, N., Dolezel, J., Bansal, K.C., Xu, X., Edwards, D., Zhang, G., Kahl, G., Gil, J., Singh, K.B., Datta, S.K., Jackson, S.A., Wang, J., Cook, D.R. (2013a). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat. Biotechnol.* **31**:240–246

Varshney, R.K., Thudi, M., Pandey, M.K., Tardieu, F., Ojiewo, C., Vadez, V., Whitbread, A.M., Siddique, K.H.M., Nguyen, H.T., Carberry, P.S., Bergvinson, D. (2018). Accelerating genetic gains in legumes for the development of prosperous smallholder agriculture: integrating genomics, phenotyping, systems modelling and agronomy. *J. Exp. Bot*.**69**:3293–3312. https://doi: 10.1093/jxb/ery088

Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, Jaganathan D, Koppolu J, Bohra A, Tripathi S, Rathore A, Jukanti AK, Jayalakshmi V, Vemula A, Singh SJ, Yasin M, Sheshshayee MS, Viswanatha KP (2014a). Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet.* **127**:445–462

Varshney, R.K, Thudi, M., Roorkiwal, M., He, W., Upadhyaya, H.D., Yang, W., Bajaj, P., Cubry, P., Rathore, A., Jian, J., Doddamani, D., Khan, A.W., Garg, V., Chitikineni, A., Xu, D., Gaur, P.M., Singh, N.P., Chaturvedi, S.K., Nadigatla, G.V.P.R., Krishnamurthy, L., Dixit, G.P., Fikre, A., Kimurto, P.K., Sreeman, S.M., Bharadwaj, C., Tripathi, S., Wang, J., Lee, S.H., Edwards, D., Polavarapu, K.K.B.,

Penmetsa, R.V., Crossa, J., Nguyen, H.T., Siddique, K.H.M., Colmer, T.D., Sutton, T., von Wettberg, E., Vigouroux, Y., Xu, X., Liu, X. (2019a). Resequencing of 429 chickpea accessions from 45 countries provides insights into genome diversity, domestication and agronomic traits. *Nat Genet.* **51**:857–864

Varshney, R.K., Close, T.J., Singh, N.K., Hoisington, D.A., Cook, D.R. (2009). Orphan legume crops enter the genomics era! *Curr. Opin. Plant Biol.* **12**, 1–9.

Verma, S., Gupta, S., Bandhiwal, N., Kumar, T., Bharadwaj, C., Bhatia, S.(2015). High-density linkage map construction and mapping of seed trait QTLs in chickpea (*Cicer arietinum* L) using Genotyping-by-Sequencing (GBS). *Sci. Rep.* **5**:17512

Von Wettberg, E.J.B, Chang, P.L., Başdemir, F., Carrasquila-Garcia, N., Korbu, L.B., Moenga, S.M., Bedada, G., Greenlon, A., Moriuchi, K.S., Singh, V., Cordeiro, M.A., Noujdina, N.V., Dinegde, K.N., Abbas Shah Sani, S.G., Getahun, T., Vance, L., Bergmann, E., Lindsay, D., Bullo, E.M., Warschefsky, E.J., Dacosta-Calheiros E., Marques, E., Yilmaz, M.A., Cakmak, A., Rose, J., Migneault, A., Krieg, C.P., Saylak, S., Teme, H., Friesen, M.L., Siler, E., Akhmetov, Z., Ozcelik H., Kholova, J., Can, C., Gaur, P., Yildirim, M., Sharma, H., Vadez, V., Tesfaye, K., Woldemedhin, A.F., Tar'an, B., Aydogan, A., Bukun, B., Penmetsa, R.V., Berger, J. Kahraman, A., Nuzhdin, S., Cook, D.R. (2018). Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. *Nat. commun.* **9:**649. DOI:10.1038/s41467-018-02867-z | www.nature.com/naturecommunications

Voorrips, R. E., (2002). Map Chart Software for the graphical presentation of linkage maps and QTLs. *J. Hered*. **93**:77–78.

Whish, J. P. M., Castor, P., Carberry, P. S. and Peake, A. S. (2007). ON-farm assessment of constraints to chickpea (*Cicer arietinum*) production in marginal areas of northern Australia. *Exper. Agri.* **43** (04): 505-520.

Wunsch, M.J., Baker, A.H., Kalb, D.W., Bergstrom, G.C. (2009). Characterization of *Fusarium oxysporum f. sp. loti* forma specialis nov., a monophyletic pathogen causing vascular wilt of birdsfoot trefoil. *Plant Dis*. **93**:58-66

Xu, Y., Crouch, J.H. (2008). Marker-assisted selection in plant breeding: From publications to practice. *Crop Sci*. **48:**391–407.

Yang, H., Y. Tao, et al. (2012). Application of next-generation sequencing for rapid marker development in molecular plant breeding: a case study on anthracnose disease resistance in Lupinus angustifolius L. *BMC Genomics* **13**: 318.

Yimer, S.M., Ahmed, S., Fininsa, C., Tadesse, N., Hamwieh, A., Cook, D.R. (2018). Distribution and factors influencing chickpea wilt and root rot epidemics in Ethiopia. *Crop Prot.* **106**:150-155, https://doi.org/10.1016

Zhang, Y. Ma, L.J.(2017). Deciphering pathogenicity of *Fusarium oxysporum* from a phylogenomic perspective. *Adv. Genet.* **100**:179-209. https://doi.org/10.1016/bs.adgen.2017.09.010.

10. Appendix

Appendix 9.1. Sample collection region, zone, district altitude and GPS points

S.No	Region	Zone	District	Location	Altitude	Latitude	Longitude
1	Amhara	East Gojam	Awobel	Yegidina	2430	10.22101	37.98501
2	Amhara	East Gojam	Awobel	Getahua	2440	10.23801	38.06009
3	Amhara	East Gojam	Awobel	Emiga	2457	10.24399	38.11962
4	Amhara	East Gojam	Guazamen	Damil	1822	10.62389	37.41582
5	Amhara	East Gojam	Guazamen	Yekuas	2183	10.39659	37.57511
6	Amhara	East Gojam	Guazamen	Digil	2228	10.35001	37.66479
7	Amhara	West Gojam	Jabi tena	Jabi	1980	10.69718	37.14028
8	Amhara	West Gojam	Jabi tena	Jalmeda	1822	10.67472	37.33030
9	Amhara	West Gojam	Jabi tena	Wengie	1802	10.65707	37.38309
10	Amhara	West Gojam	N/Achefer	Campas	1839	11.56230	37.32896
11	Amhara	West Gojam	N/Achefer	Admina	2082	11.63299	36.92491
12	Amhara	West Gojam	N/Achefer	Akumihret	2008	11.66485	36.92371
13	Amhara	West Gojam	N/Achefer	Jiwacha awura	1974	11.71142	36.96107
14	Amhara	West Gojam	N/Achefer	Golashkan	1895	11.75585	36.98505
15	Amhara	West Gojam	N/Achefer	Shankra	1927	11.79425	36.98303
16	Amhara	North Gonder	Chilga	Deserek	2096	12.39954	37.01949
17	Amhara	North Gonder	Chilga	Laysek	2180	12.47305	37.03597
18	Amhara	North Gonder	Chilga	Nora Awudard	1950	12.56331	37.11211
19	Amhara	North Gonder	Denbia	Giragie	1959	12.47008	37.37276
20	Amhara	North Gonder	Denbia	Gebeba	1810	12.40477	37.30368
21	Amhara	North Gonder	Denbia	Cherkaw	1905	12.36591	37.26150
22	Amhara	North Gonder	Denbia	Jigiba	1877	12.36003	37.18202
23	Amhara	North Gonder	Gonder Zuria	Debre selam	1919	12.17419	37.69380
24	Amhara	North Gonder	Gonder Zuria	Guzara	1895	12.22782	37.63115
25	Amhara	North Gonder	Gonder Zuria	Delanta	1965	12.28399	37.60776
26	Amhara	North Gonder	Gonder Zuria	Adoch	1956	12.33472	37.58117
27	Amhara	North Gonder	Gonder Zuria	Bruewax	1940	12.44170	37.50513
28	Amhara	North Gonder	Takusa	Saruha selam	1871	12.31818	37.09853
29	Amhara	North Gonder	Takusa	Guhal	1812	12.24829	37.08631
30	Amhara	North Gonder	Takusa	Chewaduba	1868	12.31499	37.07400
31	Amhara	South Gonder	Tachgaynt	Tsegur eyesus	2427	11.89286	37.97184
32	Amhara	South Gonder	Fogera	Alember	2070	11.91745	37.91340
33	Amhara	South Gonder	Fogera	Gazo	1998	11.92523	37.85114
34	Amhara	South Gonder	Libokemkem	Shina tseyon	1800	12.02741	37.72261
35	Amhara	South Gonder	Tachgaynt	Awrajit	2766	11.75532	38.50494

S.No	Region	Zone	District	Location	Altitude	Latitude	Longitude
36	Amhara	North Shewa	Enewari	Beler Tilk	2664	9.86943	39.17809
37	Amhara	North Shewa	M/shenkora	Bililgn	2296	8.48354	39.17000
38	Amhara	North Shewa	M/shenkora	chercha	2254	8.48511	39.18125
39	Amhara	North Shewa	M/shenkora	W/amlak	2200	8.51258	39.19475
40	Amhara	North Shewa	M/shenkora	Arerti	1764	8.55328	39.36360
41	Amhara	North Shewa	M/shenkora	Korkoro amba	1757	8.55383	39.26305
42	Amhara	North Shewa	M/shenkora	Shewa genet	1792	8.54289	39.24429
43	Amhara	North Shewa	M/shenkora	Bullo silasie	1942	8.50574	39.22518
44	Amhara	North Shewa	M/shenkora	Bullo Giorgis	1988	8.50000	39.21426
45	Amhara	North Shewa	M/shenkora	Ensira	2198	8.46492	39.17205
46	Amhara	North Shewa	Moretina jiru	Kaloli	2658	9.93128	39.19221
47	Amhara	North Shewa	Moretina jiru	Tachamba	2651	9.96724	39.22387
48	Amhara	North Shewa	Moretina jiru	Jihur	2641	9.98046	39.22947
49	Amhara	North Shewa	Moretina jiru	Robi	2661	9.59027	38.92239
50	Amhara	North Shewa	Moretina Jiru	Kallele	2600	9.46453	39.10284
51	Amhara	North Shewa	Moretina Jiru	Bollo	2656	9.56031	39.11459
52	Amhara	North Shewa	Moretina Jiru	Agul amba	2615	9.56151	39.1268
53	Amhara	North Shewa	Moretina Jiru	Weyra amba	2610	9.56566	39.12578
54	Amhara	North Shewa	Moretina Jiru	Amba	2640	9.56184	39.12132
55	Amhara	North Shewa	Siya Debir	Wele	2660	9.46483	39.11284
56	Amhara	North Shewa	Deneba	Kora	2640	9.78209	39.19478
57	Amhara	North Welo	Gubalafto	Jenetuber	1914	11.77483	39.57838
58	Amhara	North Welo	Mersa	Mersa	1645	11.61950	39.67102
59	Amhara	North Welo	Mersa	Chefemeda	1857	11.75056	39.622059
60	Amhara	North Welo	Qobo	Afasash	1497	12.1122	39.61959
61	Amhara	North Welo	Qobo	Dingay qulqul	1650	12.18452	39.62445
62	Amhara	North Welo	Raya kobo	kobo	1484	12.14338	39.64022
63	Amhara	North Welo	Wolideya	W/zuria	1884	11.81363	39.58688
64	Oromiya	Arsi	Shirka	Merhabete	2389	7.60347	39.52061
65	Oromiya	Arsi	Shirka	Hella zenbaba	2334	7.59698	39.54580
66	Oromiya	Arsi	Shirka	Hella tereta	2326	7.59060	39.58173
67	Oromiya	Arsi	Shirka	Gelebeha	2325	7.59623	39.56008
68	Oromiya	Arsi	Shirka	Burkutu	2304	7.58052	39.51770
69	Oromiya	Arsi	Shirka	Jelko	2362	7.60611	39.50603
70	Oromiya	Arsi	Shirka	Debre selam	2331	7.63155	39.52213
71	Oromiya	Arsi	Shirka	Goro	2338	7.63519	39.57496
72	Oromiya	Arsi	Xiyo	Chichti	2430	7.82102	39.09217
S. No	**Region**	**Zone**	**District**	**Location**	**Altitude**	**Latitude**	**Longitude**

73	Oromiya	Arsi	Xiyo	Tullu chebi	2344	7.83127	39.06544
74	Oromiya	Arsi	Xiyo	Abichu	2380	7.81491	39.07238
75	Oromiya	Arsi	Xiyo	Burkitu	2595	7.80798	39.14025
76	Oromiya	Bale	Adaba	adaba Zuria	2398	7.00644	39.37111
77	Oromiya	Bale	Adaba	Herer	2429	6.99251	39.23872
78	Oromiya	Bale	Adaba	Wesha	2611	7.03874	39.48768
79	Oromiya	Bale	Adaba	Adaba	2412	7.03849	39.48737
80	Oromiya	Bale	Goro	Addisalema	2052	7.01013	40.38829
81	Oromiya	Bale	Goro	Chefamana	2014	7.00785	40.41349
82	Oromiya	Bale	Goro	Meliyu	1996	7.01365	40.40505
83	Oromiya	Bale	Sinana	Shallo		7.06882	39.64987
84	Oromiya	East Harerge	Haromaya	Alemaya	2017	9.41781	42.03592
85	Oromiya	East Harerge	Haromaya	Gobe selama	2105	9.37483	42.99038
86	Oromiya	East Harerge	Haromaya	Gobe chala	2035	9.34749	41.98482
87	Oromiya	East Harerge	Jarso	Sonbe	2556	9.49636	42.24750
88	Oromiya	East Harerge	Jarso	Metokema	2001	9.45104	41.85831
89	Oromiya	East Harerge	Jarso	Gandechela	1978	9.44165	41.66310
90	Oromiya	East Harerge	Kersa	Agello	1996	9.45068	41.86469
91	Oromiya	East Harerge	Kersa	Guta woliso	2137	9.37879	41.58224
92	Oromiya	East Harerge	Kersa	Gutuware	2099	9.37656	41.58562
93	Oromiya	East Harerge	Meta	Chelenko lola	2138	9.42071	41.61528
94	Oromiya	East Harerge	Meta	Gorogutu	2114	9.384774	41.45673
95	Oromiya	East Harerge	Meta	Workshop	2064	9.39369	41.44468
96	Oromiya	East Harerge	Meta	Ifabelem	2328	9.27836	41.16406
97	Oromiya	West Harerge	Habro	Kemer	1753	8.78967	40.49142
98	Oromiya	West Harerge	Habro	Gerbi goba	1747	8.80132	40.50627
99	Oromiya	West Harerge	Habro	Lugo	1820	8.8187	40.49926
100	Oromiya	West Harerge	Habro	Hature selale	1714	8.82043	40.55502
101	Oromiya	West Harerge	Habro	Sukaye	1730	8.8356	40.59676
102	Oromiya	West Harerge	Habro	Geteta	1723	8.86153	40.64635
103	Oromiya	West Harerge	Habro	Didimina	1728	8.88596	40.71497
104	Oromiya	West Harerge	Odabultuma	Jewis	1724	8.92267	40.81709
105	Oromiya	West Harerge	Odabultuma	Arbereketie	2270	9.041743	40.90948
106	Oromiya	West Harerge	Tullo	Korkie	2340	9.25763	41.14438
107	Oromiya	West Harerge	Tullo	Worani	1942	9.24143	41.11993
108	Oromiya	West Harerge	Tullo	Lubudhekeb	1732	9.18006	41.10549
109	Oromiya	West Harerge	Tullo	Lubudhekeb	1803	9.1736	41.09300
S. No	**Region**	**Zone**	**District**	**Location**	**Altitude**	**Latitude**	**Longitude**
110	Oromiya	West Harerge	Tullo	Terkafeta	1929	9.17142	41.07368

111	Oromiya	West Harerge	Tullo	Terkafeta	2170	9.17900	41.05761
112	Oromiya	East Shewa	Ada'a	Debrezt	1900	8.4032	38.58363
113	Oromiya	East Shewa	Ada'a	Debrezt	1900	8.4032	38.58363
114	Oromiya	East Shewa	Addaa	Gerbicha	2002	8.4032	38.58363
115	Oromiya	East Shewa	Addaa	Gindebel	1966	8.41096	38.58597
116	Oromiya	East Shewa	Addaa	Gichie	1935	8.39567	38.59147
117	Oromiya	East Shewa	Addaa	Gerbabo	1923	8.39557	38.59192
118	Oromiya	East Shewa	Addaa	Gichie	1929	8.40028	38.59093
119	Oromiya	East Shewa	Addaa	Mukiye	1880	8.37586	38.55399
120	Oromiya	East Shewa	Addaa	Bekejo	1817	8.36566	38.56257
121	Oromiya	East Shewa	Gelan	Kela	2086	8.5048	38.48306
122	Oromiya	East Shewa	Gelan	Beda oda	2072	8.5022	38.48036
123	Oromiya	East Shewa	Gelan	Cholo	2098	8.50459	38.48479
124	Oromiya	East Shewa	Gelan	Sidamo	2085	8.50074	38.48328
125	Oromiya	East Shewa	Gimbichu	Ambelta	2311	8.90027	39.05750
126	Oromiya	East Shewa	Gimbichu	Habru	2426	8.94653	39.09507
127	Oromiya	East Shewa	Gimbichu	Chefe station	2436	8.95323	39.10077
128	Oromiya	East Shewa	Liben	Gulu	1938	8.32312	38.48068
129	Oromiya	East Shewa	Liben	Ashufie	1896	8.3123	38.48101
130	Oromiya	East Shewa	Liben	Agamsa	1879	8.30342	38.47532
131	Oromiya	East Shewa	Liben	Rogicha	1878	8.30186	38.4740
132	Oromiya	East Shewa	Liben	Werejarso	1927	8.37172	38.49007
133	Oromiya	East Shewa	Lume	Ejere	2240	8.78450	39.24482
134	Oromiya	East Shewa	Lume	Elebela	2279	8.78713	39.27285
135	Oromiya	East Shewa	Lume	Tullurae	2005	8.65825	39.17059
136	Oromiya	East Shewa	Lume	Ejere welkitie	2264	8.47241	39.16001
137	Oromiya	East Shewa	Lume	Nanawa	2205	8.46003	39.15130
138	Oromiya	East Shewa	Lume	Bulti Gerbi	2129	8.44255	39.12500
139	Oromiya	North Shewa	Sheno	Adaw Gore	2479	9.00814	39.52163
140	Oromiya	North Shewa	Sheno	Elesebeto	2518	9.08428	39.06594
141	Oromiya	North Shewa	Sheno	Aragecha	2913	9.36100	39.31948
142	Oromiya	South W/Shewa	Becho	Boji gefere	2237	8.61532	38.23398
143	Oromiya	South W/Shewa	Becho	kobo	2157	8.69614	38.24696
144	Oromiya	South W/Shewa	Becho	keta	2111	8.76196	38.30695
145	Oromiya	South W/Shewa	Goro	chancho	1883	8.41422	37.88969
146	Oromiya	South W/Shewa	Kersa	Godeti	2013	8.41168	38.35535
S. No	**Region**	**Zone**	**District**	**Location**	**Altitude**	**Latitude**	**Longitude**
147	Oromiya	South W/Shewa	Malima	Multialibo	2056	8.38230	38.35593
148	Oromiya	South W/Shewa	Kersamalima	Mitichole	2111	8.39221	38.35015

149	Oromiya	South W/Shewa	Kersamalima	Mutideyu	2158	8.38223	38.32569
150	Oromiya	South W/Shewa	Kersamalima	Adadi	2284	8.37417	38.30366
151	Oromiya	South W/Shewa	Lemen	Foka	2112	8.588814	38.62904
152	Oromiya	South W/Shewa	Lemen	Godeta	2109	8.67926	38.59443
153	Oromiya	South W/Shewa	Sebeta	Gejagedanba	2260	8.85929	38.66027
154	Oromiya	South W/Shewa	Sebeta	Atebela	2170	8.79791	38.64038
155	Oromiya	South W/Shewa	Sebeta	Jewe	2137	8.73608	38.64780
156	Oromiya	South W/Shewa	Sebeta	Onhikie	2113	8.48004	38.38000
157	Oromiya	South W/Shewa	Sebeta	Geja Daleti	2130	8.44194	38.38541
158	Oromiya	South W/Shewa	Seden sodo	adadiborele	2421	8.4717	38.24451
159	Oromiya	South W/Shewa	Seden sodo	tullie belekesa	2387	8.50879	38.24181
160	Oromiya	South W/Shewa	Seden sodo	uragokelecha	2291	8.56132	38.25115
161	Oromiya	South W/Shewa	Sodo dachie	Badu sonbo	2082	8.30509	38.48393
162	Oromiya	South W/Shewa	Sodo dachie	Bukie robele	1854	8.26149	38.43157
163	Oromiya	South W/Shewa	Sodo dachie	Hodokotiyo	1825	8.26002	38.43417
164	Oromiya	South W/Shewa	Sodo dachie	Gerersa	1787	8.24003	38.44299
165	Oromiya	South W/Shewa	Sododachie	Jebdu	2180	8.54147	38.61982
166	Oromiya	South W/Shewa	Sododachie	S/muchuchatu	2201	8.51201	38.63671
167	Oromiya	South W/Shewa	woiliso	hobi koji	2112	8.56807	37.99521
168	Oromiya	South W/Shewa	woiliso	doyokora	2336	8.64406	38.12547
169	Oromiya	South W/Shewa	woiliso	soyoma	2234	8.63809	38.19476
170	SNNP	Gurage	Kebena	kolakabada	1807	8.2683	37.76060
171	SNNP	Gurage	Kebena	jorgia	1817	8.26102	37.72870
172	SNNP	Gurage	kebena	fikadu	1796	8.32407	37.78682
173	SNNP	Gurage	Meskan/Sodo	Jollie	1910	8.19469	38.45824
174	SNNP	Gurage	Sodo	Ketefo	2142	8.37972	38.58878
175	SNNP	Gurage	sodo	Gogeti	1928	8.22998	38.47747
176	SNNP	Hadiya	Badewchi	Badewachi	1892	7.11279	37.94909
177	SNNP	Hadiya	Badewchi	Lalo gerbe	1935	7.1082	37.94775
178	SNNP	Siltie	Hurbaray	Kora	2037	7.7634	38.13330
179	SNNP	Siltie	Hurbaray	Fugie	2076	7.80266	38.15517
180	SNNP	Wolayita	Bolloso sore	Gurmoweyidie	1961	6.91823	37.73024
181	SNNP	Wolayita	Bolloso sore	Gurmakosha	1944	6.96379	37.74131
182	SNNP	Wolayita	Bolloso sore	Hachura	1712	7.15223	37.70060
183	SNNP	Wolayita	Damot gale	Bugie	1882	7.06693	37.94167
S. No	**Region**	**Zone**	**District**	**Location**	**Altitude**	**Latitude**	**Longitude**
184	SNNP	Wolayita	Damot gale	Kadadi	1882	7.04101	37.92882
185	SNNP	Wolayita	Damot gale	Shoya	1912	7.00439	37.89808
186	SNNP	Wolayita	Damot gale	Gidobodetti	2071	6.93849	37.84578

187	Tigray	Axum	Abiyadi	Worq Amba	1960	13.72408	38.98404
188	Tigray	Axum	Laymachew	Lesalso	2140	14.14182	38.79789
189	Tigray	Axum	Laymachew	Lesalso	2099	14.1285	38.76329
190	Tigray	Axum	Laymachew	Atsiho	2103	14.11393	38.75741
191	Tigray	Axum	Laymachew	Atsiho	2081	14.10452	38.76814
192	Tigray	Axum	Laymachew	Sefho	2080	14.11337	38.68960
193	Tigray	Axum	Laymachew	Adura	2067	14.11172	38.65326
194	Tigray	Axum	Laymachew	Meseye	2142	14.12334	38.62304
195	Tigray	Axum	Tachmachew	Wuqiromariam	2201	14.11188	38.60606
196	Tigray	Axum	Tachmachew	Mahberawi	2202	14.1226	38.54203
197	Tigray	Axum	Abiyadi	Qaqa	1842	13.68205	38.97824
198	Tigray	East Tigray	Gemad	Tsedenaal	2406	14.90661	38.59361
199	Tigray	East Tigray	Tsedeaba	Maymegerta	2453	14.07928	38.56894
200	Tigray	East Tigray	Wuqero	Nejash	2233	13.86253	39.60371
201	Tigray	North W/Tigray	T/ Machew	Aqebe	2185	14.11914	38.52666
202	Tigray	North W/Tigray	Asgetsibila	Klteno	1744	13.92822	38.18222
203	Tigray	North W/Tigray	Asgetsibila	Lemlem	1673	13.86381	38.17735
204	Tigray	North W/Tigray	Asgetsibila	Selam	1850	14.04913	38.22661
205	Tigray	North W/Tigray	Medebayzena	Adeqemaleh	1965	14.10646	38.4462
206	Tigray	North W/Tigray	Medebayzena	Adeqemaleh	1966	14.10857	38.44426
207	Tigray	North W/Tigray	Medebayzena	Adeqemaleh	1974	14.10916	38.46928
208	Tigray	North W/Tigray	Shire	Adigdad	1936	14.08232	38.36607
209	Tigray	North W/Tigray	T/Qoraro	Adigdad	1925	14.07818	38.34615
210	Tigray	North W/Tigray	T/ Qoraro	Madirash	1920	14.10851	38.32285
211	Tigray	North W/Tigray	T/Qoraro	Kelfem	1885	14.09074	38.26422
212	Tigray	South Tigray	Enderta	Dedeba	2225	13.37968	39.522897
213	Tigray	South Tigray	Quha	Maytsede	2261	13.4482	39.55167
214	Tigray	West Tigray	Dega Tenbel	Muhubal	2602	13.61858	39.13054
215	Tigray	West Tigray	Dega Tenbel	Seret	2626	13.58802	39.11171
216	Tigray	West Tigray	Enderta	Mahberegenet	1926	11.57418	39.4366
217	Tigray	West Tigray	Meserit	Lalabla	2626	13.57152	39.11536

Appendix 9.2.Selected 166 *Fusarium*isolates for whole genome sequencing

S. No	Sample ID	Strain-ID	Location	GPS coordinate	Sequence statistics			
				(Latitude; Longitude)	Raw Reads	Draft Size	Scaffold	Mean Coverage

1	EtdFoc-1	Fo-Et-0000	Alemaya	9.41781 N; 42.03592 E	11044678	59054520	4660	17.9
2	EtdFoc-10	Fo-Et-0001	Chelenko	9.42071 N; 41.61528 E	21966650	54543889	2293	41.74
3	EtdFoc-100	Fo-Et-0002	Habru	8.94653 N; 39.09507 E	24203702	75200031	12189	33.01
4	EtdFoc-102	Fo-Et-0003	Adaw	9.00814 N; 39.52163 E	9034666	54627506	14476	15.06
5	EtdFoc-103	Fo-Et-0004	Elesebeto	9.08428 N; 39.06594 E	5621298	6811359	867	31.36
6	EtdFoc-105	Fo-Et-0005	Kora	9.78209 N; 39.19478 E	10093332	10757812	93	92.02
7	EtdFoc-107	Fo-Et-0006	Kaloli	9.93128 N; 39.19221 E	7586240	10694988	74	37.97
8	EtdFoc-108	Fo-Et-0007	Tachamba	9.96724 N; 39.22387 E	13608626	11667414	1570	112.57
9	EtdFoc-109	Fo-Et-0008	Jihur	9.98046 N; 39.22947 E	8970056	53410403	5367	15.24
10	EtdFoc-11	Fo-Et-0009	Gorogutu	9.384774 N; 41.4567 E	5650628	9274307	5375	46.71
11	EtdFoc-110	Fo-Et-0010	Robi	9.59027 N; 38.92239 E	3244056	7207149	12044	4.07
12	EtdFoc-113	Fo-Et-0011	Abichu	7.81491 N; 39.07238 E	9779214	61094291	24916	12.06
13	EtdFoc-115	Fo-Et-0012	Merhabete	7.60347 N; 39.52061 E	9850656	11445420	908	56.94
14	EtdFoc-116	Fo-Et-0013	Hella	7.59698 N; 39.5458 E	7183200	24862369	25228	19.1
15	EtdFoc-117	Fo-Et-0014	Hella	7.5906 N; 39.58173 E	15210714	78059024	34197	17.89
16	EtdFoc-118	Fo-Et-0015	Gelebeha	7.59623 N; 39.56008 E	5344774	6390296	73	36.68
17	EtdFoc-119	Fo-Et-0016	Burkutu	7.58052 N; 39.5177 E	5996472	22918040	1119	23.45
18	EtdFoc-12	Fo-Et-0017	Workshop	9.39369 N; 41.44468 E	6525782	36143656	5286	9.19
19	EtdFoc-120	Fo-Et-0018	Jelko	7.60611 N; 39.50603 E	3570526	6751378	863	39.86
20	EtdFoc-121	Fo-Et-0019	D/slam	7.63155 N; 39.52213 E	5207224	18150618	5615	8.41
21	EtdFoc-122	Fo-Et-0020	Goro	7.63519 N; 39.57496 E	7612442	6900225	827	96.34
22	EtdFoc-123	Fo-Et-0021	Adaba	7.00644 N; 39.37111 E	6394302	10686188	30	61.32
23	EtdFoc-124	Fo-Et-0022	Herer	6.99251 N; 39.23872 E	6515600	10735952	40	59.98
24	EtdFoc-125	Fo-Et-0023	Shallo	7.06882 N; 39.64987 E	2106064	10774329	510	17.24
25	EtdFoc-126	Fo-Et-0024	Addisalma	7.01013 N; 40.38829 E	5428524	6402389	74	73.88
26	EtdFoc-127	Fo-Et-0025	Chefamana	7.00785 N; 40.41349 E	4385894	10675573	58	16.48
27	EtdFoc-128	Fo-Et-0026	Meliyu	7.01365 N; 40.40505 E	6674320	34797777	29188	15.45
28	EtdFoc-129	Fo-Et-0027	Wesha	7.03874 N; 39.48768 E	7821814	17597834	13127	22.64
29	EtdFoc-13	Fo-Et-0028	Ifabelem	9.27836 N; 41.16406 E	27629788	53327092	3220	52.09
30	EtdFoc-130	Fo-Et-0029	Adaba	7.03849 N; 39.48737 E	5223728	34968080	31934	7.96
31	EtdFoc-132	Fo-Et-0030	Debrezt	8.80969 N; 39.0038 E	4122104	10828894	17470	5.14
32	EtdFoc-133	Fo-Et-0031	Ejere	8.7845 N; 39.24482 E	3516248	14331005	12926	12.13
33	EtdFoc-136	Fo-Et-0032	Ejere	8.77241 N; 39.16001 E	7828302	54339620	6486	16.05
34	EtdFoc-137	Fo-Et-0033	Nanawa	8.76003 N; 39.1513 E	6450294	12676675	3008	18.83
35	EtdFoc-138	Fo-Et-0034	B/Gerbi	8.74255 N; 39.125 E	6679154	26950178	20603	21.46
36	EtdFoc-139	Fo-Et-0035	Bililgn	8.78354 N; 39.17 E	5076192	50299589	34611	9.23
S. No	**Sample ID**	**Strain-ID**	**Location**	**GPS coordinate**	**Raw Reads**	**Draft Size**	**Scaffold**	**Mean Coverage**
37	EtdFoc-142	Fo-Et-0036	Arerti	8.75328 N; 39.3636 E	5674204	34624598	32077	12.74
38	EtdFoc-143	Fo-Et-0037	K/ amba	8.75383 N 39.26305 E	7721298	52127303	28306	13.71
39	EtdFoc-145	Fo-Et-0038	B/silasie	8.70574 N 39.22518 E	4490402	17561203	25985	4.59

40	EtdFoc-146	Fo-Et-0039	B/ Giorgis	8.7 N 39.21426 E	4629592	15806191	24262	7.35
41	EtdFoc-147	Fo-Et-0040	Ensira	8.76492 N 39.17205 E	5190530	37518216	37553	7.13
42	EtdFoc-148	Fo-Et-0041	Wele	9.76483 N 39.11284 E	9671268	6041551	938	118.98
43	EtdFoc-149	Fo-Et-0042	Kallele	9.46453 N 39.10284 E	7212434	12037647	2106	27.04
44	EtdFoc-15	Fo-Et-0043	Worani	9.24143 N 41.11993 E	5393710	32454851	31191	13.5
45	EtdFoc-152	Fo-Et-0044	W/amba	9.56566 N 39.12578 E	2803460	18704931	24435	7.35
46	EtdFoc-155	Fo-Et-0045	Beda oda	8.5022 N 38.48036 E	26594356	55038620	1955	46.93
47	EtdFoc-158	Fo-Et-0046	Gerbicha	8.4032 N 38.58363 E	12059566	52171121	3041	25.62
48	EtdFoc-159	Fo-Et-0047	Gindebel	8.41096 N 38.58597 E	6470314	9936768	5179	29.23
49	EtdFoc-16	Fo-Et-0048	L/dhekeb	9.18006 N 41.10549 E	9384118	58362368	6001	16.36
50	EtdFoc-160	Fo-Et-0049	Gichie	8.39567 N 38.59147 E	15880478	54355301	5145	27.97
51	EtdFoc-167	Fo-Et-0050	Agamsa	8.30342 N 38.47532 E	23943996	56700943	1380	41.86
52	EtdFoc-169	Fo-Et-0051	Werejarso	8.37172 N 38.49007 E	4720368	6981390	1257	46.9
53	EtdFoc-17	Fo-Et-0052	Lubudheke	9.1736 N 41.093 E	18740872	63090211	27796	30.28
54	EtdFoc-174	Fo-Et-0053	Mitichole	8.39221 N 38.35015 E	7095938	52754620	26005	10.7
55	EtdFoc-175	Fo-Et-0054	Mutideyu	8.38223 N 38.32569 E	5546568	14180083	12076	28.6
56	EtdFoc-176	Fo-Et-0055	Adadi	8.37417 N 38.30366 E	7639758	55199200	24921	12.82
57	EtdFoc-177	Fo-Et-0056	Badu	8.30509 N 38.48393 E	5673926	33675945	36286	4.93
58	EtdFoc-178	Fo-Et-0057	Bukie	8.26149 N 38.43157 E	9998810	59607700	7822	16.99
59	EtdFoc-18	Fo-Et-0058	Terkafeta	9.17142 N 41.07368 E	5915656	45387964	29413	10.48
60	EtdFoc-181	Fo-Et-0059	Mersa	11.6195 N 39.67102 E	13028964	55314610	8631	21.94
61	EtdFoc-182	Fo-Et-0060	Chefemeda	11.75056 N 39.62205 E	12470050	52983582	28820	23.84
62	EtdFoc-183	Fo-Et-0061	Jenetuber	11.77483 N 39.57838 E	17835570	56224456	6677	29.09
63	EtdFoc-184	Fo-Et-0062	W/zuria	11.81363 N 39.58688 E	15799598	59317194	2161	27.31
64	EtdFoc-185	Fo-Et-0063	Afasash	12.1122 N 39.61959 E	10637116	59084494	4752	18.73
65	EtdFoc-186	Fo-Et-0064	Dingay	12.18452 N 39.62445 E	29791544	59301844	1518	51.91
66	EtdFoc-187	Fo-Et-0065	Mahbere	11.57418 N 39.4366 E	13253610	10721526	89	125.33
67	EtdFoc-189	Fo-Et-0066	Seret	13.58802 N 39.11171 E	12619854	58828178	3167	20.52
68	EtdFoc-19	Fo-Et-0067	Terkafeta	9.179 N 41.05761 E	13118714	59244928	2427	22.52
69	EtdFoc-190	Fo-Et-0068	Lalabla	13.57152 N 39.11536 E	15532108	62784420	6395	24.6
70	EtdFoc-191	Fo-Et-0069	Qaqa	13.68205 N 38.97824 E	29288664	58169497	1548	49.49
71	EtdFoc-192	Fo-Et-0070	Worq	13.72408 N 38.98404 E	16237044	58343401	1851	28.34
72	EtdFoc-193	Fo-Et-0071	Lesalso	14.14182 N 38.79789 E	18341184	60838255	7069	31.02
73	EtdFoc-194	Fo-Et-0072	Lesalso	14.1285 N 38.76329 E	26755628	17818805	1722	143.83
S. No	**Sample ID**	**Strain-ID**	**Location**	**GPS coordinate**	**Raw Reads**	**Draft Size**	**Scaffold**	**Mean Coverage**
74	EtdFoc-195	Fo-Et-0073	Atsiho	14.11393 N 38.75741 E	28632940	59420532	2774	47.33
75	EtdFoc-198	Fo-Et-0074	Adura	14.11172 N 38.65326 E	25643898	63508565	10899	40.47
76	EtdFoc-199	Fo-Et-0075	Meseye	14.12334 N 38.62304 E	16558288	10672987	78	154.27
77	EtdFoc-2	Fo-Et-0076	Gobe	9.37483 N 42.99038 E	7236188	52759367	12365	11.72

78	EtdFoc-20	Fo-Et-0077	Kemer	8.78967 N 40.49142 E	8543680	52139755	25867	15.16
79	EtdFoc-201	Fo-Et-0078	Mahberawi	14.1226 N 38.54203 E	13971674	22257187	21164	57.9
80	EtdFoc-203	Fo-Et-0079	Adigdad	14.07818 N 38.34615 E	15866178	55460660	2701	29.24
81	EtdFoc-204	Fo-Et-0080	Madirash	14.10851 N 38.32285 E	11818380	60520689	3891	20.04
82	EtdFoc-206	Fo-Et-0081	Klteno	13.92822 N 38.18222 E	12508264	60595094	5751	20.86
83	EtdFoc-208	Fo-Et-0082	Selam	14.04913 N 38.22661 E	25467334	57038902	2431	42.5
84	EtdFoc-209	Fo-Et-0083	Adeqemale	14.10646 N 38.4462 E	8898680	56503834	8052	15.68
85	EtdFoc-213	Fo-Et-0084	Maymegert	14.07928 N 38.56894 E	7104188	52490317	9839	13.36
86	EtdFoc-214	Fo-Et-0085	Tsedenaal	14.11914 N 38.52666 E	18108356	58145279	843	31.65
87	EtdFoc-215	Fo-Et-0086	Nejash	13.86253 N 39.60371 E	29980438	6373036	201	465.67
88	EtdFoc-216	Fo-Et-0087	Maytsede	13.4482 N 39.55167 E	15451030	52480651	1802	30.77
89	EtdFoc-218	Fo-Et-0088	Ketefo	8.37972 N 38.58878 E	17849610	53007463	1881	34.26
90	EtdFoc-219	Fo-Et-0089	Fugie	7.80266 N 38.15517 E	17991468	52444643	1694	35.76
91	EtdFoc-22	Fo-Et-0090	Lugo	8.8187 N 40.49926 E	27997278	56733622	1113	50.44
92	EtdFoc-220	Fo-Et-0091	Badewachi	7.11279 N 37.94909 E	10189106	58202203	5736	17.16
93	EtdFoc-221	Fo-Et-0092	Bugie	7.06693 N 37.94167 E	11565672	53640518	2947	22.44
94	EtdFoc-222	Fo-Et-0093	Kadadi	7.04101 N 37.92882 E	12558198	59466241	3743	21.91
95	EtdFoc-223	Fo-Et-0094	soyoma	8.63809 N 38.19476 E	17219882	53588006	1909	33.36
96	EtdFoc-228	Fo-Et-0095	Awrajit	11.75532 N 38.50494 E	6498908	54499074	18823	11.33
97	EtdFoc-229	Fo-Et-0096	Tsegur	11.89286 N 37.97184 E	10185474	52565236	2800	20.48
98	EtdFoc-23	Fo-Et-0097	Hature	8.82043 N 40.55502 E	16463792	53375230	1935	32.14
99	EtdFoc-233	Fo-Et-0098	Laysek	12.47305 N 37.03597 E	17779154	56846089	5521	31.7
100	EtdFoc-234	Fo-Et-0099	Nora	12.56331 N 37.11211 E	15306826	52240984	1565	30.09
101	EtdFoc-236	Fo-Et-0100	Y/Campas	11.5623 N 37.32896 E	18353112	53923284	1912	36
102	EtdFoc-237	Fo-Et-0101	Jabi	10.69718 N 37.14028 E	5625572	50464994	23539	10.1
103	EtdFoc-24	Fo-Et-0102	Sukaye	8.8356 N 40.59676 E	15224364	7753232	2547	178.63
104	EtdFoc-240	Fo-Et-0103	Getahua	10.23801 N 38.06009 E	20982752	40275449	294	53.56
105	EtdFoc-243	Fo-Et-0104	Debrezt	8.8097 N 39.0038 E	10771766	57906179	6581	19.11
106	EtdFoc-245	Fo-Et-0105	Kela	8.5048 N 38.48306 E	11859946	53859140	3403	21.42
107	EtdFoc-247	Fo-Et-0106	Cholo	8.50459 N 38.48479 E	9809976	62352654	7753	15.57
108	EtdFoc-248	Fo-Et-0107	Sidamo	8.50074 N 38.48328 E	10270250	50545352	2494	18.54
109	EtdFoc-25	Fo-Et-0108	Geteta	8.86153 N 40.64635 E	7243696	45963372	30013	5.67
110	EtdFoc-250	Fo-Et-0109	Rogicha	8.30186 N 38.474 E	16648554	62795809	11774	26.44
S. No	**Sample ID**	**Strain-ID**	**Location**	**GPS coordinate**	**Raw Reads**	**Draft Size**	**Scaffold**	**Mean Coverage**
111	EtdFoc-253	Fo-Et-0110	Multialibo	8.3823 N 38.35593 E	6064634	50242981	15576	11.14
112	EtdFoc-256	Fo-Et-0111	H/kotyo	8.26002 N 38.43417 E	10865734	53730727	2953	21.24
113	EtdFoc-258	Fo-Et-0112	Muhubal	13.61858 N 39.13054 E	13122374	51394591	1689	26.5

114	EtdFoc-259	Fo-Et-0113	Adeqemale	14.10916 N 38.46928 E	13179630	53088910	2305	25.37
115	EtdFoc-26	Fo-Et-0114	Didimina	8.88596 N 40.71497 E	10392196	8511408	3494	110.11
116	EtdFoc-27	Fo-Et-0115	Jewis	8.92267 N 40.81709 E	5536846	17283326	1464	19.18
117	EtdFoc-28	Fo-Et-0116	Arbreketie	9.041743 N 40.90948 E	26972956	25130766	5098	100.46
118	EtdFoc-29	Fo-Et-0117	G/gedanb	8.85929 N 38.66027 E	8069462	55776947	11694	11.66
119	EtdFoc-3	Fo-Et-0118	G/chala	9.34749 N 41.98482 E	17205508	58147600	1572	30.43
120	EtdFoc-30	Fo-Et-0119	Atebela	8.79791 N 38.64038 E	18623266	52242363	1771	37
121	EtdFoc-34	Fo-Et-0120	Jebdu	8.54147 N 38.61982 E	6857940	51933932	18767	10.67
122	EtdFoc-35	Fo-Et-0121	Seden	8.51201 N 38.63671 E	8695784	52667448	18966	7.57
123	EtdFoc-37	Fo-Et-0122	Gogeti	8.22998 N 38.47747 E	4379454	31141220	33871	7.24
124	EtdFoc-38	Fo-Et-0123	Jollie	8.19469 N 38.45824 E	22976570	59159101	2337	39.13
125	EtdFoc-39	Fo-Et-0124	Kora	7.7634 N 38.1333 E	18512660	51702086	1290	36.97
126	EtdFoc-4	Fo-Et-0125	Sonbe	9.49636 N 42.2475 E	8556100	52337530	15291	8.68
127	EtdFoc-42	Fo-Et-0126	L/gerbe	7.1082 N 37.94775 E	7376062	12450855	2306	53.05
128	EtdFoc-45	Fo-Et-0127	Shoya	7.00439 N 37.89808 E	7917060	12265702	3012	28.97
129	EtdFoc-46	Fo-Et-0128	Gidobodetti	6.93849 N 37.84578 E	13731050	53151639	2398	24.74
130	EtdFoc-47	Fo-Et-0129	Gurmoweyi	6.91823 N 37.73024 E	9248680	52071680	3804	17.53
131	EtdFoc-48	Fo-Et-0130	Gurmakosh	6.96379 N 37.74131 E	14908574	54518816	2806	28.05
132	EtdFoc-49	Fo-Et-0131	Hachura	7.15223 N 37.7006 E	8996424	52407112	3909	16.69
133	EtdFoc-5	Fo-Et-0132	Metokema	9.45104 N 41.85831 E	10198758	52204541	2297	20.92
134	EtdFoc-50	Fo-Et-0133	kolakabada	8.2683 N 37.7606 E	4547708	6709947	633	28.9
135	EtdFoc-52	Fo-Et-0134	fikadu	8.32407 N 37.78682 E	8120346	53547926	8137	13.58
136	EtdFoc-53	Fo-Et-0135	C/soyoma	8.41422 N 37.88969 E	10991958	6516041	365	48.05
137	EtdFoc-55	Fo-Et-0136	doyokora	8.64406 N 38.12547 E	13515224	54837589	3245	24.74
138	EtdFoc-58	Fo-Et-0137	Tullie	8.50879 N 38.24181 E	14166562	61392647	3653	22.68
139	EtdFoc-6	Fo-Et-0138	Gandechela	9.44165 N 41.6631 E	22181844	57563866	4183	40.01
140	EtdFoc-61	Fo-Et-0139	kobo	8.69614 N 38.24696 E	11059370	52490910	3848	19.94
S. No	**Sample ID**	**Strain-ID**	**Location**	**GPS coordinate**	**Raw Reads**	**Draft Size**	**Scaffold**	**Mean Coverage**
141	EtdFoc-62	Fo-Et-0140	keta	8.76196 N 38.30695 E	31340130	1.12E+08	15957	28.05
142	EtdFoc-63	Fo-Et-0141	kobo	12.14338 N 39.64022 E	16321678	61096964	5206	25.59
143	EtdFoc-67	Fo-Et-0142	Gazo	11.92523 N 37.85114 E	4823614	6255750	102	30.6
144	EtdFoc-68	Fo-Et-0143	S/tseyon	12.02741 N 37.72261 E	19814494	59899555	3082	33.04

145	EtdFoc-69	Fo-Et-0144	Debreslam	12.17419 N 37.6938 E	6378372	51125001	23251	9.82
146	EtdFoc-7	Fo-Et-0145	Agello	9.45068 N 41.86469 E	9343254	52777434	3802	18
147	EtdFoc-72	Fo-Et-0146	Adoch	12.33472 N 37.58117 E	14380424	53952115	5220	14.06
148	EtdFoc-74	Fo-Et-0147	Giragie	12.47008 N 37.37276 E	11992152	84489735	41162	12
149	EtdFoc-75	Fo-Et-0148	Gebeba	12.40477 N 37.30368 E	6512334	54042209	34312	9.27
150	EtdFoc-76	Fo-Et-0149	Cherkaw	12.36591 N 37.2615 E	12823030	64614985	3883	21.21
151	EtdFoc-79	Fo-Et-0150	Guhal	12.24829 N 37.08631 E	40952132	53165667	2737	74.7
152	EtdFoc-8	Fo-Et-0151	G/woliso	9.37879 N 41.58224 E	14442428	52626114	1899	26.97
153	EtdFoc-80	Fo-Et-0152	Chewaduba	12.31499 N 37.074 E	6358920	12413535	10253	10.65
154	EtdFoc-81	Fo-Et-0153	Deserek	12.39954 N 37.01949 E	11708114	64516085	6292	18.37
155	EtdFoc-85	Fo-Et-0154	Admina	11.63299 N 36.92491 E	17269604	59535740	5062	29.18
156	EtdFoc-86	Fo-Et-0155	Akumihret	11.66485 N 36.92371 E	8615694	53728502	6332	15.55
157	EtdFoc-87	Fo-Et-0156	Jiwacha	11.71142 N 36.96107 E	12843340	52857531	2192	24.15
158	EtdFoc-88	Fo-Et-0157	Golashkan	11.75585 N 36.98505 E	16864766	52779487	1794	33.05
159	EtdFoc-89	Fo-Et-0158	Shankra	11.79425 N 36.98303 E	8989330	56779199	30424	12.87
160	EtdFoc-9	Fo-Et-0159	Gutuware	9.37656 N 41.58562 E	18039418	1.08E+08	11738	17.31
161	EtdFoc-92	Fo-Et-0160	Wengie	10.65707 N 37.38309 E	13153606	59308767	12344	8.31
162	EtdFoc-93	Fo-Et-0161	Damil	10.62389 N 37.41582 E	13114782	1.07E+08	35146	11.85
163	EtdFoc-94	Fo-Et-0162	Yekuas	10.39659 N 37.57511 E	8658910	53110502	10094	11.95
164	EtdFoc-95	Fo-Et-0163	Digil	10.35001 N 37.66479 E	7279220	33289044	31438	14.54
165	EtdFoc-96	Fo-Et-0164	Yegidina	10.22101 N 37.98501 E	7512504	12686891	313	24.71
166	EtdFoc-99	Fo-Et-0165	Ambelta	8.90027 N 39.0575 E	6100644	52160747	17061	11.58
Average					***12151247***	***43447573***	***9371.97***	***33.24873***