ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPETITIONAL SCIENCE

DEPARTEMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY

Characterization of Ethiopian Chickpea (*Cicer arietinum* L): Genotypic Diversity, Population Structure, Cold Tolerance, and Assessment of Change of Variability over Time

By Sintayehu Admas Mekonnen

A Dissertation Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the degree of Doctor of philosophy in Biology (Applied Genetics)

December, 2021

Addis Ababa, Ethiopia

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Supervisors: Kassahun Tesfaye (PhD, Assoc. Prof.)
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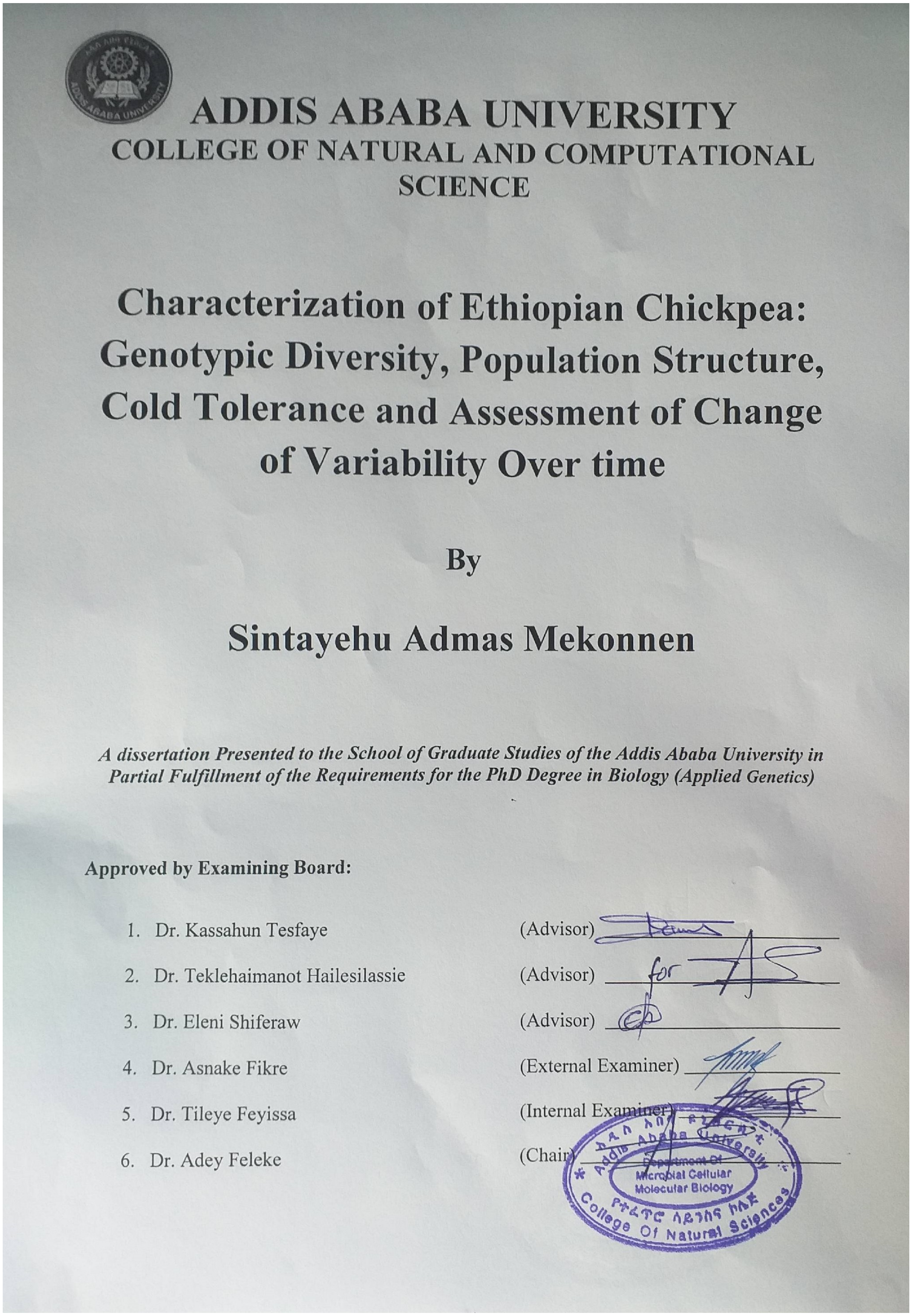

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Declaration

I, the undersigned, declare that the thesis submitted to the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) is my own work and has not been submitted at another university. The materials obtained from other sources are duly acknowledged in the thesis.

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Dedication

This PhD dissertation is dedicated to Ethiopian farmers who have been preserving crop landraces for future generations to come.

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Lists of Acronyms and Abbreviations

ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
AGP-II	Agricultural growth program phase two
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
CAPS	Cleaved amplified polymorphic sequences
CISP	Conserved intron spanning primers
CLUMPAK	Cluster Markov Package Across K
CSA	Central Statistical Agency
CTAB	Cetyltriethylammonium bromide
CV	Coefficient of variation
DA	Nei's unbiased genetic distance
DAP	Diammonium phosphate
DArT	Diversity arrays technology
DBARC	Debre Birhan Agricultural Research Center
DF	Degrees of freedom
DMRT	Dunkan's multiple range test
dNTPs	Deoxynucleoside Triphosphates
DTF	Days to 50% flowering
DTM	Days to 90% maturity
DTP	Days to 50% podding
DZARC	Debre Zeit Agricultural Research Center
EARCs	Ethiopian Agricultural Research Centers
EBI	Ethiopian Biodiversity Institute
EDTA	Ethylene diamine tetra-acetic acid
EIAR	Ethiopian Institute of Agricultural Research
ESTs	Expressed sequence tags
FAO	Food and Agriculture Organization
FC	Flower color
Fis	Inbreeding coefficient
Fit	Fixation index

FPPP	Fertile pods per plant
FTR	Freezing tolerance rate
FTR	Frost tolerance rate
FW	Fresh weight
GA	Genetic advance
GBS	Genotyping-by-sequencing
GCV	Genotypic coefficient of variation
GD	Gene diversity
GE	Genetic erosion
GLM	General linear model
GRNF	Growth rate of number of foliage during frost treatment
GRNPB	Growth rate of number of primary branch during frost treatment
GRPLH	Growth rate of plant height during frost treatment
GWAS	Genome-wide association study
GY	Grain yield
H	Heritability
H'	Shannon-Weaver diversity index
He	Expected heterozygosity
Ho	Observed heterozygosity
I	Shannon's information index
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPCA	Interaction principal component axes
IPPP	Infertile pods per plant
ISSR	Inter simple sequence repeat
K	Kinship similarity
Kg ha^{-1}	Kilogram per hectare
LSD	Least significant difference
LT50	The temperature that kills 50% of the plants
MAF	Major allele frequency
MCMC	Markov Chain Monte Carlo
MLM	Mixed linear model
MoA	Ministry of Agriculture
MSE	Mean square of error

Na	Number of alleles
Ne	Effective number of alleles
NFAF	Number of foliage after frost treatment
NFBF	Number of foliage before frost treatment
NGS	Next generation sequencing
Nm	Pairwise gene flow
NPA	Number of private alleles
NPB	Number of primary branches
NPPP	Number of pods per plant
NSB	Number secondary branches
PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PLH	Plant canopy height
QTLs	Quantitative trait loci
RAPD	Random amplified polymorphic DNAs
RFLP	Restriction fragment length polymorphism
SC	Seed color
SLP	Stem/leaf pigmentation
SNNPR	Southern Nations, Nationalities and Peoples Region
SNP	Single nucleotide polymorphism
SR	Frost survival rate
SSRs	Simple sequence repeats
SSS	Seed shriveling score
STMS	Sequence tagged microsatellite sites
TBE buffer	Tris/Borate/EDTA
TSW	Thousand Seed weight
UPGMA	Unweighted Pair Group Method with Arithmetic Average

Abstract

Characterization of Ethiopian Chickpea (*Cicer arietinum* L): Genotypic Diversity, Population Structure, Cold Tolerance, and Assessment of Change of Variability over Time

Sintayehu Admas, Addis Ababa University, December, 2021

Plant genetic resources are valuable source of desirable agronomic traits that can be used in crop improvement. These resources are constantly threatened by both natural and human-caused risks. Chickpea (*Cicer arietinum* L.) germplasm is one of the crops affected by these factors. Regular chickpea diversity monitoring in farmers' fields, as well as characterization and evaluation of Ethiopian chickpea germplasm in ex-situ, are essential in order to build viable conservation strategies and undertake breeding initiatives. However, in Ethiopia chickpea, the above-mentioned information is limited. As a result, this research began with the general objective of determining the diversity of Ethiopian chickpea germplasm and its potential for frost resistance, as well as determining the degree to which chickpea on-farm diversity has changed over time. In the study, molecular analysis was conducted to determine the genetic diversity, population structure, and relationships of 152 chickpea genotypes using simple sequence repeats (SSR) markers. There were 133 alleles observed using 23 polymorphic SSR markers, with a mean of 5.8 alleles per locus. Pairwise population Nei's genetic distance, heterozygosity (0.4), Shannon's information index (1.2), Gene Diversity (0.67), polymorphic information content (0.63), and percent polymorphism (99.5%) were among the genetic-based statistics used in the study. These studies demonstrated that there was high genetic variation within and among chickpea genotypes. The 152 genotypes were divided into two groups on UPGMA tree developed based on Nei's genetic distances. The exotic genotypes were all grouped together in one cluster, showing that they are distinct from Ethiopian genotypes. However, seed exchange caused inconsistencies in the patterns of grouping Ethiopian chickpea genotypes by geographic location. Two separate populations were discovered using model-based population structure clustering. These findings can be used to create efficient conservation approaches and breeding initiatives to enhance the genetic diversity of chickpeas. To assess the potential of Ethiopian chickpea for frost resistance, a frost screening study was carried out in a natural field and in controlled conditions. Six hundred seventy-three genotypes were characterized for two years (2018/2019 to 2019/2020) using an augmented design at Bakelo, Debre Birhan, Ethiopia, a place as national frost screening site. A significant ($p<0.01$) variability amongst genotypes was recorded for all agronomic traits considered. In terms of agronomic performance, 94 (12.6%) of the genotypes examined outperformed the frost susceptible genotypes. Stem/leaf pigmented genotypes had a better response to frost stress than non-pigmented genotypes. The majority of black seeded chickpeas were adapted well to frost stress, when compared to brown and white seeded genotypes. According to the freezing tolerance rate (FTR) and plant survival rate (SR), 83 (12.3%) and 85 (12.6%) genotypes were identified as frost tolerant. Grain yield had a significant ($p<0.01$) correlation with FTR, SR, seed shriveling score, stem/leaf pigmentation, and seed color. Based on the field experiment genotypes performances, 72 genotypes were selected and evaluated further for cold tolerance variability at seedling stage under controlled environment using a complete randomized design with two replications. The analysis of variance revealed a significant ($P<0.01$) difference among genotypes for plant height, number of foliage, number of primary branches and fresh weight. Cold tolerant genotypes had a higher growth rate than susceptible genotypes, which had a slower growth rate or completely failed. According to SR, 31 (43.1%) genotypes scored above 0.8 values. Based on FTR, 37 (51.4%) and 31 (43.1%) genotypes were rated at a score of 1 to 3 in freezing test 1 (T1) and freezing test 2 (T2), respectively indicating better frost tolerance than others. Fresh biomass yields had a high negative correlation ($p<0.01$) with SR, but a substantial positive correlation ($p<0.01$) with FTR value. At seedling stage, based on the combined results of FTR and SR scores, 26 genotypes were shown to be cold tolerant genotypes at temperatures as low as -5°C. Therefore, the field and controlled environment experiments results revealed that Ethiopian chickpea landraces have a good genetic potential for cold tolerance traits for use in breeding programs. In addition, a diversity monitoring research was conducted to look into the differences in phenotypic diversity between the present (2017) and prior chickpea collections (1979-1983). The experiment was conducted using simple lattice design with two replications at Debre Zeit Agricultural Research Center for two consecutive years (2018/2019 to 2019/2020). The result showed that chickpea has been under genetic erosion ranging from 30.4% to 100% during the last 35 years. During this time, estimations of Shannon-Weaver diversity index for black seeded and ivory white seeded chickpea kinds have declined from 1.99 to 0.69 and 1.33 to 0.0, respectively. In previous collections, 25.8% of black coated genotypes and 6.5% of white coated genotypes were found, but these chickpea varieties are now rare (black 6.5%) or unobtainable (white 0.0%). Cluster analysis revealed that genotypes clustered according to the time of collections regardless of their geographic origins. The genotypes of chickpeas in the current and prior collections were different. Landraces of black and white seeded chickpeas were vulnerable to genetic erosion and chickpea genotypes at farmer's field are found to be losing their variability. Black and white seeded chickpea are missing at farmers' fields and are under greater threat of extinction. So, implementation of immediate restoration of chickpea landraces is required to recover and maintain the lost chickpea landraces in the studied region and we strongly recommended revision of the current conservation strategies.

Key words: Chickpea diversity change, Ethiopian chickpea germplasm, Freezing test, Frost survival rate, Frost tolerance rate, Genetic erosion, Germplasm characterization, SSR markers

Chapter 1

1. Introduction

1.1. Background and Justification

Agriculture in Ethiopia is the foundation of the country's overall socioeconomic structure, and it has a significant impact on all other economic sectors and development processes for the following reasons: It employs roughly 85% of the population, contributes to about 50% of the country's GDP, generates about 90% of the country's export revenues, and supplies around 70% of the raw material requirements of agricultural-based domestic businesses (Lulseged Tamene, 2017). In Ethiopia, agriculture is carried out in a variety of agroecologies, each of which is suited to the cultivation of different crops. Ethiopian highland constitutes around 33% the total arable land (Mulugeta Assefa *et al*., 2014) and mixed farming is the typical agricultural practice in this area.

Crop production in the typical highland areas is unprofitable because frost damage is one of the most important factors, which constrains farmers from growing many varieties of crops as they would in other areas. They solely produce high input requiring cereal crops mainly barley and wheat without practicing crop rotation for a long period of time. Earlier, they used to include legume crops in the crop rotation to restore the fertility of the soils through nitrogen fixation. The current agricultural practices, which are dominantly cereal based, impair soil fertility, resulting in lower crop output and contributing to the region's food insecurity challenges. Farmers gradually increase the amount of chemical fertilizer used to enhance grain yield to fulfill rising food demands. This farming system causes land degradation, which occurs as a result of excessive grazing and over cultivation caused by the use of high agricultural inputs combined with keeping a large number of cattle beyond the carrying capacity of the area. The impact of the factors listed above is exacerbated in the highland agroecology accompanied by climate change.

Food and nutrition security is becoming a major challenge in Ethiopia due to the country's rapidly growing population, which necessitates meeting rising food demand. It is crucial to increase crop productivity to meet the growing demand without harming the environment or contributing minimally to climate change by implementing climate mitigation and adaptation strategies in the agricultural sectors. Reducing climate emissions and increasing carbon sequestration in arable soils are two of these techniques (Franzluebbers, 2005; Stagnari *et al.*, 2017). This emphasizes the significance of sustainable agriculture, and leguminous crops play an important role in this regard.

Legume crops offer a wide range of benefits to the society. It has an immense potential to reduce malnutrition by providing an abundant sources of protein, fiber, complex carbohydrates, vitamins, and minerals (Upadhyaya *et al.*, 2002). Its production does not require a large amount of nitrogen fertilizer, which is environmentally friendly and reduces fertilizer costs not only for legumes but also for succeeding crops (Siddique *et al.*, 2005; Flowers *et al.*, 2010; Stagnari *et al.*, 2017). Its production helps to minimize greenhouse gas emissions from agriculture by reducing fossil energy usage, increasing carbon immobilization in the soil, and providing essential raw material for biorefineries (Franzluebbers, 2005; Stagnari *et al.*, 2017). Legumes like chickpea and grass pea also have the potential to thrive on leftover moisture, allowing farmers to engage in double cropping (Asnake Fikre *et al.*, 2018) and they have a high market demand both domestically and internationally, which encourages smallholder farmers to produce excess and earn additional income to improve their livelihoods and send their children to school.

Significant efforts have been made to improve the country's investment environment in order to attract both domestic and foreign direct investments, with a particular focus on the agricultural sector, where high-value crops, like legume crops, have been given top priority for intensive farming. Furthermore, smallholder farmers have been encouraged to participate in the production of these crops in order to be competitive in both domestic and export markets (MoFED, 2016). Ethiopia is well-suited to legume crop production due to the country's diverse agroecological zones. It has also geographical proximity advantage for easy access to the international market as it is close to the Middle East and Europe, where significant volumes of pulses are in demand, providing a great potential for Ethiopian pulse growers to export their products for the mutual benefit of these countries. Furthermore, Ethiopia has been pursuing a green economy development strategy since 2010. Legume crops are critical to achieving these goals by playing a key role in the development of sustainable agriculture, which is a concern for many countries throughout the world. As a result, investment in the pulse development program will clearly have a direct influence on the income of legume growing farmers while also addressing the issue of sustainable agriculture.

Among the grain legume, Chickpea (*Cicer arietinum* L.) is rapidly becoming one of Ethiopia's most important export commodity crops, for making foreign exchange profits (Bekele Shiferaw and Hailemariam Teklewold, 2007), hence expanding chickpea production into new areas is critical. Chickpea production in lowland areas employing irrigation, as well as chickpea introduction to selected areas of the Southern Nation, Nationality and People Region (SNNPR) have recently showed good results in increasing chickpea production (Nigusie Girma *et al.*, 2017). Furthermore, chickpea production could be expanded into '*dega*' agroecological zones, where frost is very common and with an altitudinal range of 2,300 and 3,200 meters above sea level based on traditional agroecological divisions (Dereje Gorfu and Eshetu Ahmed, 2003) and Chickpea productivity can be increased also in some '*Woina Dega'* areas (1,500-2,300 m.a.s.l.) where its production constrained by cold stress that occurs during the late vegetative and reproductive stages (personal communications), if cold tolerant chickpea varieties made available to farmers.

Until recently, the national breeding program had not begun breeding for cold tolerance, and no cold tolerant variety of chickpea has yet been developed. Lack of setting goals to develop frost resistant varieties for frost prone areas is a major limitation of the national chickpea program. In Ethiopia, there is a huge potential to expand chickpea production into these frost prone areas of the *'dega'* Ethiopian highlands. It is also very important to introduce chickpea in highland areas making it an alternative crop for the farmers which can serve in crop rotation, and also as sources for protein for the community. Furthermore, the straw of the crop will serve as a feed for their cattle. Therefore, variety development targeting frost prone areas is mandatory to maximize chickpea production in highlands of Ethiopia.

Since 1976, the current Ethiopian Biodiversity Institute (EBI), originally called Plant Genetic Resources Center, Ethiopia (PGRC/E), has been mandated with exploring, collecting, characterizing, and conserving the country's crop genetic resources for sustainable use through tapping Ethiopian genetic resources from various agro-ecological zones. Effective crop genetic resource conservation in Ethiopia, benefits not only the country, but also all crop-growing countries. Until 2020, a total of 68,014 and 6,700 accessions of various field and horticultural crops have been conserved for long term in cold rooms and field gene banks, respectively (EBI report unpublished). These germplasms were collected from various locations in Ethiopia. Chickpea collections make up only 1,223 accessions of the overall collections out of which, 69% and 14% of the chickpeas were collected from 1977 to 1991 and 1992 to 2015, respectively. For the remaining 17% the chickpea accessions, no years of collection were reported (Table 1). The vast majority of the conserved chickpea germplasm dates back three decades and also regular chickpea diversity monitoring has not been undertaken in these areas where these genotypes were collected originally to understand how diversity changes over the years in farmers' fields.

Diversity of farmers' varieties must be monitored on a regular basis to determine the state of genetic loss or gain (Barry *et al*., 2008). Because of the changing nature of agricultural production systems, the richness and range of diversity of these farmers' varieties are now threatened (Mazhar, 1997; Barry *et al*., 2008). The widespread adoption of modern varieties, which are the result of formal plant breeding, is one key aspect (Hammer and Yifru Teklu, 2008). Genetic erosion is a term used to explain the loss of variety in crops as a result of agricultural modernization (Willemen *et al*., 2007). Genetic erosion is also defined as the loss or reduction of genetic variety across and within populations of the same species over time (Jarvis *et al*., 2000), and it is most commonly caused by agricultural, economic, and social changes (Jarvis *et al*., 2000).

Genetic erosion can occur at three different levels: crop, variety, and allele (Willemen *et al*., 2007). Individual genes or combinations of genes, such as those present in regionally adapted landraces and wild species, can also be lost (Brush *et al*., 1995). The degree of genetic erosion can be assessed using molecular data analysis (Provan *et al*., 1999), allozyme analysis (Akimoto *et al*., 1999), by comparison of the number of species/cultivars still in use by farmers today to those found in previous studies (Hammer *et al*., 1996), by using the genetic assessment model

(Guarino, 1999), and using a risk factor checklist (de Oliveira and Martins, 2002). The data gathered through diversity monitoring will aid in identifying harmful evolutionary processes, developing appropriate and effective conservation programs, and assessing the success level of conservation policies. However, such knowledge is scarce in Ethiopian chickpea productions, necessitating immediate action.

In addition to diversity monitoring activities, extensive molecular characterization and evaluation for desirable agronomic traits such as disease resistance, insect pest resistance, potassium consumption capacity features, and so on are required to make conserved germplasms more useful to breeders and farmers (Rao, 2004; Rabara *et al.*, 2015; Castañeda-Álvarez *et al.*, 2016). So far, the effort put into germplasm collection is significantly greater than that put into germplasm molecular characterization or evaluation. Molecular characterization and evaluation should be part of germplasm collections and conservation efforts if these resources are to be used effectively in breeding programs (Rao, 2004; Kumar *et al.*, 2016).

Molecular characterizations and evaluation of existing germplasm not only reveal the magnitude and pattern of genetic diversity accessible for conservation, but also allow for the identification of important genes in the germplasm and the possible progress that can be made through future breeding activities (Arumuganathan and Earle, 1991; Hayward and Breese, 1993). Ethiopian chickpea has been thoroughly characterized using morpho-phenotypic features based on chickpea descriptor lists (Gemechu Keneni *et al.*, 2012a; Nigusie Girma *et al.*, 2017; Sintayehu Admas and Gize Abeje, 2017; Vishnyakova *et al.*, 2017; Asnake Fikre *et al.*, 2018; Awol Mohammed and Bulti Tesso, 2018), however there has been little work done on describing the germplasm at molecular level.

The use of molecular data analysis to characterize chickpea germplasm in terms of genetic structure, diversity, and linkages of chickpea genotypes is particularly significant for generating genetic information that can be used to build efficient conservation strategies and crop breeding programs (Choudhary *et al.*, 2012). In terms of germplasm evaluation, Ethiopian chickpea germplasms were screened for important traits by limited authors (Yadeta Anbessa and Geletu Bejiga, 2002; Gemechu Keneni *et al.*, 2011; Gemechu Keneni *et al.*, 2012a). There have been also few studies that investigated the ability of Ethiopian chickpea germplasm to withstand low temperatures stressors, which will be addressed in this study. Therefore, the present study provided to researchers, students, and farmers a deeper understanding of Ethiopian chickpea germplasm in which very little or no information is available so fare. These include the diversity, population structure and relationship of chickpea accessions at molecular level, their potential for cold tolerance response variability, the status of on-farm diversity changes occur over time, and the severity of genetic erosion.

Table 1. Ethiopian Biodiversity Institute chickpea germplasm collections from 1977 G.C to 2017 G.C

No	**Year of collection**	Collection age	No accessions	**No**	**Year of collection**	Collection age	No accessions
1	1977	41	6	**16**	1992	26	14
2	1978	40	16	**17**	1993	25	5
3	1979	39	38	**18**	1994	25	16
4	1980	38	38	**19**	1995	23	35
5	1981	37	13	**20**	1996	22	9
6	1982	36	209	**21**	1997	21	48
7	1983	35	240	**22**	2001	17	1
8	1984	34	128	**23**	2002	16	9
9	1985	33	21	**24**	2003	15	6
10	1986	32	19	**25**	2004	14	14
11	1987	31	7	**26**	2010	8	1
12	1988	30	77	**27**	2012	6	2
13	1989	29	16	**28**	2014	4	7
14	1990	28	15	**29**	2015	3	2
15	1991	27	3		**2-26 Years Old**		**169 (14%)**
	27-41 Years Old		**846 (69%)**		**Unknown collection date**		**208 (17%)**

1.2. Objectives

1.2.1. General objective

- To study the diversity of Ethiopian chickpea germplasm conserved *in situ*at Ethiopian Biodiversity Institute's gene bank and to analyzeon farm chickpea diversity change over time
- To assess the potential of the Ethiopian chickpea germplasm for cold tolerances, and to identify germplasm that could be used to improve chickpea resilience to low temperature stress

1.2.2. Specific objectives

- To assess the nature of genetic structure and the level and patterns of genetic diversity and relationships within and between Ethiopian chickpea genotypes, improved chickpea varieties and breeding lines by using microsatellite (SSR) markers;
- To assess the genetic diversity for cold tolerance amongst Ethiopian chickpea genotypes under field and controlled environment and identify cold tolerant genotypes for further breeding work;
- To assess changes in chickpea on-farm genetic diversity and its agronomic performances in the Amhara Regional State during a 35-year period, from 1979 (when the first collections were made) to 2017 (when the current collections were carried out);
- To estimate the level and extent of chickpea genetic erosion by evaluating the temporal changes of population genetic structure and to provide some suggestions for conservation actions in the Amhara Regional State.

Chapter 2

2. Literature Review

2.1. The Chickpea Crop

2.1.1. Biology and Plant Growth Habit

Chickpea (*Cicer arietinum* L.) is an annual self-pollinated grain legume crop (Muehlbauer and Abebe Tullu, 1997; Sajja *et al.*, 2017), with just 0 to 1% chance of cross-pollination (Singh, 1987). It has a chromosome number of 2n = 2x = 16 (van der Maesen, 1987) and a genome size of 740 Mbp (Arumuganathan and Earle, 1991). Chickpea leaves are petiolate and compound, with simple leaves in some genotypes (Singh and Diwakar, 1995). The plant has a strong root system and is a resilient crop with its roots growing deep up to more than 120 cm in Vertisols (Sheldrake and Saxena, 1979). The plant has a strong root system and is a hardy crop (Sheldrake and Saxena, 1979). Depending on the compatibility of the growing environment, the crop can reach a height of 20-100 cm (Sheldrake and Saxena, 1979). On both sides of the stem are little feathery leaves (Muehlbauer and Abebe Tullu, 1997). In most cases, only one seed is produced per pod (Van der Maesen *et al.*, 2007).

There are two main types of chickpeas currently grown in Ethiopia, namely desi and kabuli. However, rarely pea-shaped chickpea types are also available (Muehlbauer and Tullu, 1997). Kabuli seed types (Macrosperma) are large (100-seed mass >25 g), round or ram head, and cream-colored. It has medium to tall plant height, large leaflets and white flowers, and it contains no anthocyanin. The desi chickpea types (Microsperma) are characterized by pink, purple or blue flower color, darker seeds with a rough seed coat. It has small and angular seed shape. There are 2-3 ovules per pod, however only 1-2 seeds are generated on average within each pod. The plants are anthocyanin-rich and have tiny leaves and purplish blooms. Pea-shaped chickpea type is characterized by medium to small seed size and creamy color (Cubero, 1975; Singh and Diwakar, 1995; Upadhyaya *et al*., 2008). Moreover, it may be a result of a cross between desi and kabuli types that has resulted in a sort of intermediate types (Muehlbauer and Abebe Tullu, 1997). Chickpea are well adapted to a subtropical or tropical climate with well drained fertile soils having pH of 5.5-8.6 (Muehlbauer and Abebe Tullu, 1997).

2.1.2. Taxonomy

The genus *Cicer* belongs to the Fabaceae family and it comprises 46 species, with 10 annual species, 36 perennial species (van der Maesen, 1987; Toker *et al*., 2021). Due to their different physical characteristics, life cycle, and geographic distribution, the annual species are further classified as Monocicer and Chamaecicer (van der Maesen, 1987; Muehlbauer, 1993). Only one annual species (*Cicer chorassanicum*) belongs to the Chamaecicer group, whereas nine annual species (*C. turcicum, C. arietinum, C. reticulatum, C. echinospermum, C. bijugum, C. judaicum, C. pinnatifidum, C. yamashitae, C. cuneatum,* and *C. chorassanicum*) belong to the Monocicer group (Muehlbauer, 1993; Toker *et al*., 2021). *Cicer arietinum* is the only domesticated and cultivated species among the 46 species (Millan *et al*., 2006). Based on investigations of cytogenetics and seed protein analysis *C. reticulatum* is the wild progenitor of the cultivated chickpea (Ladizinsky and Adler, 1976), yet a research by Ohri and Pal (1991) did not support this claim. The genus belongs to the Cicereae Alef. monogeneric tribe, subfamily Papilionoideae, family Leguminoseae (Van der Maesen *et al*., 2007). It was previously classified as a member of the Vicieae Alef legume family (Van der Maesen *et al*., 2007).

2.1.3. Origin and Domestication of Chickpea

Chickpea is thought to be originated in the South-Eastern Turkey and the adjoining areas of Syria because of the widespread presence of chickpea wild relatives, namely *C. reticulatum* and *C. echinospermum* in these places (Ladizinsky and Adler, 1976; van der Maesen, 1987; Singh and Ocampo, 1997). According to Zeven and De Wet (1982), chickpea had different secondary centers of diversity in at least four regions: the Near East Region (which includes the Fertile Crescent), Hindustani Region (present India and East Pakistan), Central Asian Region (Afghanistan, Western Pakistan, Iran, and the former Soviet Union's south), and Mediterranean Region (including Lebanon and Palestine). Ethiopia is also regarded as one of the chickpea's secondary diversity hotspots (van der Maesen, 1987).

Chickpea was first domesticated in the Middle East before the late Neolithic period (as early as 3,500 BC) in Turkey, according to archeological findings (Tanno and Willcox, 2006). Even chickpea seeds dating back to roughly 7000 BC have been discovered in Syria and Turkey (Van der Maesen, 1987). According to Zohary and Hopf (2000), chickpea was domesticated in the Fertile Crescent 12,000-10,000 years ago in conjunction with other crops as part of the evolution of agriculture. Chickpea was domesticated in Turkey and it eventually spread into the Middle East, the Mediterranean region, India and Ethiopia and it has been introduced to Australia, New Zealand, the United States and Canada (Ladizinsky, 1975; van der Maesan, 1987).

2.2. Chickpea Cultivation

Chickpea is a part of the West Asian Leolithic crop assemblage, which is related with the origin of agriculture in the Fertile Crescent approximately 10,000 years ago. It is one of the oldest (older than 9,500 BC) and commonly farmed pulse crops in many nations throughout the world

(Lev-Yadun *et al*., 2000). It is a popular pulse crop grown throughout the tropics, subtropics, and temperate zones, including Ethiopia, India, Mexico, Myanmar, Pakistan, and Turkey (Singh, 1997; Croser *et al*., 2003). According to data from FAOSTAT (2021) chickpea is the second most important legume crop in the world, after dried beans and it is farmed in 50 countries in 2019. Chickpea was harvested in 14.26 million tons from 13.72 million hectares of land from these countries, with an average yield of 1,655.14 kg/ha (FAOSTAT, 2021). Notably, about 87.3% of the world's chickpea is produced in India (69.7%), Turkey (4.4%), Russian Federation (3.5%), Myanmar (3.5%), Pakistan (3.1%) and Ethiopia (3.1%) (FAOSTAT, 2021). In Africa, it is primarily grown in East Africa (Sudan, Eritrea, Ethiopia, Kenya, Tanzania) and Malawi in tropical Africa, where it is grown in locations with a distinct cool season (Geletu Bejiga and van der Maesen, 2006; Asnake Fikre *et al.*, 2021). Amongst the varieties cultivated, Kabuli types are primarily planted in the Mediterranean basin and Central Asia (Castro *et al*., 2011), whereas desi varieties are primarily grown in East Africa and India (Malhotra *et al*., 1987).

Chickpea has been planted in Ethiopia since 1,520 BC (Joshi *et al*., 2001), and it is presently the third largest food legume crop in terms of area covered and production volume next to faba bean and haricot bean. It occupies roughly 239,786.13ha (163067.24 ha for desi and 76718.89 ha for Kabuli types) of land annually and producing 459,173,187 Kg (330,153,198 from desi and 129,019,989 from kabuli type chickpea) with an average productivity of 2,025 kg/ha for desi and 1,682 kg/ha for kabuli type chickpea (CSA, 2019). Amhara (Gonder, Gojam, Wollo, and North Shewa Zones) and Oromia (East, West, and Northwest Shewa, and Arsi Zones) contribute 49.84% and 44.27% of total chickpea production, respectively, while Tigray and SNNPR account for roughly less than 6% of total chickpea production (CSA, 2019).

Within Ethiopia, chickpea is the second most important export commodity after haricot beans, accounting for approximately a quarter of all legume export revenues (Setotaw Ferede *et al.*, 2018). According to Setotaw Ferede *et al.* (2018), the local market accounts for 85% of the country's total yearly grain trade volume, while the export market accounts for only 15%. During the years 2010-2017, Ethiopia exported a total of 456,290 tons of chickpea grain, earning 304.6 million dollars (Setotaw Ferede *et al.*, 2018). The kabuli variety accounted for more than 56% of total export volume, with the desi type accounting for the remainder (Setotaw Ferede *et al.*, 2018).

2.3. The Importance of Chickpea

The issue of addressing food security is becoming a major challenge in developing countries like Ethiopia because of the ever increasing population demand for adequate food. To fulfill rising demand, crop production must be increased while causing little or no environmental damage and contributing minimally to climate change by applying climate mitigation and adaptation techniques (Stagnari *et al.*, 2017). These strategies include reducing climate emissions and increasing carbon sequestration in arable soils (Franzluebbers, 2005). This necessitates the integration of leguminous crops into the country's agricultural system to ensure sustainable agriculture, which leguminous crops play a key part in. Legume crops provide a variety of other economic benefits to the society which includes serving as human food, feed, and cash crops, while requiring little external inputs and labor-intensive crop.

Grain legumes are sometimes referred to as the poor man's meat (Maya and Maphosa, 2020) because they are a cheap and vital source of protein for individuals who cannot afford animal protein or who are mostly vegetarian (Upadhyaya *et al.*, 2002). Furthermore, they are a good source of minerals (i.e., calcium, phosphorus, magnesium, zinc, and iron), fiber, complex carbohydrates, vitamins, unsaturated fatty acids, fiber, and -carotene, all of which can aid in the treatment of malnutrition (Güler *et al*., 2001). Grain legumes are important sources of nutrition, and when mixed with cereals, they give a nutritionally balanced amino acid composition with a ratio that is close to the ideal requirement for humans (Saxena, 1990).

By humans, grain legumes are prepared and consumed in a variety of ways based on the cultural traditions of the various communities. Across India, for example, chickpea grain is crushed into flour that may be used to produce a variety of snacks (Hulse, 1991), whereas chickpea is used to make stews, soups, and salads in Asia and most of Africa. The seed is also consumed in boiled, salted, roasted, and fermented forms (Gecit, 1991). Chickpea grain is used as food in Ethiopia in several forms. The green immature seed is eaten as a green vegetable. Chickpea grains consumed as ‘Kolo' prepared by soaking in water followed by roasting, or ‘nifro' prepared by boiling the grain. Sauces (‘wot') are made with chickpea grain powder (‘shiro'). It's also employed in traditional food processing techniques for infant food formulation.

Legume crops have the potential to be one of the most important agricultural export commodity crops in terms of export earnings due to high demand in international markets. Nevertheless, most grain legumes are consumed locally (Bekele Shiferaw and Hailemariam Teklewold, 2007). This is due to low grain production volumes that cannot satisfy local and international markets (Setotaw Ferede *et al*., 2018) leading to weak integration of farmers into the market economy (Bekele Shiferaw and Hailemariam Teklewold, 2007). Among grain legumes in Ethiopia, advances in chickpea breeding and crop management practices over the last four decades have resulted in the development and release of technology that have significantly improved productivity, adaptability, and production, ultimately benefiting small holder farmers' livelihoods

(Asnake Fikre and Dagnachew Bekele, 2020). Recently, chickpea is a crop that has progressed from a basic predecessor to the primary crop of Ethiopian households (Asnake Fikre *et al.*, 2018). As a result of the aforementioned factor, chickpea production has continuously increased, and Ethiopia has risen to become one of the top 10 chickpea producing countries in the world, as well as Africa's leading producer (FAOSTAT, 2021).

Legume crops, which serve as rotational crops in cereal-based crop production systems, are critical for agricultural systems that provide sustainable crop production. It has various advantages for agricultural production systems, including the capacity to raise soil nitrogen levels through biological nitrogen fixation, increased weed control, increased yield, and as a break crop for cereal diseases (Siddique *et al.*, 2005; Flowers *et al.*, 2010; Stagnari *et al.*, 2017). Legume crops are also used for roughage, green manure, hay and silage (Rowland *et al.*, 1994). Crop rotation with legumes and cereals can boost cereal output and protein content (Rowland *et al.*, 1994).

Chickpea, as one of the legume crops, is vital for soil fertility maintenance and require little nitrogen chemical fertilizer. Chickpea crops, for example, may fix nitrogen at rates of up to 140 kg/ha/year (Flowers *et al.*, 2010), and they require little nitrogen fertilizer since symbiotic N_2 fixation meets 70% of their demand (Siddique *et al.*, 2005). Legume production does not necessitate a large amount of nitrogen fertilizer, which is environmentally friendly and reduces fertilizer input costs not only for legumes, but also for succeeding crops (Flowers *et al.*, 2010). In addition, legumes minimize greenhouse gas emissions from agriculture by reducing fossil energy usage, increasing carbon immobilization in the soil, and providing essential raw material for biorefineries (Stagnari *et al.*, 2017).

2.4. Chickpea Production Constraint

The main problems for chickpea growers are to increase chickpea yield in a consistent manner while maintaining acceptable seed quality across years and locations. This is owing to several chickpea production limits, which can be classified as biotic and abiotic stresses, as well as the crop's restricted genetic potential (Singh *et al*., 1994)

The major biotic stressors that cause drastic yield reduction and instability are those caused by fungal, bacterial and viral diseases, insect pests, parasitic nematodes (Geletu Bejiga *et al*., 1996; Basandrai *et al*., 2011), and parasitic weeds of chickpea (Cubero *et al*., 1986). Generally, 5-10% and 50-100% yield loss was estimated due the damage incurred by biotic factors (i.e. insect pest, diseases or weeds) in temperate and tropical regions, respectively (van Emden, 1988). The most destructive foliar and root diseases in chickpea are Ascochyta blight (caused by *Ascochyta rabiei*) and Fusarium wilt (caused by *Fusarium oxysporum*), both of which cause significant yield loss (Singh *et al*., 2008). Wilt and root rots cause significant crop loss and can have a significant impact on chickpea output in the Nile valley and Red Sea countries (Ethiopia, Egypt, Sudan, and Yemen) as well as North Africa, especially Tunisia (Geletu Bejiga *et al*., 1988). Chickpea wilt has caused a 30% crop loss in Ethiopia (Meki Shehabu *et al*., 2008). Pod borer (*Helicoverpa armigera*) and seed beetle or bruchid (Callosobruchus spp.) are the most common insect pests that decimate chickpea crops (Winch, 2006). In Ethiopia, pod borer damages more than 80% of early-sown chickpea pods (Geletu Bejiga *et al*., 1996).

Abiotic stressors are one of the most significant negative environmental variables limiting plant productivity (Doupis *et al*., 2011). The most common abiotic stressors affecting chickpea production are drought, cold, terminal heat, soil salinity, water logging, soil acidity and soil metal toxicity. These stressors adversely affect chickpea growth and development and ultimately lead to physio-morphological, biochemical, and molecular changes in the plant (Ryan, 1997; Millan *et al*., 2006; Potters *et al*., 2007). According to Ryan (1997), yield losses caused by abiotic stress may exceed 6.4 million tons while yield losses due to biotic stresses could reach up to 4.8 million tons. Specifically, drought/heat, cold, and salinity-related economic losses in chickpea were projected to be 1.3 billion, 186 million, and 354 million US dollars, respectively, causing great worry among chickpea-growing countries.

Drought is one of the most significant abiotic factors limiting chickpea production in many parts of the globe resulting in up to 40-50% yield reduction (Ahmad *et al*., 2005; Tuberosa and Salvi, 2006; Toker *et al*., 2007). However, in severe drought conditions, full chickpea crop failure can occur (Boyer, 1982; Saxena *et al*., 1993; Singh and Saxena, 1996). A rise in temperature above a specific ideal level is known as terminal heat stress (Wahid *et al*., 2007). Chickpea is a cool-season crop that can suffer significant production loss if exposed to high temperatures (30-35 ^{o}C) for a few days during flowering (Saxena *et al*., 1988). Summerfield *et al*. (1984) found a negative association between high temperature during the reproductive phase and chickpea grain yield. Due to its relevance for this PhD research work, low temperature stress will be reviewed in better detail in the section below.

2.5. Low Temperature Stress

Chickpea is a chilling-sensitive legume, and one of the most significant hurdles to chickpea production is low temperature (Singh *et al*., 1984). The second most important limiting factor in chickpea production is low temperature stress (Sassenrath *et al*., 1990; McKersie and Bowley, 1997). Chilling injury occurs when the temperature remains above freezing/frost injury (0°C to

15°C) and freezing injury occurs when the temperature drops below freezing point (below -1.5^{o}C) (Croser *et al.*, 2003; Yadav, 2010; Jha *et al.*, 2014), both of which have overlapping effects on chickpea growth and production (Croser *et al.*, 2003; Yadav, 2010; Jha *et al.*, 2014)

2.5.1. Low Temperature Impacts on Agronomical Traits

The effects of cold temperatures on chickpea crop growth have been studied at various stages of the crop, including seed germination, vegetative, flowering, poding, and seed setting. The degree of damage varies depending on the chickpea genotypes and the severity of the frost stress. Seed germination and seedling emergence are critical for a successful crop establishment; however, soil conditions and air temperature influence seed germination (Chen *et al.*, 1983).

According to reports, the minimal soil temperature for chickpea seed germination is 0°C, and any temperature below that hinders seed germination (Siddique *et al.*, 1983; Ellis *et al.*, 1986). Various authors have found that in chickpea, seed germination percentage and seed development stages are extremely sensitive to frost stress, resulting in reduced crop growth and, in some sensitive genotypes, in complete plant necrosis and plant mortality (Srinivasan *et al.*, 1998; Croser *et al.*, 2003; Maphosa *et al.*, 2020). Although susceptibility to cold is greater at the late vegetative stage than at the seedling stage (Singh *et al.*, 1984), chilling stress mostly impacts mean germination time among cultivars, which is strongly correlated with shoot length and dry weight of the plant (Matthews *et al.*, 2011). Cold temperatures enhance susceptibility to soil-borne infections during chickpea germination, slowing plant growth and lowering dry matter output (Wery *et al.*, 1994). One of the most important causes of physiological damage to seed at planting is imbibitional chilling injury when dry seeds imbibe cold water in the field (Cal and Obendorf, 1972).

Low field temperatures hamper vegetative development and vigor, delay all phenological stages, and result in some vegetative damages such as poor crop stand establishment, chlorosis, wilting, necrosis of leaf tips, reduced plant height and branches, full leaf curling, and plant death (Croser *et al*., 2003; Nayyar *et al*., 2005a; Kumar *et al*., 2010). It also reduces assimilate partitioning to the vegetative sections, resulting in a lower harvest index (Siddique and Sedgley, 1986; Saxena, 1990). When chickpea is subjected to low temperatures throughout its growth period, a significant yield loss is encountered (Singh *et al*., 1993, Croser *et al*., 2003, Nayyar *et al*., 2005a).

Low temperatures can restrict chickpea growth and vigor at all phenological stages, but the damage is more severe if it happens during reproductive stages, resulting in a large yield loss (Kumar *et al*., 2010). Particularly, temperature stress at or around flowering is considered a major challenge to yield in many chickpea production areas (Srinivasan *et al.,* 1998; Clarke and Siddique, 2004; Bakht *et al*., 2006). This is because, the reproductive stage represents the most vulnerable phase resulting in damaging events, such as bud abscission, floral abortion, reduced pollen viability and stigma receptivity, inhibited pollen tube growth, poor pod set, infertile pods, reduced seed size, deteriorated seed quality and seed yield that substantially limit the production potential of this crop (Srinivasan *et al*., 1998; Croser *et al*., 2003; Clarke and Siddique, 2004; Nayyar *et al*., 2005a; Kumar *et al*., 2007; Kumar *et al*., 2010).

According to Clarke and Siddique (2004), pollen viability and pollen germination on the stigma were the key reasons why pod set in chickpea was reduced during low temperature stress. Several authors demonstrated also that low temperature did not affect the pistil function, i.e., esterase activity; however, pollen tube growth on the styles of sensitive genotypes was restricted due to cold temperatures (Savithri *et al*., 1980; Srinivasan *et al*., 1999; Clarke and Siddique, 2004). Cold stress can cause yield losses of up to 50% or complete plant failure in chickpeas (Saxena and Johansen, 1990), depending on the degree of the stress and the genotype's sensitivity.

Low temperature also affects nutritional and processing quality of chickpea seed. The quality of chickpea seeds is harmed by environmental stressors during seed development (Behboudian *et al*., 2001). Cold stress reduces the rate and duration of grain filling period in chickpeas, resulting in smaller seeds (Nayyar *et al*., 2007; Kaur *et al*., 2008). Chickpea average seed weight and size fell by 41% and 24%, respectively, at 13/5°C compared to 28/17°C, owing to a reduction in seed filling duration from 20 days without cold-stress to 14 days with cold-stressed (Nayyar *et al*., 2005b). Similarly, in Northern New South Wales, Australia, low post-anthesis temperatures lowered yield by 1.3t/ha in 2009 (Moore *et al*., 2010).

Abiotic stresses such as altered carbohydrate and nitrogen metabolism in source and sink plants (Nayyar *et al*., 2005a), impaired photosynthesis (Nayyar *et al*., 2005a), senescence of source leaves (Yang *et al*., 2001), hormonal imbalance (Nayyar and Walia, 2004), vascular restrictions, and reduced assimilate uptake into seeds can all inhibit seed growth. According to Nayyar *et al*. (2005b), grain sugar concentration increased in chickpeas during cold stress, whereas storage proteins, starch, and many amino acids declined. The stage of seed development, however, had an impact on the result. When cold stress occurred in late pod-filling stages compared to early pod-filling stages, there was a larger loss in carbohydrates, proteins, soluble sugars, fat, crude fiber, and store protein fractions (Kaur *et al*., 2008). Freezing can diminish seed size, most likely due to stress circumstances impacting plant resource mobilization. Furthermore, the seed coat can become discolored (Croser *et al*., 2003).

2.5.2. Low Temperature Impacts on Physiological and Biochemical Traits

The effect of low temperature on physiological and biochemical features in chickpea has been studied in a number of ways. Frost stress lowers leaf water status and chloroplast membrane stability, resulting in the loss of respiration and photosynthesis (Heerden and Krüger, 2000; Croser *et al*., 2003; Yadav, 2010). Ice formation in plant tissues causes severe damage such as dehydration and electrolyte imbalances when temperatures drop below freezing (Croser *et al*., 2003; Yadav, 2010).

Different modifications in crops, such as soluble carbohydrate content, soluble lipids, protein content, and degree of fatty acid content in the shoot cells, have been found in biochemical studies to complement cold adaptation (Lee *et al*., 1997; Bakht *et al*., 2006; Thakur *et al*., 2010). Frost stress raises the levels of abscisic acid (ABA) and calcium in the frost-resistant plant (Lang *et al*., 1994; Knight *et al*., 1996), while it lowers the accumulation of starch, proteins, lipids, crude fiber, protein fractions (i.e., albumins, globulins, prolamins, and glutelins), sucrose accumulation, and enzyme activity levels such as starch synthase, sucrose synthase, and invertase in the seeds of freezing sensitive genotypes (Bakht *et al*., 2006).

Bakht *et al*. (2013) investigated the applications of ABA to frost-sensitive chickpea and found out that it can reduce the effect of low temperature by preventing or lowering cell lysis due to an increased level of desaturation fatty acid in plasma membrane, which is an important component for cell membrane integrity. Similar findings were reported from Nayyar *et al*., (2005a) that exogenous ABA increases tolerance to cold stress by boosting survival rate through the reproductive stage too. The application of glycine betaine at budding and podding stages can improve cold tolerance in chickpea by increasing chlorophyll retention, pollen germination, pollen viability, pollen tube growth, stigma receptivity, ovule viability flower retention and pod set, which in turn increases seed weight, grain yield, number of seeds/pod and decrease in infertile pod (Nayyar *et al*., 2005a; Kumar *et al*., 2007). It has also been reported that antioxidative enzymes such as catalase, ascorbate peroxidise, glutathione reductase and sucrose synthase can protect seeds and pod walls from cold stressors (Kaur *et al*., 2009). Therefore, the manipulation of ABA and glycine betaine contents could improve cold tolerance in chickpea.

2.6. Cold tolerances and Screening Methods

Cold tolerance refers to a genotype's capacity to establish pods and seeds, while limiting excessive vegetative development, which results in less lodging, lower pest and disease incidence, and a higher harvest index at low temperatures (Saxena *et al*., 1988). Agronomic, physiological, and biochemical techniques have been used to investigate the sensitivity of chickpea genotypes to low temperatures at vegetative or reproductive stages in both field and controlled environments (Saxena *et al*., 1988).

Chickpea genotypes responded differently to cold stress at different phonological stages and in distinct tissues (lower and upper leaves of the plant canopy, stems, meristems, or roots) (Croser *et al*., 2003). During the evolutionary process, plants develop an adaptation to endure cold, known as "cold hardening," which is triggered by low non-freezing temperatures (Levitt, 1980). Cold hardening occurs in natural situations when the temperature gradually drops to $0^{o}C$ over several weeks (Levitt, 1980; Croser *et al*., 2003). Cold hardening, also known as acclimation, is a non-heritable alteration of structures and functions as a reaction to cold that reduces damage and increases a plant's fitness (Levitt, 1980; Croser *et al*., 2003).

When plants are not acclimated to the cold, they will suffer irreparable harm such as the disintegration of cell membrane systems or the loss of photosynthetically active tissue (Chen *et al*., 2014; Menon *et al*., 2015). Ice forming within the intercellular gaps is the most common cause of freezing injury. With lowering temperature, the stiff ice lattice structure expands and may penetrate cellular walls and membranes to an amount that normal cell processes cannot repair (Andrews, 1996). Many plant species can withstand extracellular ice generation, but intracellular ice development is harmful (Guy, 1990). According to Gilmour *et al*., (1988), cold-acclimated plants resisted frost stress better than non-acclimated plants. Nezami *et al*. (2012) created a stepwise low temperature acclimatization technique for chickpea seedlings to promote acclimation under control conditions, and discovered an increase in vegetative cold tolerance. It is unclear whether exposing chickpea plants to low positive temperatures before reproductive stages would improve their tolerance to frost occurring at reproductive-stage (Croser *et al*., 2003).

The cold tolerance mechanism involves a series of biochemical and physiological changes. These include an increase in ABA levels (Morgan and Drew, 1997), increases membrane fluidity, an increase in antioxidants and osmotic adjustment (Fridovich, 1986; Buddenhagen and Richards, 1988; Wery *et al*., 1993), super cooling without ice nucleation (Olien and Smith, 1981), and changes in cell membrane lipid composition (Graham and Patterson, 1982). Increased amounts of carbohydrates, soluble proteins, prolines, and organic acids, as well as the development of novel protein isoforms and changed lipid membrane composition, are among the cellular and metabolic changes that occur during cold adaptation (Hughes and Dunn, 1990).

Identifying cold tolerant chickpea is a necessary component for increasing yield during frost seasons, both at freezing and chilling temperatures, which has an impact on the entire crop development process from germination to maturity (Croser *et al.*, 2003). From germination to flowering, phenological stages should be taken into account when measuring a genotype's cold tolerance. Given the importance of pollen in altering freezing-tolerance, chickpea pollen at the gametophytic stage was chosen as an effective visible marker during genotype selection, and flower color was chosen as an effective visible marker during genotype selection (Clarke *et al.*, 2004).

The hardening circumstances have a significant impact on the degree of freezing-tolerance. Exposing the plants to a temperature low enough to cause harm and finding either the temperature that kills 50% of the plants (LT50) or the percentage of plants that survive is how cold tolerance is measured. Re-growth, electrolyte leakage, browning, or chlorophyll fluorescence can all be used to calculate the LT50 (lowest test temperature required to kill 50% of the plant population) (Levitt, 1980). Because cold injury disrupts cell plasma membranes, harm can also be assessed by tissue electrical conductivity or leaking of different chemicals. Methods such as vital staining or comparing the ability of plants to re-grow after a recovery time are also extensively utilized (Levitt, 1980).

An efficient and repeatable screening process for huge collections of germplasm is required to reliably pick accessions and develop innovative inbred lines with enhanced germination under cold conditions. To produce cold tolerant cultivars, the impact of cold stress on chickpea during the vegetative and reproductive phases must be explored by observing agronomic, physiological, and biochemical properties (Kaloki *et al.*, 2019). Several non-destructive approaches for evaluating chickpea cold tolerance have also been proposed, including visual scoring of freezing damage in leaves, detecting re-growth or leaf conductivity, and evaluating chlorophyll fluorescence. In previous investigations, a scoring scale of 1-9 was employed to assess cold stress injury during the early vegetative or seedling stages (Singh *et al*., 1989) under natural and controlled environments.

2.6.1. Natural Field Environments

Genotypes are tested in the field and chosen once natural frost appears at each phenological crop stage, such as germination, seedling, vegetative, or reproductive growth. Srinivasan *et al*. (1999) investigated the morpho-physiological basis for cold tolerance variation in early reproductive stages in both field and laboratory environments. They looked at anther dehiscence on the day of flower opening, as well as the number of flowers with fully dehiscent anthers, pollen presence, and stigma load. The score scale was 1 to 5, with 1 being the lowest and 5 being the highest. The cold tolerance of a group of 30 chickpea accessions were assessed in the field under natural conditions on a scale of 1 to 9 (Singh *et al*., 1989), and the scoring was completed after the death of the vulnerable check. The problem with field screening is the unpredictability of the severity and frequency of low temperatures in the field; it also necessitates land, labor, and, most importantly, suitable freezing conditions for seedling screening (Maqbool *et al*., 2010). This method is sometimes referred to as the opportunistic method due to the unpredictability of low temperature intensity and frequency (Tóth *et al*., 2004).

2.6.2. Controlled Environments

In a controlled setting, such as a greenhouse or a plant growth chamber, artificial freezing emulating circumstances can be established. Seedlings as early as the first, second, or third leaf stage are sampled and exposed to various freezing temperature ranges to screen for vegetative stage low temperature-tolerance. The controlled environment screening method overcomes the limitations of the field environment by ensuring that frost stress is induced at the proper developmental stage at which chickpea plants are susceptible to frost, and that the stress is evenly distributed across the trial area or the plants (Chen *et al*., 2009). This screening process allows for much more exact control of frost treatment timing and intensity (Wu *et al*., 2014). It is also low-cost, easier, fast, highly repeatable, and avoids many of the issues that occur in the field (Nezami *et al*., 2012). The controlled environment method lacks a real-world environment that plants are subjected to in addition to frost damage (GRDC, 2009). For several crops such as winter wheat (Zhu *et al*., 2014), chickpea (Nezami *et al*., 2012), faba bean (Sallam *et al*., 2016), and field pea (Sallam *et al*., 2016), this method was employed. Generally, it is advisable to employ natural and controlled environment simultaneously to complement the short comings of each other and produce reliable information.

2.6.3. QTLs and other Indirect Methods

This strategy relies on the use of indicators like genes linked to cold tolerance. DNA markers may be used for labeling genes that are resistant to freezing and chilling temperatures (Maphosa *et al*., 2020). Most cool-season legumes, such as field pea (Burstin *et al*., 2001), lentil (Kahraman *et al*., 2004), and faba bean (Arbaoui and Link, 2008), have had quantitative trait loci (QTLs) identified for winter hardiness during the vegetative stage. However, few investigations on QTLs for cold tolerance in chickpea have been conducted. Mugabe *et al*. (2019) used single nucleotide polymorphism markers and sequencing technologies to map three potential QTLs for cold tolerance characteristics for the first time. Chickpea breeders will be able to use marker assisted selection in their breeding program to generate frost tolerant genotypes as a result of their research.

2.7 Genetics of Cold Tolerance in Crops

Studies on the genetics of cold tolerance in chickpea at various phenological phases (germination, seedling, vegetative, flowering, podding, grain filling) are limited. Malhotra and Singh (1990) investigated the mode of freezing-tolerance inheritance in chickpea seedlings to early vegetative stages. They argued that the mode of inheritance is additive x additive and dominance x dominance interactions with duplicate epistasis.

Furthermore, the inheritance research revealed that cold tolerance is dominant over sensitivity and that it is highly heritable, allowing for visual selection in the field. Due to the high heritability of the trait and the small number of genes influencing cold tolerance inheritance, Malhotra and Singh (1991) argue that selection in early generations should be effective. Selection, according to Singh *et al.* (1994), should be delayed until the F_3 or later generations, when dominance effects are diminished and additive and additive additive gene effects are fixed. After a few generations of selfing, Crosser *et al*. (2003) concurred that selection is more effective when dominance and epistatic effects are minimized. Mugabe *et al*. (2019) investigated the QTL analysis for cold tolerance in chickpea and discovered three significant QTLs, CT Ca-3.1, CT Ca-8.1, and CT Ca-1.1(B) on linkage groups 3, 8, and 1B respectively.

2.8. Chickpea Germplasm Management

Plant genetic resources are the bases for variety development as they contain various agronomic and nutritional quality traits. It is mandatory to protect the genetic resources from being lost (Upadhyaya *et al*., 2008). The main goals of genetic conservation is collection of plant genetic resources primarily aiming at tapping of cultivated germplasm and their wilds relatives' from different agro-ecological regions and conserve these genotypes for sustainable use (Kumar *et al*., 2016). So far, in Ethiopia, a large number of germplasm have been collected and conserved in Ethiopian Biodiversity Institute gene bank using *ex-situ* and *in-situ* conservation strategies (Unpublished EBI report, 2021). *Ex-situ* conservation is performed outside the original habitat of plants (Khoury *et al*., 2010), while *in-situ* conservation is the maintenance of plant genetic resource in their natural habitats that they exist (Meilleur and Hodgkin, 2004). The effort given to germplasm collections is however, by far more intense than the effort given to characterization, evaluation, and diversity monitoring works in most gene banks (Upadhyaya *et al*., 2008). The number of accessions conserved is growing faster than they can be effectively characterized, evaluated, and utilized because of lack of adequate capital resources, and limited use of advanced technology.

Characterization is the recording of distinctly identifiable characteristics which are heritable, while, evaluation is the recording of a limited number of agronomic traits considered to be important in crop improvement (Upadhyaya *et al*., 2008; Kumar *et al*., 2016). Adequate germplasm characterization and evaluation for agronomic and morphological traits is necessary to facilitate utilization of germplasm by breeders. These activities will ensure to establish germplasm diagnostic characteristics and identify duplicates, classify groups of accessions using sound criteria, identify accessions with desired agronomic traits and select entries for more precise evaluation, develop interrelationships between, or among traits and between geographic groups of cultivars, and estimate the extent of variation in the collection (Upadhyaya *et al*., 2008; Kumar *et al*., 2016). These can be achieved by using various techniques, namely morphological characterization/evaluation (Hayward and Breese, 1993), biochemical (Tanksley and McCouch, 1997), and DNA (or molecular) marker analysis (Govindaraj *et al*., 2015).

2.8.1. Morphological Markers

Morphological markers are based on visually observable characters which can be qualitative or quantitative traits. These traits include plant height, number of tillers, maturity days, flower color, seed shape, growth habits, pigmentation, etc. It requires an intensive work in the field for recording or measuring the phenotypic data. The accuracy of phenotypic data is essentials and it can be improved through various techniques like by replicating the trials across locations and over years, by using appropriate experimental design and blocking, on data recording and using appropriate statistical methods for data analysis (Zhu *et al*., 2008; Govindaraj *et al*., 2015).

2.8.2. Biochemical Markers

Storage proteins and allelic variant of enzymes (isozymes) have been used to assess the genetic diversity of plant species (Govindaraj *et al*., 2015). The variability can be examined using electrophoresis and specific staining chemicals. These markers are codominant in nature. They have simple inheritance and detect variability at the functional gene level. It can be detected with very little plant material. However, there are only a few enzyme markers available, and the resolution capacity for genetic diversity analysis is restricted (Govindaraj *et al*., 2015).

2.8.3. Molecular Markers

Genotyping has been practiced using different kinds of DNA markers to analyze the genetic and molecular variation among and within populations. Molecular markers are located near to genes that control the traits and do not affect the phenotypic expression of the given traits. A number of DNA markers are available, and they are unaffected by environmental conditions or the stage of growth of the plant (Winter and Kahl, 1995). DNA-based molecular markers (such as Restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), random amplified polymorphic DNAs (RAPD), and amplified fragment length polymorphism (AFLP) have been widely utilized for molecular characterization (Collard *et al*., 2005; Baloch *et al*., 2015). However, for a big number of accessions, their analysis technique is arduous and time consuming, and some of them have problems with reproducibility among laboratories (Basel *et al*., 2014). Recently, characterization using next generation sequencing (NGS) is being used.

One of the greatest revolutionary influences on biological studies has been the examination of entire or partial DNA sequences. It is helpful in understanding the roles, network, and evolutionary relationships of genes (Bevan and Uauy, 2013). Genome sequencing costs have been reduced in the past several years. It is now inexpensive to gather genome sequence information in large numbers of samples in a short period of time, even shorter than any crop's life cycle (Graner and Killian, 2012). The advancement in genome sequencing technology gives an opportunity to assess the genetic makeup of an organism, down to base pair resolution with fast and cheap methods. Recently, the genomic sequence data have been generated for most crops and even reference genome sequences have been developed for key species (Edwards *et al*., 2012). The information is readily available in public databases for free use for different studies, like to study sequence variation within species. In addition to this, de novo assembly of sequence data can be used to detect all differences at nucleotide level (Henry, 2014). Therefore, the opportunities genomic characterization will bring to the conservation and use of plant genetic resources have been reported by various scientists for several years (McCouch *et al*., 2013).

Application of genomics sciences in chickpea have been increasing, and currently, the advanced draft genome assembly of a desi type chickpea (Parween *et al*., 2015) and draft genome sequence of *Cicer reticulatum* L. (the wild progenitor of chickpea) (Gubta *et al*., 2016) are publically available. Kujur *et al* (2015) identified 44,844 high-quality SNPs by sequencing 92 diverse chickpea accessions belonging to a seed and pod trait-specific association panel using reference genome- and de novo-based genotyping-by-sequencing (GBS) assays. They also employed genome-wide association study (GWAS) for desi and kabuli type chickpea. Varshney *et al.* (2014) identified 28,000 genes and several million genetic markers from genome sequencing of 90 chickpea genotypes. This recent advancement in chickpea genomics is beneficial for gene bank managers for efficient germplasm conservation and utilization of germplasm, and also for breeders.

2.8.4. Population Structure

Population structure is defined as the arrangement of genetic variation, which is created by the combined effects of evolutionary processes such as recombination, mutation, genetic drift, demographic history, and natural selection (Tibayrene, 2017). It is a complicated phenomenon, and no single statistical analysis can fully reflect to understand the structure of a population. This requires a combination of model based statistical methods (Meirmans and Hedrick 2010; Lawson, 2018). Many model based population structure analysis are available in order to infer historical population changes, including the occurrence of population bottlenecks, admixture events, or population divergence dates, however the methods rely on simple population models (Evanno *et al*., 2005). For example, The STRUCTURE software, the Bayesian model-based clustering methods, can be used to analyze the presence of population structure, identify separate genetic populations, assign individual genotypes to populations, and identify admixed individuals (Pritchard *et al*., 2000). The software assumes a model in which there are K populations contributing to individual genotypes with varying proportions. And, it can be determined using a web-based Structure Harvester program developed by (Earl and Holdt, 2012).

2.9. Chickpea National Breeding Program in Ethiopia

According to Asnake Fikre and Dagnachew Bekele (2020), chickpea breeding in Ethiopia has been going on since 1970, and during that time the focus of breeding was on local germplasm. Later, the program shifted from indigenous germplasm to exotic/introduced material from other sources outside Ethiopia to identify desirable traits. The major agro-morphological traits prioritized by the breeding program include productivity, seed size, plant phenology and type, and resistance to key biotic and abiotic stressors prevalent in the country; particularly resistance to wilt/root rot diseases complex, ascochyta blight, major insect pests, drought, moisture and heat stresses. The exotic genotypes have been introduced from international institutes/organizations, primarily the International Center for Agricultural Research in the Dry Areas (ICARDA) or the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), as well as exchanging material with colleague breeders and breeding programs.

So far, a significant result has been accomplished, with the program developing 27 (13 desi type and 14 kabuli type) improved varieties aimed towards the aforementioned goal (MoA, 2018). These varieties were developed after years of intensive evaluation and critical selection of introduced germplasm lines for a variety of agro-morphological features in a variety of agro-ecologies and places. This means that the improved varieties released in Ethiopia have an exotic genetic background, and Ethiopian chickpea germplasm were used very sparingly, with only three old varieties (DZ-10-4 released in 1974, DZ-10-11 4 released in 1974, and Dubie 4 released in 1978) developed from local germplasm selection (Asnake Fikre and Dagnachew Bekele, 2020). It is apparent that the national chickpea breeding program is entirely reliant on exotic material for the development of chickpea varieties. This is demonstrated by the fact that the majority of Ethiopia's released varieties can be traced back to ICARDA or ICRISAT introductions (MoA, 2018).

The high level of dependence of the national chickpea breeding program on exotic genotypes has its own limitations. First, the continuity of receiving germplasm from international research organizations may not be sustainable due to a lack of funding to operate the organization as it has in the past. Second, the access and benefit sharing of germplasm by all countries may be fully implemented in the near future, affecting free access to germplasm internationally. Third, improved varieties are being adopted at an increasing rate, which will eventually replace local germplasm, resulting in local chickpea genetic degradation. Fourth, breeders' neglect of Ethiopian chickpea material results in an inability to assess the merits of Ethiopian genotypes. Therefore, these issues should be considered in future chickpea breeding programs, and the use of Ethiopian chickpea germplasm should be of equal importance, requiring detailed characterization and evaluation, conservation and monitoring of diversity.

Chapter 3

3. Genetic Variability and Population Structure of Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm

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3.1. Abstract

Evaluation of the genetic diversity and an understanding of the genetic structure and relationships of chickpea genotypes are valuable to establish efficient germplasm conservation strategies and crop breeding programs. Information is limited, in these regards, for Ethiopian chickpea genotypes. Therefore, the present study was carried out to estimate the genetic diversity, population structure, and relationships of 152 chickpea genotypes using simple sequence repeats (SSR) markers. Twenty three SSR markers exhibited polymorphism producing a total of 133 alleles, with a mean of 5.8 alleles per locus. Analyses utilizing various genetic-based statistics included pairwise population Nei's genetic distance, heterozygosity, Shannon's information index, polymorphic information content, and percent polymorphism. These analyses exemplified the existence of high genetic variation within and among chickpea genotypes. The 152 genotypes were divided into two major clusters based on Nei's genetic distances. The exotic genotypes were grouped in one cluster exclusively showing that these genotypes are distinct to Ethiopian genotypes, while the patterns of clustering of Ethiopian chickpea genotypes based on their geographic region were not consistent because of the seed exchange across regions. Model-based population structure clustering identified two discrete populations. These finding provides useful information to design efficient conservation strategies and breeding programs for widening the genetic base of chickpea.

Keywords: Chickpea; Genetic diversity; Population structure; Principal coordinate analysis; SSR markers

3.2. Introduction

Chickpea (*Cicer arietinum* L.) belongs to the family Fabaceae (formerly Leguminoseae) and subfamily Faboideae. It is a diploid self-pollinated crop species having chromosome number of 2n = 2x = 16 (Van der Maesen, 1987; Kujur *et al*., 2015) with a comparatively small genome size of 740 Mbp (Arumuganathan and Earle, 1991). Chickpea is believed to have originated from the South Eastern Turkey and the adjoining areas of Syria (Kujur *et al*., 2015), where chickpea was domesticated and later spread to the secondary centers of diversity: north-east Africa, Mediterranean Europe and the Indian sub-continent and more recently to Mexico and Chile (Harlan, 1992; Van der Maesen *et al*., 1972; Zohary and Hopf, 2000).Based on the presence of wild relative (*Cicer Cuneatum*) found in Northern Ethiopia (Yadeta Anbessa and Geletu Bejiga, 2002) and the Archaeological evidence from Lalibela caves in Ethiopia with seed samples dated at over 2500 years (ICB, 2012), Asnake Fikre *et al*. (2020) suggested for reconsideration of Ethiopia as the origin of chickpea. However, with the current consensus, Ethiopia is considered as the secondary center of diversity for chickpea (Harlan, 1992).

Chickpea is one of the first domesticated, cool season autogamous grain legume cultivated in more than 50 countries in subtropical and temperate regions throughout the world (Upadhyaya *et al*., 2011). Chickpea is also one of the major crops cultivated in Sub-Saharan Africa and its area of production and productivity trend has shown steady increases since 1961 because it serves as a cash crop, break crop for crop diseases managements, a rotational crop for soil fertility restoration, food for human beings, feed for animals, and it is suitable for sustainable intensification and to adapt climatic shocks (Asnake Fikre *et al*., 2020). In Ethiopia, chickpea is the third largest food legume crop in sowing area of cultivation and production and the second major export commodity next to white pea beans generating nearly 25% of the total legumes export earnings (Setotaw Ferede *et al*., 2018).

Knowledge of genetic diversity, structure and relationship among germplasm collections are vital to design appropriate germplasm conservation strategies and potential breeding programs (Choudhary *et al*., 2012). These activities aid in the maintenance of highly diversified germplasm which provides ample opportunity to breeders to look for desirable traits for developing new and superior varieties. Comprehensive information on genetic diversity and structure can be generated from morphological and molecular data. Ethiopian chickpea germplasm have been characterized extensively for phenotypic characteristics (Kebede Teshome, 2012; Gemechu Keneni *et al*., 2012a; Nigusie Girma *et al*., 2017; Sintayehu Admas and Gize Abeje, 2017;

Vishnyakova *et al*., 2017; Asnake Fikre *et al*., 2018; Awol Mohammed and Bulti Tesso, 2018).Although morphological markers allow the identification of genetic variation, it is disguised by factors in the environment, and minimized by a paucity of discernible morphological markers (Park *et al*., 2009). This shortcoming of phenotypic characterization has to be complemented by a molecular approach using molecular markers on which reliable and repeatable information can be generated for diversity assessment (Vieira *et al*., 2016; Powell *et al*., 1996). In chickpea, various markers have been used for diversity analysis which includes microsatellite or simple sequence repeats (SSRs), sequence tagged microsatellite markers (STMS), expressed sequence tags (ESTs), single nucleotide polymorphism (SNP), cleaved amplified polymorphic sequences (CAPS), conserved intron spanning primers (CISP) and diversity arrays technology (DArT) markers (Jacked *et al*., 2001; Varshney, *et al*., 2006; Bharadwaj *et al*., 2010; Thudi *et al*., 2011; Upadhyaya *et al*., 2016).

Simple sequence repeats (SSRs) are short tandem repetitive DNA sequences with a repeat length of few (1-6) base pairs which are abundant, dispersed through the genome and are highly polymorphic in comparison with other molecular markers (Vieira *et al*., 2016; Choudhary *et al*., 2009). SSRs have been the most widely used markers for genotyping chickpea because they are highly informative, co-dominant, reproducible and transferable among related species, multi-allelic, and have high degree of polymorphism and extensive genome coverage (Powell *et al*., 1996; Tadesse Sefera *et al*., 2011; Choudhary *et al*., 2012; Gemechu Keneni *et al*., 2012b). Moreover, SSR markers are three times as efficient as dominant markers for intraspecific analysis and are as efficient as other dominant markers in detecting interspecific variability (Nybom, 2004). In chickpea, large numbers of SSR markers have been characterized, identified and utilized extensively to study genetic diversity and relationships to identify genetically diverse germplasm with beneficial traits for use in chickpea genome analysis, germplasm characterization, phylogenetic analysis and genetic diagnostics (Powell *et al*., 1996; Tadesse Sefera *et al*., 2011; Choudhary *et al*., 2012; Gemechu Keneni, *et al*., 2012b). Kebede Teshome (2012) has tried to establish core collections of Ethiopian chickpea using SNPs markers. The use of SSR markers for characterizing Ethiopian Chickpea has been implemented, however, the number of genotypes characterized so far were small (Gemechu Keneni *et al*., 2012b; Tsegaye Getahun *et al*., 2021) in number as compared to the total number of genotypes conserved in the Ethiopian Biodiversity Institute (EBI) gene bank. The aim of this study was, therefore, to assess the nature of genetic structure and the level of genetic diversity and relationships within and between Ethiopian Chickpea genotypes, improved chickpea varieties and breeding lines by using 25 SSR markers.

3.3. Materials and Methods

3.3.1 Plant materials

One-hundred fifty-two chickpea genotypes were considered for this study (Table 2). One-hundred thirty-eight are Ethiopian genotypes, eight are nationally released varieties from Ethiopian agricultural research centers and six were breeding lines accessed from the International Center for Agricultural Research in the Dry Areas (ICARDA). The genotypes were grown at Bakelo Research Station of Debre Birhan Agricultural Research Center in the 2018/2019 cropping seasons. Two weeks after planting, approximately equal amounts of bulk leaf samples (two expanded leaves per plant) were collected from five plants of each genotypes as suggested by Gilbert *et al.* (1999) and then the leaves were stored in plastic locking bags containing Silica gel.

Table 2. List of chickpea genotypes used for this study

Region	Zone	District	# of genotype	Name of genotypes
Amhar	*East Gojjam1	Awabel, Dejen, Enarj enawga, Enemay, Goncha siso enese, Guzamn	22	228290, 240050, 207728-A, 207736-B,30287-C, 30288-A, 30289-A, 30289-B, 30290-A, 30300-A, 41021-A, 41029-B, 41080-B, 41086-A, 41222-B, 41222-B, 41228-A, 41230-A, 41231-B, 41234-C, 41247-A and 41247-B
	*East Gojjam2	Debay telatgen, Hulet ej enese, Mota, Shebel berenta, Gonji, Yilmana densa, D/dare zuria, Dega damot, Adet	23	207638, 212685-B, 212685-B, 215289-A, 30307-D, 30307-D, 30308-B, 30309-A, 30311-A, 30313-C, 30314-A, 30314-B, 30316-A, 41020-A, 41075-C, 41078-B, 41090-A, 41245-A, 41257-A, 41258-A, 41265-B, 41270-B and 41320-A
	North Gonder	Alefa, Belesa, Chilga, Dabat, Debark, Este, and Wegera	16	207136-A, 207136-A, 207167-A, 207173-B, 207175-A, 207609-B, 207617-A, 225884-A, 227152-A, 227160-B, 227161-B, 24159-C, 241800-A, 241801-A, 41301-A and 9646-A
	Central Gonder	Gonder zuria, Kemkem, Mirab belesa, Mirab dendia, Misrak belesa	14	30333, 207753-B, 236475-A, 30317-A, 30318-B, 30319-B, 30326-A, 30326-C, 30334-B, 30335-B, 30335-B, 30336-B, 30337-A and 41043-B
	North Shewa	Ankober, Debre Birhan, Efratana gidim, Mezezo Mojana, Mama lalo midir, Minjarna Shenkora, Siadebr and Tegulet	22	207652, 215067-B, 215067-C, 235036-B, 235036-C, 237055-B, 30348-C, 41093-C, 41094-B, Dbarc-black 1, Dbarc-black 2, Dbarc-black 3, Dbarc-red 4, enewari1, enewari2, enewari3, enewari4, enewari5, enewari6, enewari7, tegulet1 and tegulet 2
****Tigray**		Rayaazebo, Medebay zana, Axum, Maychew and Wukro	5	16586-A, 234050-B, 235391-A, 236459-B and 236467-A
Oromia	West Shewa	Ambo, Alem gena, Becho, Ejerie (addis alem), Jeldu, Akaki and Ada'a chukala and Kersana kondaltiiti	12	207684, 207712, 207714, 207691-B, 207765-B, 207769-A, 209026-A, 228197-E, 41169-C, 41200-B, 41200-C and 41206-B
	Arsi-Bale	Bekoji, Chole, Jeju, Goro, Robe, Robe market, and Girawa	12	207670, 207664-A, 207679-B, 230796-C, 231331-A, 28741-A, 41035-C, 216854-C, 41136-A, 41030, 41034, and 41153-A
****SNNP**		Konso special district	1	225741-C
Exotic Genotypes	ICARDA Genotypes		6	125231***, 128699***, 140294***, 69757***, 70788*** and 9003***
	Improved Varieties		8	Dalota, Dhara***, Dubie, Ejere***, Mastewal, Minjar, Shahso*** and Teji***

*East Gojjam was grouped in two; **Due to small number of genotypes, Tigray collections was grouped with North Wollo collections, Konso and Hareregie with Arsi-Bale collections, *** Kabuli types chickpea, while the rest are desi type chickpea

3.3.2. DNA extraction and quantification

Genomic DNA was extracted using the cetyltriethylammonium bromide (CTAB) technique (Doyle and Doyle, 1987) with slight adjustments at Ethiopian Biodiversity Institute's Molecular Laboratory. The leaf samples were ground into fine powder using pestle and mortar using 250 µl DNA extraction buffers (0.35M sorbitol, 0.1M Tris–HCl (pH 7.6), 0.005M EDTA, and 0.2M Tris-HCl, 0.05M EDTA, 2M NaCl and 2% CTAB, mixed in equal amounts). About 100 mg of ground leaf sample was transferred to 2 ml microcentrifuge tubes and 750 µl of extraction buffer was added. Tubes were maintained at 65°C for one hour followed by chloroform-isoamyl alcohol (24:1) extraction. The DNA pellet was air dried and dissolved in 100 µl of 1× TE buffer. The quality and quantity of all DNA samples were checked using Nano Drop Spectrometer (ND-2000). In addition, agarose gel (0.8%) was used for checking the quality of the DNA by taking 30 genotypes selected systematically based on the result from the NanoDrop. The working DNA sample was diluted to obtain a final DNA concentration of 30 - 50 ng μL^{-1}.

3.3.3. Polymerase chain reaction (PCR) and electrophoresis

PCR reaction and electrophoresis activities were undertaken at Ethiopian Biodiversity Institute's Molecular Laboratory. Twenty-three SSR markers were used to carry out amplifications of the target DNA portion (Table 3). The SSR markers were purchased from Invitrogen Thermo Fisher from Scientific Life Technologies Europe BV, Nederlaenderna filial Sverige, Lindhagensgatan, Stockholm in 2020. The markers were selected based on polymorphic information content (PIC), allelic richness and herozygosity reports from various studies (Qadir *et al*., 2007; Choudhary *et al*., 2009; Choudhary *et al*., 2012; Tadesse Sefera, *et al*., 2011; Gemechu Keneni *et al*., 2012b; Amina *et al*., 2020). These SSR markers were developed from sequence information obtained by various authors (Hüttel *et al*., 1999; Winter *et al*., 1999; Winter *et al.,* 2000; Sethy *et al*., 2003;

Millan *et al*., 2006; Qadir *et al*., 2007; Radhika *et al*., 2007; Upadhyaya *et al*., 2008; Choudhary *et al*., 2009; Nayak *et al*., 2010). The list and description of the primers is indicated in Table 3.

PCR reaction was performed with a Hybaid PCR express thermal cycler (Hybaid, UK) after optimizing the amplification conditions for each primer pair in a total volume of 10 µl containing 50 ng DNA,1.5mM $MgCl_2$, 0.2mM dNTPs, 0.4 mM each of the forward and reverse primers and 0.05U/µl Taq polymerase. The PCR was programmed at an initial denaturation step of 3 min at 94°C followed by 35 cycles of 20 s denaturation at 94°C, annealing at 55 to 60°C (depending on the primer) for 50 s, initial extension at 72°C for 50 s, and final extension at 72°C for seven mins. Before determining polyacrylamide gel staining, the amplified products were checked for the reproducibility of PCR products using a 2% agarose gel stained with ethidium bromide in a TBE buffer and were visualized on a UVITEC gel doc (UVITEC, UK). The resolution of PCR products was done on 6% polyacrylamide gel in 0.5x TBE buffer with a 6x DNA loading dye. Electrophoresis was carried out on a vertical electrophoresis set up using a standard DNA ladder (100 bp, Solis Biodyne, Estonia). The vertical electrophoresis was run with 100V for two hrs and 30mins, and stained using silver staining developed by Huang *et al*. (2018). Then gel pictures were taken using digital camera. The band sizes were determined using UVITEC (UVITEC, Cambridge, UK) software. Primer bands that were unclear or absent were sorted and repeated. Non-polymorphic, missing, faint and distorted gels were disregarded at scoring and only records of 23 primers with clear polymorphic bands were considered for statistical analysis.

Table 3. List and description of SSR primers used for the molecular characterization of 152 chickpea genotypes

SSR Locus	**Primer Sequence (5' to 3')**		**Repeat motif**	**Size (bp)**	**Linkage Group**	**References**
	Forward	**Reverse**				
CaSTMS 11	GTATCTACTTGTAATATTCTCTTCTCT	ATATCATAAACCCCCCAC	(GA)20	232	4	Gemechu Keneni, *et al*., 2012b
CESSR 42	TGGTTGAAGAAAAGAAGGTAGTG	CGGTTCACTAATGCAAAAACCT	(ACC)5	298		Choudhary *et al*., 2009
CESSR 62	ACCAGCTGCTAGACCTGATGTT	GCAATAAAACAAAATCCTCACACC	(TGA)5, (TAT)3	245		Choudhary *et al*., 2009
CESSR 71	TTGTAGTTCTCCTCTCTCTCTCTC	CATCAAAACCAAACCTATGGAG	(CT)C(CT)8, (CT)6, (CT)11	295		Choudhary *et al*., 2009
CESSRDB 45	AGATGGTTTGAATGTTGAGG	CACTTGACCCTTTGATTGTT	(AT)7(AG)5	295		Choudhary *et al*., 2009
CESSRDB 54	AGTGTTGTGGGTTTCATTTC	TTGATTTGCCAAAGTACACA	(TTA)5	221		Choudhary *et al*., 2009
GA 11	GTTGAGCAACAAAGCCACAA	TTCTTGTCTGGTTGTGTGAGC	(CT)21	159	3,1, 2, 6	Winter *et al*., 1999; Gemechu Keneni, *et al*., 2012b
GA 24	TTGCCAAAACCAATAACTCTG	TCCCTTTTACACAAGGCCAG	(GA)19	203	1,2,4	Winter *et al*., 1999; Gemechu Keneni, *et al*., 2012b
GA-20	TATGCACCACACCTCGTACC	TGACGGAATTCGTGATGTGT	(CT)23	174	2,6	Winter *et al*., 1999; Bharadwaj *et al*., 2011
NCPGR 100	CCATTTTCTACAATCTCATGTCT	GTAGAAAGAGCCAAGAGGCA	CT)15N42(CT)2CC(CT)5TT(CT)6AT(CT) 7	263	1	Gemechu Keneni, *et al*., 2012b
NCPGR 45	TGTTTTCAAATCAAACAGGC	GATACACACCAAGGCACAGT	(CT)2GTCAT(CT)5CC(CT)2CC(CT)17	223	2	Gemechu Keneni, *et al*., 2012b
NCPGR 53	CCCTCCTTCTTGCTTACAAA	TAATGGTGAACGAATCATGG	(CT)5CA(CT)CA(CT)10CA(CT)4CA(CT)TA(CT)4GTCA(CT)12	194	1	Gemechu Keneni, *et al*., 2012b
NCPGR 94	GGTTTGATGTGTTCTTGGCT	CCCTCAATTCCCTCGATTTA	(CT)25	176	5	Gemechu Keneni, *et al*., 2012b
SSR 1	TGAATTTTGTTTACCACCCCTC	TTTGGCTTATTCTGTTCTTCCC	(AG)20	157		Qadir *et al*., 2007
SSR 22	GCTTTCCCTTTACTTCTTGGGT	TGCTATTCAAGTCTCCCTCCTC	(AATG)5	275		Qadir *et al*., 2007
SSR 4	GACAAAACAACCTCCCAAGAAA	AACAACGACAACAACAACAACG	(TTG)6	279		Qadir *et al*., 2007

Table 3: Continued....

SSR Locus	Primer Sequence (5' to 3')		Repeat motif	Size (bp)	Linkage Group	References
	Forward	**Reverse**				
SSR 5	GAGCCCTGAAATGAAGAAAGAA	CACCTTTGAGCCCTAGTCTGTT	(AAAT)5	387		Qadir *et al*., 2007
SSR 60	GGTCATGTTGATTTCTCACCAA	GAACTTTCCGCACACGTTATG	(AAAT)6	337		Qadir *et al*., 2007
TA 144	ATTTTAATCCGGTGAATATTACCTTT	GTGGAGTCACTATCAACAATCATACAT	(TAA)27	241	5,6,8	Winter *et al*., 1999; Gemechu Keneni, *et al*., 2012b
TA 18	AAATAATCTCCACTTCACAAATTTTC	ATAAGTGCGTTATTAGTTTGGTCTTGT	(TAA)24	147	7,5,6	Winter *et al*., 1999; Bharadwaj *et al*., 2011; Gemechu Keneni, *et al*., 2012b
TR 1	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGAAAGT	(TAA)31	224	5,6	Winter *et al*., 1999; Gemechu Keneni, *et al*., 2012b
TR 2	GGCTTAGAGTTCAAAGAGAGAA	AACCAAGATTGGAAGTTGTG	(TAA)36	210	3	Winter *et al*., 1999; Gemechu Keneni, *et al*., 2012b
TR 29	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG	(TAA)8TAGTAATAG(TAA)32	197–251	7,5,1,3	Winter *et al*., 1999; Bharadwaj *et al*., 2011; Gemechu Keneni, *et al*., 2012b

3.3.4. Scoring SSR data and statistical analysis

Allelic data were recorded for each of the microsatellites markers for each genotype with the help of UVITEC software as well as visually. The allelic data scores locus-based diversity indices including the number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's information index (I), number of privet alleles (NPA), fixation index, percent polymorphism and unique alleles were recorded using GenAlEx v.6.502 (Peakall *et al*., 2012). Estimates of genetic differentiation were computed by analysis of molecular variance (AMOVA) to partition total genetic variation into within and among population subgroups using GenAlEx 6.502 (Peakall *et al*., 2012). PowerMarker 3.25 (Liu *et al*., 2005) was used to estimate major allele frequency (MAF), Gene Diversity (GD), and polymorphic information content (PIC).

The allelic data scored was used to analyze principal coordinate analysis (PCoA) using GenAlEx v.6.502 (Peakall *et al*., 2012). Dendrogram tree was constructed based on Nei's genetic distance using PowerMarker 3.25 and the tree was visualized using Molecular Evolutionary Genetic Analysis (MEGA 6) (Tamura *et al*., 2013). Dendrogram was constructed by the unweighted pair-group method with arithmetic averages (UPGMA) (Nei *et al*., 1983). The structure of the population was analyzed based on the Bayesian model-based clustering method using Structure 2.3.4 software as suggested by Pritchard *et al.* (2000). This software assumes a model in which there are K populations, which contribute to the genotype of each individual. Burning period of 50,000 and 100,000 Markov Chain Monte Carlo (MCMC) iterations were used with independent replications of 10 times for each K value (K = 1 to 10) assuming an admixture model and uncorrelated allele frequencies. A web-based Structure Harvester program (Evanno *et al*., 2005) was employed to determine the most likely value of K for each test (Earl and Holdt, 2012).

3.4. Results

3.4.1. Microsatellite repeats locus diversity

The polyacrylamide gel electrophoresis pictures and the estimated genetic diversity parameters of the SSR locus diversity are indicated in Figure 1 and Table 4, respectively. The Visual observations on the gels of the amplification products of the respective markers revealed the existence of low (Figure 1 A and B) to high (Figure 1 C and D) level of polymorphism in the Ethiopian and exotic genotypes depending on the types of primer used. Among 152 chickpea genotypes a total of 133 alleles with an average value of 5.8 alleles per SSR were recorded. The allelic richness (Na) per locus varied widely among markers, ranging from two (CESSRDB 45, SSR22, and SSR 5) to 16 (TR 1). The number of effective alleles (Ne) ranged between 1.3 (CESSRDB 45) and 7.6 (TR 29), with an overall mean of 3.2. Shannon's information index (I) ranged from 0.4 (CESSRDB 45) to 2.1 (TR 1 and TR 29) with mean of 1.2. The average observed heterozygosity (0.4) was lower than the expected heterozygosity (0.6) and unbiased expected heterozygosity (0.6). The inbreeding coefficient (Fis) and fixation index (Fit) values ranged from -1.1 to 1.0 and -1.0 to 1.0, respectively. The major allele frequency varied from 0.11 (TR 29) to 0.88 (CESSRDB 45) with an average of 0.49. Polymorphic information content (PIC) values ranged from 0.19 (CESSRDB 45) to 0.9 (TR 1 and TR 29) with an average of 0.58. Seventeen markers (73.1%) had a PIC score of 0.5 and above. Gene diversity values ranged from 0.22 (CESSRDB 45) to 0.91 (TR 29) with an average of 0.67. Out of the total number of alleles only five alleles (0.07% of the total alleles detected) were private alleles observed in locus NCPGR 100 in genotypes 30307-A from East Gojjam2, TR 1 in genotypes Enewari1 from North Shewa, SSR 1 in genotypes enewari1 from North Shewa, SSR1 in genotypes 30347-B from North Wollo and NCPGR 94 in genotypes 41030 from Arsi Bale.

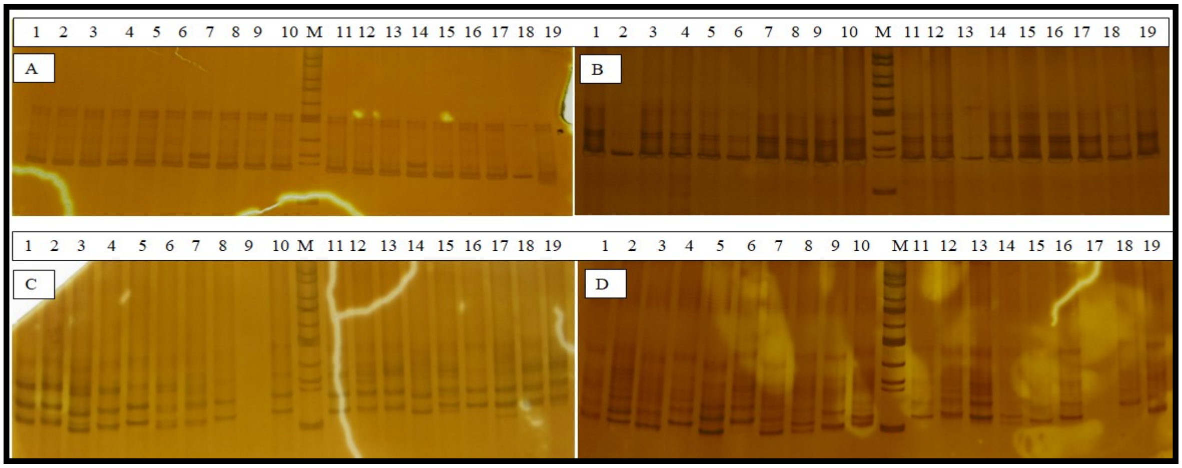

Figure 1. Polyacrylamide gel electrophoresis pictures obtained with SSR 22 (A), CESSR 42 (B), TR 29 (C) and TR 1 (D) primers detected in chickpea genotypes. The lane numbers identify serial no of genotypes and M stands for 100 bp DNA ladder.

Table 4. Estimated genetic diversity parameters of 23 SSR markers in 152 chickpea genotypes

Locus	Na	Ne	I	Ho	He	UHe	F	Ht	Fis	Fit	Fst	Nm	MAF	PIC	NPA	GD
CaSTMS 11	5	2.0	0.8	0.0	0.4	0.5	1.0	0.7	1.0	1.0	0.4	0.5	0.49	0.58	0	0.63
CESSR 42	5	2.7	1.1	0.0	0.6	0.6	1.0	0.8	1.0	1.0	0.2	1.1	0.32	0.71	0	0.75
CESSR 62	3	1.9	0.7	0.5	0.4	0.4	-0.1	0.5	-0.2	0.1	0.2	0.9	0.59	0.47	0	0.55
CESSR 71	5	2.8	1.2	0.4	0.6	0.7	0.4	0.7	0.4	0.4	0.1	1.7	0.44	0.67	0	0.71
CESSRDB 45	2	1.3	0.4	0.5	0.2	0.2	-1.4	0.2	-1.1	-1.0	0.0	6.0	0.88	0.19	0	0.22
CESSRDB 54	4	1.8	0.7	0.2	0.4	0.4	0.6	0.6	0.6	0.7	0.3	0.7	0.53	0.48	0	0.56
GA 11	6	2.8	1.2	0.3	0.6	0.6	0.6	0.7	0.5	0.6	0.1	2.0	0.42	0.66	0	0.70
GA 24	6	2.6	1.2	0.3	0.6	0.6	0.6	0.7	0.6	0.7	0.2	1.3	0.39	0.67	0	0.71
GA-20	6	3.1	1.3	0.5	0.7	0.7	0.3	0.8	0.3	0.4	0.1	1.5	0.38	0.73	0	0.76
NCPGR 100	3	1.9	0.7	0.5	0.5	0.5	0.0	0.5	0.0	0.1	0.1	3.1	0.56	0.38	1	0.49
NCPGR 45	4	2.6	1.1	0.7	0.6	0.6	-0.2	0.7	-0.2	-0.1	0.1	2.6	0.45	0.62	0	0.67
NCPGR 53	4	2.2	0.9	0.4	0.5	0.5	0.3	0.7	0.3	0.4	0.2	0.9	0.41	0.59	0	0.65
NCPGR 94	10	3.9	1.5	0.6	0.7	0.8	0.2	0.8	0.2	0.3	0.1	1.9	0.26	0.80	1	0.82
SSR 1	6	3.2	1.2	0.5	0.7	0.7	0.2	0.7	0.2	0.3	0.1	3.3	0.44	0.66	1	0.70
SSR 22	2	1.3	0.4	0.3	0.2	0.2	-0.2	0.2	-0.2	-0.2	0.0	7.4	0.85	0.22	0	0.25
SSR 4	6	4.0	1.5	0.6	0.7	0.8	0.2	0.8	0.2	0.2	0.1	2.9	0.29	0.78	0	0.80
SSR 5	2	1.8	0.6	0.0	0.4	0.4	1.0	0.5	1.0	1.0	0.2	1.4	0.55	0.37	0	0.49
SSR 60	6	3.1	1.3	0.4	0.7	0.7	0.4	0.8	0.5	0.5	0.1	1.8	0.33	0.72	0	0.75
TA 144	3	2.4	0.9	0.0	0.6	0.6	1.0	0.7	1.0	1.0	0.2	1.4	0.35	0.59	0	0.66
TA 18	8	5.2	1.8	0.6	0.8	0.8	0.3	0.9	0.3	0.3	0.1	3.6	0.20	0.84	0	0.85
TR 1	16	7.3	2.1	1.0	0.8	0.9	-0.2	0.9	-0.2	-0.1	0.1	3.7	0.14	0.90	2	0.90
TR 2	9	6.0	1.9	1.0	0.8	0.9	-0.2	0.9	-0.2	-0.2	0.1	4.7	0.22	0.86	0	0.87
TR 29	12	7.6	2.1	1.0	0.9	0.9	-0.2	0.9	-0.2	-0.1	0.1	4.5	0.11	0.90	0	0.91
Total	133	73.3	-	-	-	-	-	-	-	-	-	-	-	-	5	-
Mean	5.8	3.2	1.2	0.4	0.6	0.6	0.2	0.7	0.3	0.3	0.1	2.6	0.42	0.63	-	0.67
SE	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.4	-	-	-	-

Key: Na = number of alleles detected per locus; Ne = number of effective alleles; I = Shannon's Information Index; Ho = Observed Heterozygosity; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity; F = fixation Index; Ht = Total Expected Heterozygosity; Fis = inbreeding coefficient; Fit = fixation index; Fst = genetic differentiation; Nm = Gene flow; MAF = major allele frequency; PIC = Polymorphic Information Center; NPA = number of privet alleles; GD = Gene Diversity; and SE is standard error.

3.4.2. Genetic diversity in chickpea genotypes and population

The genetic diversity indices for chickpea genotypes based on geographic origins is summarized in Table 5. The observed numbers of alleles (Na) were in the range of 3.7 (Exotic Genotypes) to

5.3 (East Gojjam2). The number of effective alleles (Ne) ranged from 2.7 (Exotic Genotypes) to 3.6 (East Gojjam2 and North Shewa). Shannon's information index (I) ranged from 1.0 (Exotic Genotypes) to 1.3 (East Gojjam 2). The mean of the observed heterozygosity (0.4) is less than expected heterozygosity (0.6) and unbiased expected heterozygosity (0.6). The inbreeding coefficient (F) estimate ranged from 0.0 (Exotic Genotypes) to 0.3 (North Gonder, Central Gonder, North Shewa, North Wollo and West Shewa) with the average of 0.2. The mean percentage of polymorphic locus (% P) across population was 99.5% varying from 95.7 to 100%. Higher values of number of private alleles (NPA) were observed in population of East Gojjam 2 (0.04), North Shewa (0.09), North Wollo (0.04) and Arsi-Bale (0.04).

Table 5. Summary of parameters for genetic diversity in chickpea population from different geographic regions

Populations	**Population diversity parameters**										
	Na	Ne	I	Ho	He	UHe	F	% P	NPA	MAF	PIC
East Gojjam1	4.7	3.3	1.2	0.5	0.6	0.6	0.2	95.7	0.00	0.50	0.55
East Gojjam2	5.3	3.6	1.3	0.5	0.6	0.6	0.2	100.0	0.04	0.49	0.58
North Gonder	4.7	3.2	1.2	0.4	0.6	0.6	0.3	100.0	0.00	0.52	0.54
Central Gonder	4.6	3.0	1.1	0.4	0.6	0.6	0.3	100.0	0.00	0.55	0.51
North Shewa	5.1	3.6	1.2	0.4	0.6	0.6	0.3	100.0	0.09	0.49	0.57
North Wollo	4.9	3.2	1.2	0.4	0.6	0.6	0.3	100.0	0.04	0.52	0.55
West Shewa	4.4	3.1	1.1	0.4	0.6	0.6	0.3	100.0	0.00	0.52	0.54
Arsi-Bale	4.6	2.9	1.1	0.5	0.6	0.6	0.2	100.0	0.04	0.56	0.52
Exotic Genotypes	3.7	2.7	1.0	0.5	0.5	0.6	0.0	100.0	0.00	0.54	0.49
Mean	4.7	3.2	1.1	0.4	0.6	0.6	0.2	99.5	-	-	-
SE	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.5	-	-	-

Key: Na = number of alleles detected per locus; Ne = number of effective alleles; I = Shannon's Information Index; Ho = Observed Heterozygosity; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity; F = fixation Index; % P = percent polymorphism; NPA = number of private Alleles; MAF = major allele frequency; PIC = Polymorphic Information Center and SE = standard error.

3.4.3. Analysis of molecular variance (AMOVA) and genetic partitioning

The AMOVA showed that 88% of the allelic variation was attributed to individual genotypes within populations, while only 12% was distributed among populations (Table 6). The local population contributed 7% (West Shewa) to 14% (East Gojjam2), while the exotic genotypes contributed 7.6% of the total variation. The value of pairwise comparisons of population differentiation (Fst) and Gene flow (Nm) among geographical regions of chickpea population are indicated in Table 7. The highest Fst value was observed between exotic genotypes and chickpea populations from central Gonder (0.18) and Arsi-Bale (0.18), while the lowest was recorded between chickpea genotypes population of North Shewa and Central Gonder (0.05), North Wollo (0.05) versus North Shewa, and Arsi-Bale and West Shewa (0.05). Generally the exotic genotypes showed high Fst value compared to chickpea genotypes of Ethiopian origin than pairwise comparison between chickpea population within Ethiopian origin. Gene flow (Nm) between and within geographical regions was recorded from 1.16 (Arsi-Bale versus Exotic Genotypes) to 3.96 (East Gojjam2 versus North Gonder).

Table 6. Analysis of Molecular Variance (AMOVA) showing the distribution of genetic diversity within and among populations of chickpea genotypes from different sources of origins

Source of variations	**Degree of freedom**	**Sum square**	**Mean square**	**Variance Estimated variances**	**Proportion of explained variance in %**	**Statistics**	**Value**	***P value***
Among Pops	8	288.993	35.874	0.856	12			
Within Pops	295	2114.224	7.167	7.167	88	PhiPT	0.107	0.001
East Gojjam1		*306.500*			*12.8*			
East Gojjam2		*336.413*			*14*			
North Gonder		*221.688*			*9.2*			
Central Gonder		*186.607*			*7.8*			
North Shewa		*317.682*			*13.2*			
North Wollo		*219.313*			*9.1*			
West Shewa		*167.500*			*7.0*			
Arsi Bale		*177.308*			*7.4*			
Exotic Genotypes		*181.214*			*7.6*			
Total	303	2402.217		8.023	100			

Table 7. Pairwise population differentiation (Fst) values above diagonal and gene flow (Nm) below diagonal among chickpea populations from different growing geographic areas based on the probability level based on 999 permutations

Population	**East Gojjam1**	**East Gojjam2**	**North Gonder**	**Central Gonder**	**North Shewa**	**North Wollo**	**West Shewa**	**Arsi Bale**	**Exotic Genotypes**
East Gojjam1	0	0.07*	0.08*	0.13**	0.10**	0.14**	0.13**	0.11**	0.16**
East Gojjam2	3.14	0	0.06*	0.09*	0.07**	0.14**	0.10**	0.09**	0.14**
North Gonder	2.90	3.96	0	0.13**	0.07**	0.12**	0.11**	0.13**	0.12**
Central Gonder	1.69	2.57	1.74	0	0.05**	0.10**	0.09**	0.13**	0.18**
North Shewa	2.25	3.24	3.11	4.87	0	0.05**	0.08**	0.10**	0.16**
North Wollo	1.57	1.58	1.80	2.19	4.67	0	0.08**	0.11**	0.16**
West Shewa	1.66	2.37	2.12	2.63	2.96	2.79	0	0.05**	0.15**
Arsi/Bale	2.05	2.59	1.73	1.75	2.26	2.03	4.35	0	0.18**
Exotic Genotypes	1.29	1.59	1.75	1.12	1.32	1.33	1.47	1.16	0

3.4.4. Principal coordinates analysis (PCoA)

The multivariate principal coordinate analysis (PCoA) of the molecular data showed that the first 3 coordinates were important and accounted for 26.6% of the variation; PCs 1 (14.0%), 2 (6.9%), and 3 (5.7%). The PCoA plots of PC 1 versus PC 2 using factorial analysis of GenAlEx showed the exotic genotypes were clustered in quadrant I entirely, while a wide dispersion of Ethiopian genotypes across the four quadrants (Figure 2) was observed without considering their geographic origin. Genotypes collected from East Gojjam1 clustered in quadrant III (eight genotypes), and IV (13 genotypes) forming small sub-clusters in both quadrants. A single genotype from this zone falls in quadrant I. East Gojjam2 collections clustered in quadrant I (8 genotypes), III (3 genotypes), and IV (12 genotypes) sowing a tendency of forming sub-clusters in each quadrant. Genotypes of North Gonder clustered in quadrant I (5 genotypes) and IV (9 genotypes). The remaining two genotypes were grouped in cluster II and III. The majority of the genotypes collected from Central Gonder were clustered in quadrant III (8 genotypes). The remaining five genotypes and one genotype fall in quadrant II and I, respectively. Genotypes of North Shewa collection were clustered in quadrant I (3 genotypes), II (7 genotypes), III (10 genotypes), and IV (1 genotype). Genotypes from North Wollo formed two sub clusters in quadrant II (12 genotypes). The remaining one and four genotypes clustered in quadrant I and III, respectively. Genotypes from West Shewa were clustered in quadrant I (4 genotypes) and II (8 genotypes). Genotypes of Arsi Bale appeared in all quadrants with nearly equal proportion, I (4 genotypes), II (3 genotypes), III (2 genotypes), and IV (4 genotypes).

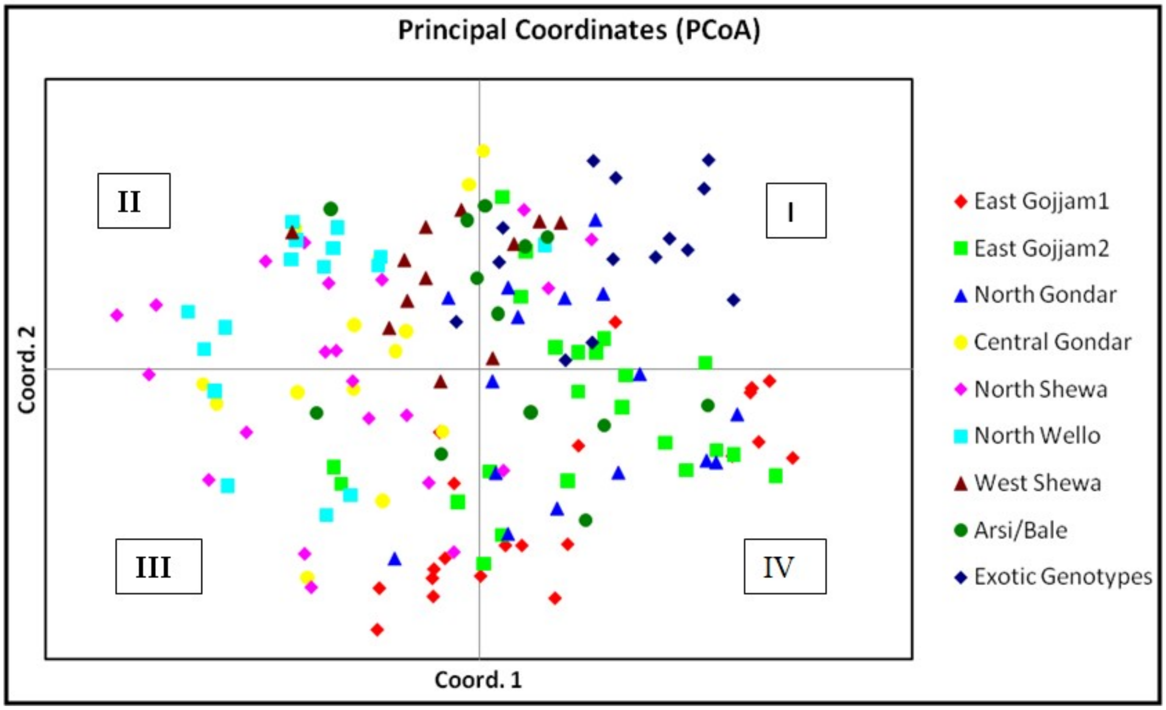

Figure 2. Two-dimensional plot obtained from principal coordinate analysis (PCoA) of 152 chickpea accessions using 23 SSR markers.

3.4.5. Genetic distance

The pairwise Nei's unbiased genetic distances (above diagonal) and unbiased genetic identity values (below diagonal) for all the chickpea populations representing the growing regions are shown in Table 8. The matrix of pairwise Nei's unbiased genetic distances between populations showed a close genetic distance between North Shewa and Central Gonder populations (0.09), North Wollo and North Shewa (0.09), and West Shewa and Arsi-Bale (0.09). On the other hand, the largest genetic distance (0.38) was obtained between population of Central Gonder and exotic genotypes. Generally, genetic distances between Ethiopian chickpea population and exotic genotypes were greater than any other combinations of paired populations within Ethiopia. The highest genetic identity value (0.92) was recorded between North Shewa population and Central Gonder population and the lowest genetic identity value (0.68) was recorded between Arsi-Bale and exotic genotypes. The genetic identity pairwise comparisons within genotypes of Ethiopian origin were more than the comparison between exotic genotypes with genotypes of Ethiopian origin.

Table 8.Pairwise Population Matrix of Nei's unbiased genetic distance (DA) above diagonal and Pairwise Population Matrix of Nei Unbiased genetic identity below diagonal among chickpea populations from different origins

Populations	**East Gojjam1**	**East Gojjam2**	**North Gonder**	**Central Gonder**	**North Shewa**	**North Wollo**	**West Shewa**	**Arsi-Bale**	**Exotic Genotypes**
East Gojjam1	*	0.15	0.15	0.23	0.21	0.25	0.25	0.21	0.34
East Gojjam2	0.86	*	0.11	0.17	0.15	0.31	0.20	0.17	0.29
North Gonder	0.86	0.89	*	0.22	0.14	0.25	0.21	0.25	0.26
Central Gonder	0.77	0.84	0.78	*	0.09	0.19	0.15	0.24	0.38
North Shewa	0.81	0.86	0.87	0.92	*	0.09	0.16	0.20	0.35
North Wollo	0.76	0.76	0.78	0.83	0.91	*	0.16	0.20	0.33
West Shewa	0.76	0.82	0.81	0.86	0.85	0.86	*	0.09	0.29
Arsi-Bale	0.81	0.84	0.78	0.79	0.82	0.82	0.91	*	0.37
Exotic Genotypes	0.71	0.75	0.75	0.69	0.71	0.72	0.75	0.68	*

3.4.6. Cluster analysis

The result from UPGMA based dendrogram shows that nine chickpea populations from different geographic origins were grouped into two major clusters (Figure 3). The first cluster contained the exotic genotype population, while cluster II consisted of the Ethiopian populations. Cluster II was divided into three sub-clusters showing the tendencies of grouping the neighboring regions together. The 152 genotypes were divided into two major clusters (Figure 4). Cluster I had 14 genotypes which were exclusively from the exotic genotypes. Cluster II was further sub divided into six distinct sub-clusters with variable number of genotypes in each sub-cluster. Sub-cluster 1 consisted of 27 genotypes with the following proportions, 22 (81.5%) from East Gojjam 1, two (7.4%) from East Gojjam 2, and three (11.1%) from Arsi-Bale. Sub-cluster 2 was composed of 25 genotypes of which 19 (76%) genotypes were from East Gojjam 2 and six (24%) genotypes from North Gonder. Sub-cluster 3 was composed of 14 genotypes of which 9 (64.3%) genotypes were from North Gonder, four (28.6%) from North Shewa and one (7.1%) from Arsi-Bale. Sub-cluster 4 contained 25 genotypes of which two genotypes (8%) were from East Gojjam 2, one genotype (4%) from North Gonder, 12 genotypes (48%) from Central Gonder, and 10 genotypes (40%)

from North Shewa. Sub-cluster 5 included 9 (64.3%) genotypes from North Wollo, three (21.4%) genotypes from West Shewa, and two genotypes (14.3%) from Arsi-Bale. Sub-cluster 6 represented a heterogeneous group which constituted 33 genotypes of which two (6.1%) genotypes were from Central Gonder, 8 (24.2%) genotypes from North Shewa, seven genotypes (21.2%) from North Wollo, nine (9%) genotype from West Shewa and seven genotypes (21.2%) from Arsi-Bale.

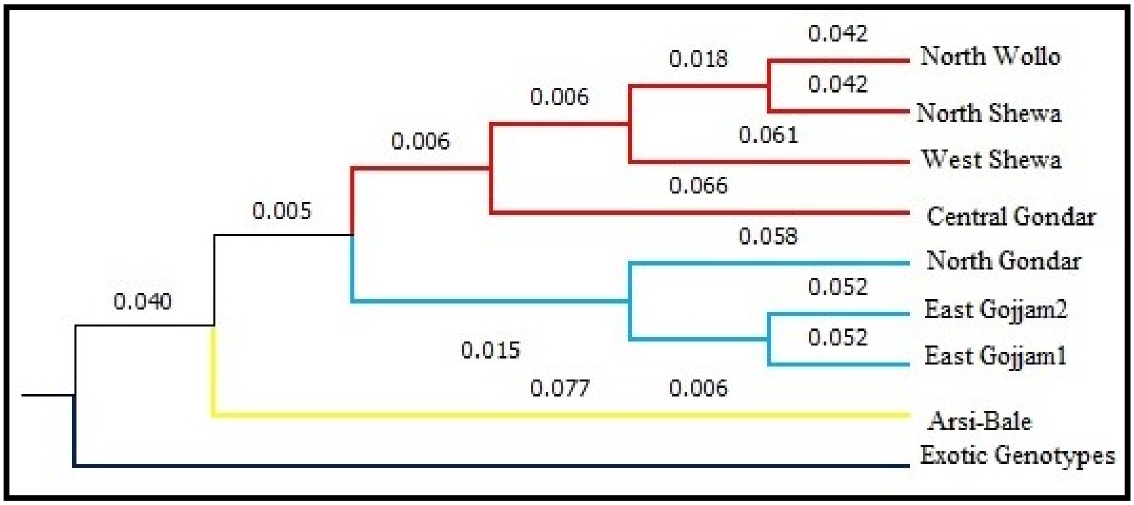

Figure 3. UPGMA dendrogram showing the genetic relationships of nine chickpea populations collection areas

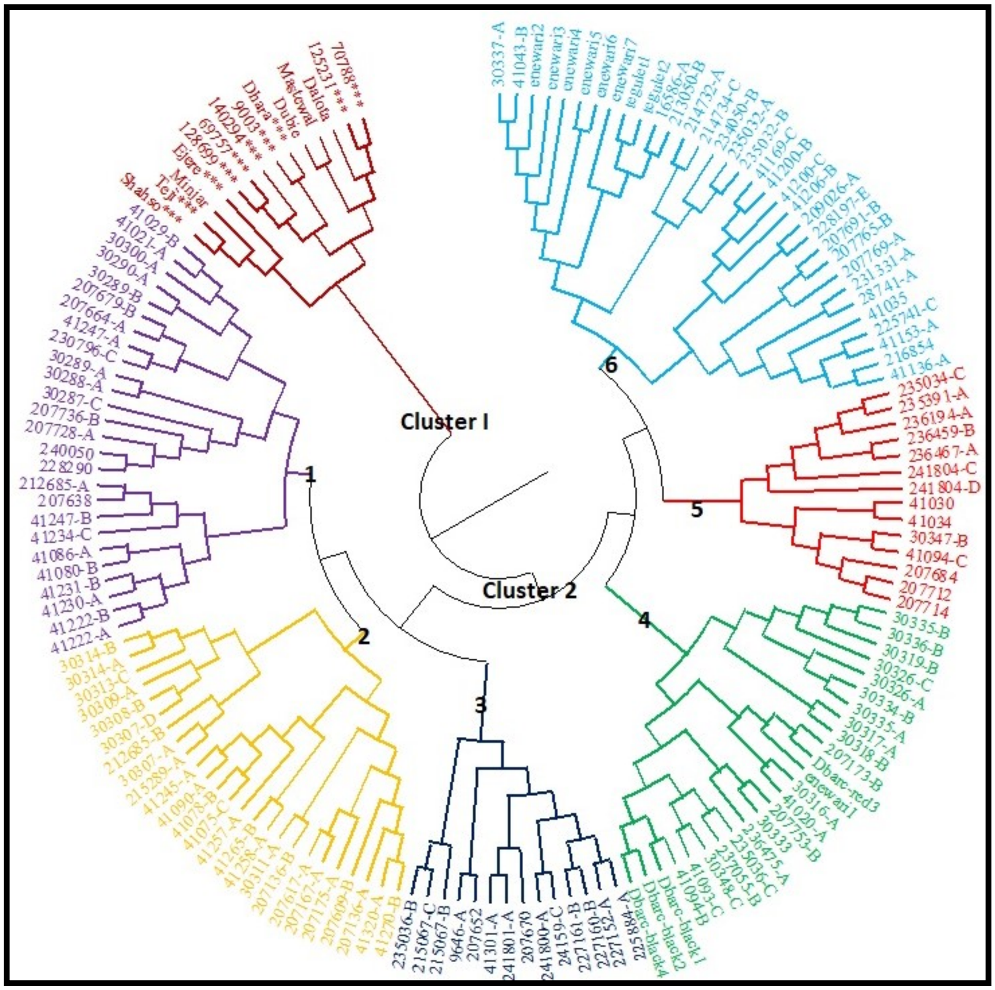

Figure 4. UPGMA based dendrogram of 152 chickpea genotypes was obtained using 23 SSR markers and Nie 1983 frequency based distance (*** Kabuli types chickpea, while the rest are desi type chickpea)

3.4.7. Population structure

The population structure of the 152 chickpea genotypes was analyzed and the results showed that the highest peak was observed at K = 2 indicating the presence of two major clusters (Figure 5 and Table 9). The result from STRUCTURE analysis further confirmed results of the UPGMA tree clustering. Based on the probable likelihood of each genotype to be grouped into any of the two distinct groups, a total of 85 genotypes (55.9%) were grouped into one of the two populations. The first cluster which was of 43 (28.3% of total genotypes) genotypes were grouped into population 1, the next 42 (27.6%) into population 2. The remaining 67 genotypes (44.1%)

were placed in the admixture group (Table 9). Cluster I was composed of 32.6%, 23.3%, 14% and 30.2 % East Gojjam1, East Gojjam2, North Gonder and the exotic genotypes, respectively. Cluster II was made from 4.8%, 7.1%, 19.0 5%, 19.0%, 26.2%, 14.3 %, 9.5% from East Gojjam2, North Gonder , Central Gonder , North Shewa, North Wollo, West Shewa, Arsi-Bale, respectively. All population contributed to admixed group with variable proportion ranging from 1.5% (Exotic Genotypes) to 19.4% (East Gojjam2).

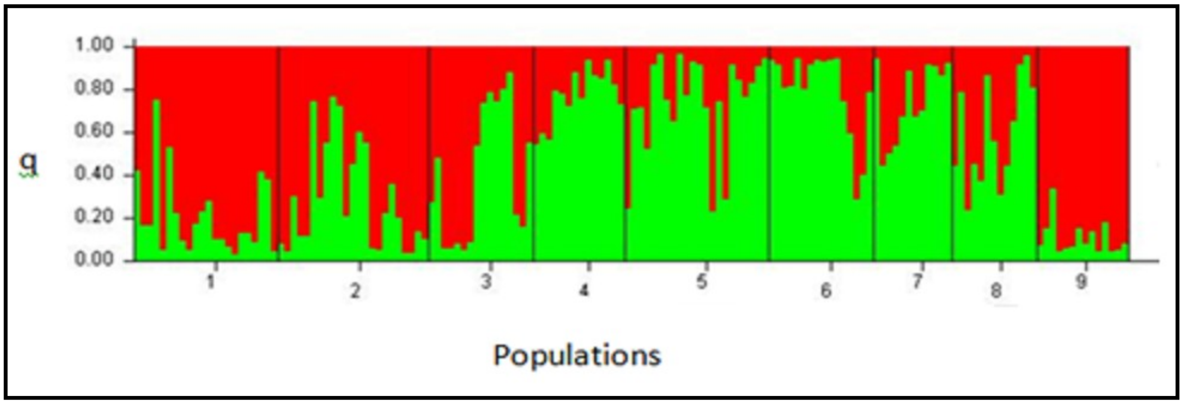

Figure 5. Structure bar plot assigning 152 chickpea genotypes in two groups (K = 2) based on 23 SSR markers analyzed by the structure program, showing proportion of the two groups in each zones, where 1 = East Gojjam1, 2 = East Gojjam2, 3 = North Gonder, 4 = Central Gonder, 5 = North Shewa, 6 is North Wollo, 7 is West Shewa, 8 = Arsi-Bale and 9 = Exotic Genotypes, q=membership coefficient

Table 9. Proportion of membership of each predefined nine populations in each of the clusters obtained at the best K (k=2)

Predefined Populations	**Total Number of genotypes**	**Admixed**		**Cluster 1**		**Cluster 2**	
		Number of genotypes	**Proportion in %**	**Number of genotypes**	**Proportion in %**	**Number of genotypes**	**Proportion in %**
East Gojjam1	22	8	11.9	14	32.6	0	0.0
East Gojjam2	23	13	19.4	10	23.3	2	4.8
North Gonder	16	7	10.4	6	14.0	3	7.1
Central Gonder	14	6	9.0	0	0.0	8	19.0
North Shewa	22	12	17.9	0	0.0	8	19.0
North Wollo	16	5	7.5	0	0.0	11	26.2
West Shewa	12	6	9.0	0	0.0	6	14.3
Arsi-Bale	13	9	13.4	0	0.0	4	9.5
Exotic Genotypes	14	1	1.5	13	30.2	0	0.0
Total	152	67	44.1	43	28.3	42	27.6
Average Distance	-	-	-	0.6565		0.6408	
Mean value of Fst	-	-	-	0.0396		0.0904	

Fst is genetic differentiation

3.5. Discussion

Efficient germplasm conservation and sustainable utilization requires a clear understanding of the genetic structure, diversity, and relationships among chickpea genotypes. This information is also helpful for breeders to identify new sources of germplasm harboring valuable alleles for improving yield, grain quality, and enhancing the level of resistance in cultivated varieties to various biotic and abiotic stresses (Choudhary *et al*., 2012; Valadez-Moctezuma *et al*., 2020).

Result from SSR analysis indicated the presence of considerable allelic richness per locus, relatively moderate to high PIC (0.63), Ho (0.4) and He (0.6) values, and the presence of private alleles. High level of genetic diversity (0.67) indicates the existence of molecular variation among the analyzed chickpea genotypes. High PIC values were also reported by Tadesse Sefera *et al* (2011) and Ghaffari *et al*., (2014) which is in agreement with the present study, however a lower number of effective alleles per locus was recorded in the present study in contrast to that of Tadesse Sefera *et al* (2011) and Tsegaye Getahun *et al*. (2021). This happened because of the different number of accessions, different number of loci examined, and the nature of markers used in each study. However, comparable results were reported from Gemechu Keneni *et al*. (2012b). The high level of PIC values were an indicator of the efficiency of the markers for diversity studies in chickpea genotypes because a locus, with an estimated PIC value greater than 0.50, is considered to be highly diverse (Botstein *et al*., 1980). Nineteen markers had a score of 0.5 and above which indicates that these markers are highly informative SSR markers that could be employed in genetic diversity studies in chickpea. The ability of SSRs to detect intraspecific as well as interspecific variation in chickpea has been demonstrated by many authors (Hüttel *et al*., 1999; Choudhary *et al*., 2012; Agarwal *et al*., 2015).

In this study, loci CaSTMS 11, CESSR 42, CESSRDB 54, GA 11, GA 24, SSR 22, and SSR 5 exhibited low-level of observed heterozygosity compared to the expected heterozygosity. Moreover, the high associated fixation index, implies that high levels of inbreeding among the assessed chickpea genotypes, which is expected because chickpea is a self-pollinated crop, previously only 0 to 1.58% of out-crossing was reported (Ghaffari *et al*., 2014). Simultaneously, loci CESSR 62, CESSR 71, NCPGR 45, NCPGR 53, NCPGR 94, SSR 1, SSR 4, TA 18, TR 1, TR 2, and TR 29 had a high-level of observed heterozygosity and low associated fixation index. This indicates that these loci could be associated with the occurrence of higher mutation rates or inbreeding depression (Choudhary *et al*., 2012). The low level of heterozyogosity observed for the majority of the SSR markers are in agreement with other studies (Choudhary *et al*., 2012; Gemechu Keneni, *et al*., 2012b). However, higher level of heterozygosity was also reported for some SSR markers (Choudhary *et al*., 2009; Datta *et al*., 2015; Hajibarat *et al*., 2015; Tsegaye Getahun *et al*., 2021). According to Ghaffari *et al*. (2014), allelic frequency of <0.03 is considered as low, 0.03–0.20 considered as common, and > 0.20 considered as most frequent. Based on this delineation, rare alleles comprised 7.5% (10 alleles) of all the detected alleles while intermediate alleles accounted for 63.9% (85 alleles). The remaining alleles accounted for 28.6% of the allelic frequency (38 alleles).

All of the nine chickpea populations had a high percentage of polymorphism among the populations with the range of 95.7% to 100% and average of 99.5%. Comparable values of Shannon's Information Index were recorded for all populations. A relatively high number of alleles, effective alleles, and Shannon's information index were recorded in East Gojjam 2, which implies that chickpea genotypes from East Gojjam 2 are more diverse than the remaining chickpea collection of other geographic regions. The low-levels of private alleles were recorded in East Gojjam 2, North Shewa, North Wollo and Arsi Bale. Matus and Hayes (2012) suggested that the occurrence of unique alleles could be an indication of the relatively high rate of mutation and diversity at SSR loci. The occurrence of unique or rare alleles has the potential to serve as a source of novel alleles for plant breeding and also provides an immense opportunity for generation of comprehensive fingerprint database for establishing genotype identity (Bharadwaj *et al*., 2010). The percentage of polymorphism among Ethiopian chickpea populations discovered by Gemechu Keneni *et al*. (2012b) and Tsegaye Getahun *et al*. (2021) were lower than the present finding. The differences in values for estimated genetic diversity parameters between studies may be explained by the different types and numbers of genotypes, different numbers and types of loci examined and perhaps the nature of markers used in each study.

AMOVA results indicate much of the variation was accounted for by the variation within population, probably undertake high seed exchanges, rather than among populations, suggesting that individual variation was more important for chickpea breeding programs. The low-level of molecular variation among population indicates that the presence of a high number of shared alleles among populations collected from different origins (Mulugeta Atnaf *et al*., 2017). The exotic genotypes contributed 7.6% to the total molecular variation which could provide an opportunity to expand the chickpea gene pool of Ethiopian origin, if there is no complete replacement of local germplasm with the improved one. A low-level of molecular variation among chickpea populations were also reported from Gemechu Keneni *et al*. (2012b) and Tsegaye Getahun *et al*. (2021) for Ethiopian genotypes and Valadez-Moctezuma *et al*. (2020) for Mexican chickpea. According to the combination of Fst rating by Wright (1977), Fst value of 0.00 to 0.05 indicates low, 0.05–0.15 indicates moderate, 0.15–0.25 indicates high, and >0.25 indicates a very high-level of differentiation. Based on this delineation, the Fst score for the present study could be rated as low to moderate level of differentiation among populations with an increased level of admixtures which is the possible reason for the existence of the low-level of molecular variation among populations. Similar observation was made in cowpea (Selamawit Ketema *et al*., 2020). The lower level of variation among populations might be attributed to germplasm exchange among regions and this is further confirmed from the result of pairwise gene flow (Nm) values among populations which were scored within the range of 1.12 to 4.87 exhibiting gene exchange among populations. The Nm value greater than 1 is considered an indicator of adequate gene flow among populations (Wright, 1931).

The genetic distance results showed that the genetic distance between each of the Ethiopian populations (eight populations) and the exotic population was higher than any pair of combinations within Ethiopian populations. This indicates that the genetic similarity between the exotic genotype and the Ethiopian populations is low, implying that Ethiopian populations are distantly related to exotic genotypes. However, close distance was estimated among Ethiopian populations collected from different regions, indicating that the highest genetic similarity was existed among Ethiopian chickpea genotypes. These results are in agreement with findings from Gemechu Keneni *et al.* (2012b) and Tsegaye Getahun *et al*. (2021). In addition, UPGMA dendrogram tree of nine chickpea populations based on origins showed tendencies to be grouped together which indicates that the patterns of genetic relationships are among proximity areas of collections.

PCoA result indicates that the Ethiopian genotypes were uniformly distributed in the four quadrants regardless of their geographic origin, while the exotic genotypes were grouped in quadrant I forming sub-clusters which are distinct from the local genotypes. Genotypes from East Gojjam 2, North Shewa, and Arsi-Bale were highly diverse because they were evenly distributed in the three quadrants regardless of their geographic origins. However, some Ethiopian genotypes and the exotic genotypes appeared to follow geographic origins from which the genotypes were obtained. This result is supported by earlier studies using SSR markers (Bharadwaj *et al*., 2011; Choudhary *et al*., 2012; Gemechu Keneni, *et al*., 2012b; Tsegaye Getahun *et al*., 2021). The distinct identity of the exotic genotypes could be a consequence of deliberate selection criteria followed by the breeders in the development of these varieties (Choudhary *et al*., 2012).

The dendrogram tree constructed using the UPGMA clustering algorithm, clearly delineated the chickpea genotypes into two major clusters, Cluster I and Cluster II. Cluster II was sub-divided into six sub-clusters, each consisting of variable number of genotypes. The exotic genotypes were grouped in single cluster. Results generated from dendrogram were also in agreement with those of the PCoA result. The patterns of genotypes clustering based on their geographic region were not consistent meaning that the distribution of genotypes of similar origin into different clusters. This indicates that the existence of genotypes diversity within the populations of origin. This situation implies no parallelism between genetic diversity and geographical distribution. Similar trends were reported by earlier works in chickpea (Choudhary *et al*., 2009; Valadez-Moctezuma *et al*., 2020). The most probable reason could be seed exchange, and/or trade between farmers, leading to gene flow across boundaries within those areas. The dendrogram did not indicate any clear divisions between desi and kabuli type chickpea in the exotic genotypes. This may be due to the fact that markers used for this experiment were not directly related with the characteristics that differentiate kabuli from desi type chickpea (Valadez-Moctezuma *et al*., 2020). However, various authors have reported that the clustering of chickpea genotypes appears to follow geographic distribution from where these germplasm lines were obtained (Bharadwaj *et al*., 2011; Ghaffari *et al*., 2014; Agarwal *et al*., 2015; Hajibarat *et al*., 2015). Tadesse Sefera *et al*. (2011) and Tsegaye Getahun *et al*. (2021) showed SSR markers had the potential in discriminating kabuli genotypes with that of desi genotype.

Applications of model-based clustering methods in the STRUCTURE software is helpful to demonstrate the presence of population structure, identify distinct genetic populations, assign individuals to populations, and identifies admixed individuals (Pritchard *et al.*, 2000). In the present study, a structured population in chickpea was revealed, and was divided into two groups. The analysis of population structure revealed similarity with the results obtained from UPGMA clustering. The chickpea genotypes used for this study evolved from two population types showing varying degrees of introgression of the two types into respective genotypes. Structure is considered to be uniform when more than 80% of the accessions in one group have more than 80% membership of the group (Pritchard *et al.*, 2000; Choudhary *et al.*, 2012). There were no genotypes showing uniform structure with 100% membership in their cluster, indicating that the existence of gene flow or introgression was apparent. Gene introgression is critical for breeders for variety development programs because it provides essential trait combinations such as improved agronomic features, high resilience to environmental challenges, diseases, and insects, as well as other benefits such as improved nutritional quality (Choudhary *et al.*, 2012). It is also applicable to broaden the genetic base of chickpea genotypes through crossing programs.

3.6. Conclusions and recommendations

The magnitude and pattern of genetic variation was estimated, which indicated that a considerable genetic diversity existed in Ethiopia chickpea genotypes. The results also further confirmed the efficiency and effectiveness of SSR markers to study genetic diversity in chickpea. This result will have a direct applicability for efficient and systematic conservation and sustainable utilization of germplasm. For example, conservation strategy should focus on individual accessions than population origin or subpopulation, because the molecular variations within populations were by far higher than among populations. This result can also assist chickpea breeders in selecting diverse parental materials for crossing activities to take the advantage of heterosis value. The results are also helpful for gene bank managers because there are large numbers of genotypes clustering in one group collected from the same locality and it seems that these genotypes are duplicated genotypes which are the major problems in germplasm conservations. To reduce the high amount of redundancy in germplasm collections, techniques including deliberate bulking and the establishment of core collections must be implemented. Though this work provided preliminary information regarding the existences of genetic diversity, studies related to marker traits association are required. Therefore, a comprehensive study to map the associations of the markers with agronomic traits of economic importance is highly required.

Chapter 4

4. Evaluation of Chickpea (*Cicer arietinum* L.) Genotypes for Cold Tolerance under Natural Condition in Ethiopia

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4.1. Abstract

Cold stress is one of the most significant abiotic factors affecting chickpea (*Cicer arietinum* L.) production in the Ethiopian highlands. To investigate the cold tolerance of chickpea, 673 genotypes (Ethiopian accession, improved varieties and ICARDA genotypes) were characterized using an augmented design at Bakelo, Debre Birhan, Ethiopia for two years. A significant ($p<0.01$) variability amongst genotypes was recorded for all agronomic traits considered in response to cold stress. Ninety four genotypes better performing over the cold susceptible genotypes were identified for agronomic traits. Stem/leaf pigmented genotypes showed a better reaction to cold stress than non-pigmented genotypes. The majority of black seeded chickpea adapted well under cold stress when compared to brown and white seeded genotypes. According to the freezing tolerance rate (FTR) and plant survival rate (SR), 83 (12.3%) and 85 (12.6%) genotypes were identified as cold tolerant. There was a strong correlation ($p<0.01$) in grain yield with FTR, SR, seed shriveling score, stem/leaf pigmentation and seed color. Based on our findings, Ethiopian chickpea genotypes have a good genetic potential for cold resistance traits for use in future breeding programs.

Key words: chickpea; Ethiopian landraces; frost survival rate; frost tolerance rate; germplasm characterization

4.2. Introduction

Chickpea (*Cicer arietinum* L.) cultivation and utilization are profoundly notable within Ethiopian culture and produced by smallholder farmers under rain fed condition (Setotaw Ferede *et al*., 2018). The chickpea production in Ethiopia is one of the most widespread legumes in terms of both area and volume. Across Ethiopia chickpea cultivation occupies 239,786.13 hectares of land annually with an estimated production of 4,5917,318.7 kg (CSA, 2019). Both the land dedicated to chickpea production and the volume of production itself has been increasing over the last decade in Ethiopia (Asnake Fikre and Dagnachew Bekele, 2020; Asnake Fikre *et al*., 2018). Ethiopia is thus the largest producer, consumer, and exporter of chickpea in Africa, and is among the top ten most vital chickpea producers in the world (FAO, 2020).

Chickpea production is suited to areas having vertisol-dominated soil with an altitudinal range of 1400 to 2300 meters above sea level (Geletu Bejiga *et al*., 1996). Nevertheless, it is cultivated across a wide range of productions zones (Asnake Fikre *et al*., 2018). Moreover, Ethiopia is considered to be the secondary center of diversity hotspot of chickpea amongst major chickpea growing countries (Van der Maesen, 1987). Taking both the immense variability among the chickpea germplasm and many agroecological zones as well as the increased demand for animal feed and processed foods into consideration (Asnake Fikre *et al*., 2020; Muoni *et al*., 2019; Bekele Shiferaw and Hailemariam Teklewold, 2007), Ethiopia features great potential to expand chickpea production into '*dega*' agroecological zones, where frost is very common and with an altitudinal range of 2,300 and 3,200 meters above sea level based on traditional agroecological divisions (Dereje Gorfu and Eshetu Ahmed, 2003). Moreover, chickpea productivity can be increased also in some '*Woina Dega'* areas (1,500-2,300 m.a.s.l.) where its production constrained by cold stress that occurs during the late vegetative and reproductive stages (personal communications)if chickpea varieties that are resistance to cold stress could be identified.

Chickpea is important for Ethiopian highland cultivation and is preferably sown in early- to mid-September. Previously, mid-August was considered the appropriate sowing date, but due to the "*belg"* rainy season, chickpea cultivation was heavily impacted by root rot problem. Root rot issues can be avoided by planting in mid-September, leading to higher yields. However, the late sowing presents a new phenomenon, due to the elevation of highlands, which is cold stress. The cold stress takes place late in the podding and flowering stages. Cold stress during these stages causes developmental problems such as flower abortion, poor pod set, and impaired pod filling, leading to a drastic reduction in yield and quality (Croser *et al*., 2003). These stressors can be classified as chilling (0^{o}C to 12^{o}C) or freezing/frost (<0^{o}C) temperatures (Gogoi *et al*., 2018; Toker *et al*., 2007). Moreover, temperatures lower than 10°C at flowering can reduce grain yield by 15–20% (Chaturvedi *et al*., 2009). Therefore, the need for improving cold tolerance in chickpea has become evident which requires characterization of chickpea germplasm for cold tolerance.

Determining the nature of genetic diversity and variability existing among chickpea genotypes for cold resistance is mandatory to identify promising genotypes that are productive in Ethiopian highlands with late sowing dates. However, few studies have been conducted so far in this regard. Hence, research is needed to further understand the optimal utilization of landraces as sources of novel traits for cold resistant chickpea varietal development. Therefore, the aim of this research is to identify chickpea genotypes that are both highly productive and cold resistant through use of field screening of genotypes for cold tolerance. The long-term goal is to establish highly productive and cold tolerant chickpea varieties supporting Ethiopian highland farmers to enhance food security and improve rural livelihoods.

3.1 Material and methods

4.3.1. Experimental Site

The experiment was conducted at Bakelo, Debre Birhan Agricultural Research Center experimental site (Debre Birhan, Ethiopia) for two consecutive growing seasons (2018/19 and 2019/20). The experimental site is located 147 km away from Addis Ababa at N 09°41'42" latitude and E 39°37'20" longitude. Its altitude is 2,837 meter above sea level and receives an annual mean precipitation of 965.25 mm. The temperature ranges from 6.5°C to 20.1°C with mean annual temperature of 13.3°C. The dominant soil type of Bakelo is black vertisol. The daily minimum and maximum temperature values are indicated in Figure 6.

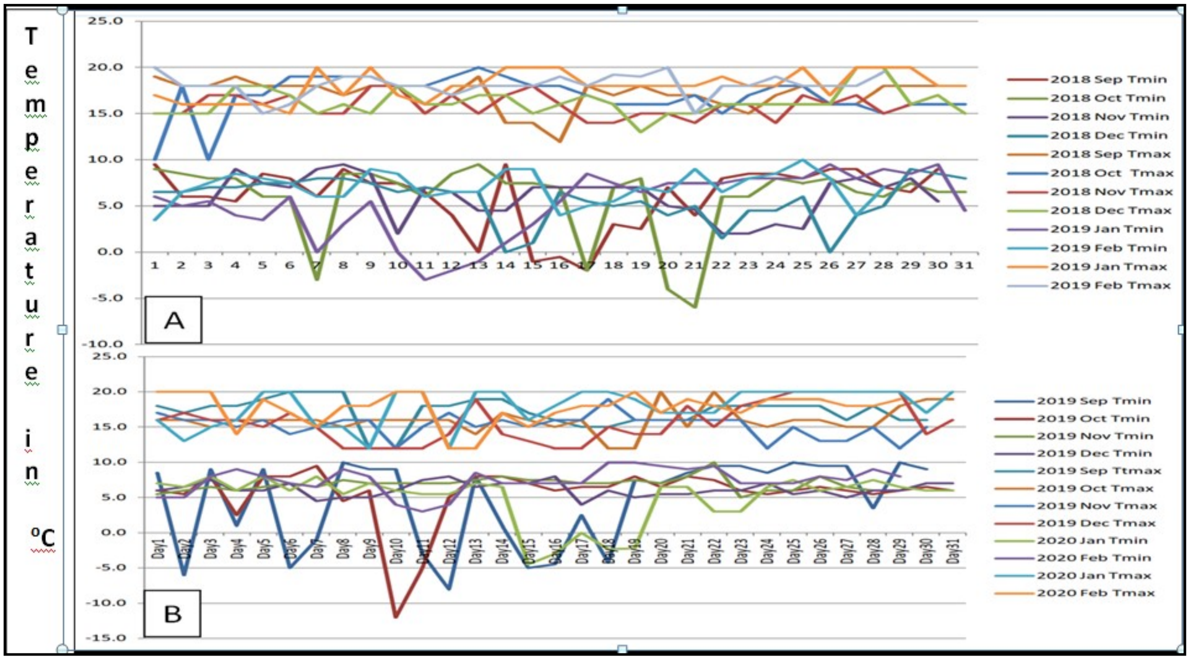

Figure 6. Daily maximum and minimum temperature of Bakelo, Debre Birhan during 2018/2019 (A) to 2019/2020 (B) growing seasons (Source: Debre Birhan Agricultural Research Center)

4.3.2. Plant Materials

A total of 673 genotypes of which 559 (83%) Ethiopian genotypes from the Ethiopian Biodiversity Institute (EBI), 83 (12%) elite cold resistant genotypes from the International Center for Agricultural Research in the Dry Areas (ICARDA), 28 (4%) improved chickpea varieties originated from imported germplasm lines and released from Ethiopian Agricultural Research Centers, and three (0.1%) susceptible local checks were screened for their tolerance against cold stress under field condition at Bakelo, Debre Birhan, Ethiopia, which is a cold prone area, (national and regional cold resistance screening site) (see Appendix 1 for further details). The parameters used were freezing tolerance rate, plant survival rate and other cold resistant-related agronomic traits. The geographical origin of the Ethiopian chickpea germplasm used in the study is indicated in Figure 7.

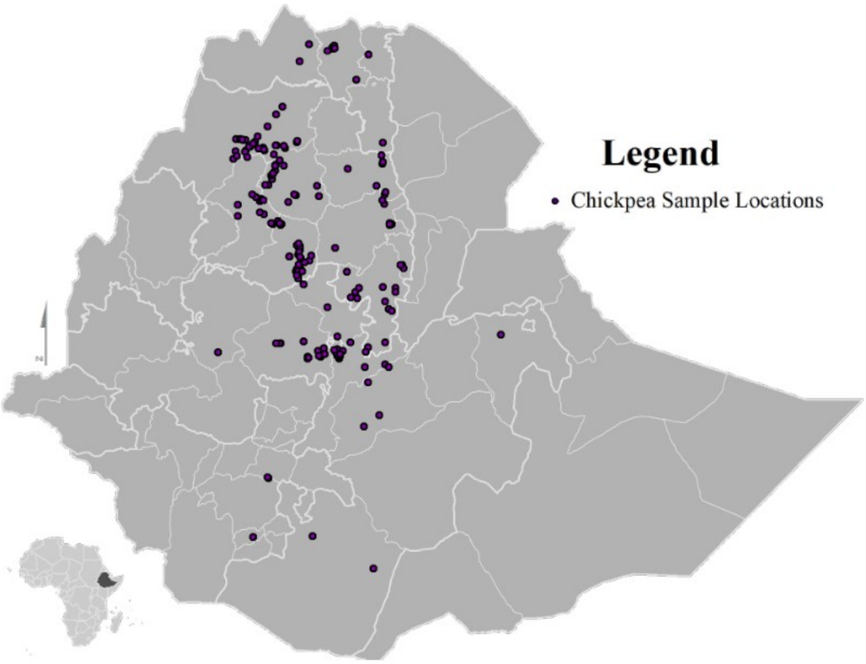

Figure 7. Map showing the geographical distribution of Ethiopian chickpea germplasm

4.3.3. Experimental Design

The trial was arranged in augmented design. The tested genotypes were sown without replications, while the checks were replicated 10 times. Each of the genotypes was sown in two rows with 3 m row length and 0.2 m spacing between rows and 0.1 m between plants. Diammonium phosphate fertilizer (100 $kgha^{-1}$) and other appropriate management practices were applied. Five individual plants were tagged randomly from each genotype per plot and they were used for morphological data collection. Recording agronomic characteristics were conducted following the procedure described by chickpea descriptor (IBPGR, ICRISAT and ICARDA 1993).

4.3.4. Data Collection

Qualitative and quantitative morphological traits were recorded as per the description in Table 10

Table 10. List of qualitative and quantitative characters recorded, their codes and descriptions

Characters	Description
Qualitative traits	
Stem/Foliage Pigmentation (SLP)	0=No Anthocyanin, 1=Low Anthocyanin 2=Medium Anthocyanin3=High Anthocyanin
Seed Color (SC)	1=Black, 2=Brown, 3=White
Flower Color (FC)	0=White, 1=Pink
Quantitative traits	
Plant Height (cm) (PLH)	Average canopy height of five representative plants taken at maturity stage
Days to 50 % Flowering (DTF)	Number of days from sowing until 50 % of the plants have started to flower
Days to 50 % Podding (DTP)	Number of days from sowing until 50 % of the plants have started to podding
Days to 90 % Maturity (DTM)	Number of days from sowing until 90 % of the pods have matured and turned yellow
Number of Primary Branches (NPB)	Average number of basal primary branches per plant taken from five representative plants
Number Secondary Branches (NSB)	Average number of secondary branches per plant taken from five representative plants
Number of Fertile Pods per Plant (FPPP)	Average number of fertile pods taken from five representative plants taken at maturity stage
Number of Infertile Pods per Plant (IPPP)	Average number of infertile pods taken from five representative plants taken at maturity stage
Thousand Seed weight (TSW)	Thousand seeds were counted and weighted at 12 % moisture content on a 0.1 g sensitive balance in milligram
Grain Yield (GY in kg ha^{-1})	Dried weight (kg) of seed per plot at 12 % moisture content
*Freezing tolerance rate (FTR)	Scored on 1-9 scale bases (Singh *et al.*, 1989): where, 1= No visible symptoms of damage; 2= Highly tolerant, up to 10 % leaflets show damage; 3= Tolerant, 11-20 % leaflets show damage; 4= Moderately tolerant, 21-30 % leaflets and up to 20 % branches show withering and drying, but no killing; 5= Intermediate, 41-60 % of leaflets and 21-40 % branches show withering and drying, up to 5 % plant killing; 6= Moderately susceptible, 61-80 % leaflets and from 41-60 % branches show withering and drying, 6-25 % plant killing; 7= Susceptible, 81-99 % leaflets and 41-80 % branches show withering and drying, 26-50% plant killing; 8= Highly susceptible, 100% leaflets and 81-99 % branches show withering and drying, 51-99 % plant killing; and 9=100 % plant killing
Plant survival rate (SR)	Calculated by dividing the number of surviving plants after the frost period by the number of emerged plants after sowing was calculated (Heidarvand *et al.*, 2011)
Seed shriveling score (SSS)	Visual measurement and estimating the kernel's condition (1= plump, 3=intermediate and 5=shriveled

*= Frost score was recorded when susceptible checks showed sign for frost damages or completely died.

4.3.5. Data Analysis

The data collected for each trait were subjected to statistical analysis of variance using augmentedRCBD R Packages version 0.1.3 (Aravind *et al.*, 2020). The analysis helps us to partition the variance into different sources (phenotypic, genotypic and environmental variance) and genetic parameters to see if the difference among genotypes is statistically significant or not for each trait considered (Singh and Chaudhary, 1977). Pearson correlation coefficients between variable was estimated and tested for significance using MINITAB 10 statistical package (MINITAB, 1998).

4.4. Result

4.4.1. The Effect of Cold Stress Agronomic Traits

The combined ANOVA analysis of the two seasons (2018/19 and 2019/20) gave high value of the error term coefficient for recorded traits because the intensity of cold stress differed between the two seasons. So, individual ANOVA analysis was performed for the two seasons separately. There was a significant difference ($p<0.01$) among genotypes for plant canopy height, number of primary branches, number of secondary branches, fertile pods per plant, infertile pods per plant, days to 50% flowering, days to 50% podding, days to 90% maturity, thousand seed weight, and grain yield (Table 11). The Fisher's least significant difference (LSD) result indicated that there was a significant difference ($p<0.05$) among genotypes for the mean value of agronomic traits examined in this study. The wide ranges of mean values were recorded for the traits examined. The LSD means and range of values of the traits for chickpea genotypes examined is presented in Appendix 2 for further details.

4.4.1.1. Seedling and Vegetative Stage

The cold stress that occurred in both seasons and genotypes had shown uniform germination and seedling establishment (Figure 8A). However, during the vegetative stage, 60 (8.9 %) genotypes (One improved variety, Eight EBI genotypes and 51 ICARDA genotypes) were killed by cold stress (Figure 8B) in both growing seasons. The lists of genotypes killed by cold stress are shown in Appendix 3.

4.4.1.2. Number of Branches and Plant Height

The number of primary and secondary branches has been significantly affected by cold stress in both seasons where a wide range was recorded. The range of number of primary branches was 0 to 16.1 in 2018/2019 and 0 to 27.2 in 2019/2020 growing season and for number of secondary branches it was 0 to 25.6 in 2018 and 0 to 46.5 in 2019. The majority of the genotypes scored below five for primary and secondary branches in both growing seasons. However, 69 (10.3%) and 71 (10.6%) genotypes produced better number of primary branch (>7) in 2018/2019 and 2019/2020 growing seasons, respectively. The response of genotypes to the effect of cold stress for plant height development was variable. A wide range of plant height was observed in both cropping seasons (20.3 to 58 cm in 2018/2019 and 17.2 to 57 cm in 2019/2020). One hundred two (15.2%) and 89 (13.2%) genotypes had a record of less than 35 cm plant height in 2018/2019 and 2019/2020 cropping season, respectively. Genotypes 132663 (58 cm) and 140294 (57.04 cm) had shown better plant height.

4.4.1.3. Reproductive Stages

Seventeen genotypes (2.5%) (Seven EBI genotypes and 10 from ICARDA) were killed by cold stress during reproductive stages (Figure 8C and 8D). The range recorded for days to flower, days to pod and days to mature for 2018/2019 growing season were from 47.7 to 87.54, 54.2 to 89.6 and 118.7 to 160, respectively, while for 2019/2020 cropping season, the mean ranges recorded were from 48 to 77.7, 55 to 99.6 and 99.9 to 171.2, respectively. The range of fertile pods per plant was 0 to 237.5 and 0 to 162.7 for 2018/12019 and 2019/2020 cropping seasons, respectively. The range of infertile pods per plant was 0 to 77.3 and 0 to 116 for 2018/2019 and

2019/2020 cropping seasons, respectively. The genotypes 227152-A (237.5) and 41301-B (162.7) produced the highest number of fertile pods in 2018/2019 and 2019/2020 cropping seasons, respectively (Figure 8F).

4.4.1.4. Thousand Seed Weight and Grain Yield

The tested genotypes had wide mean performances for thousand seed weight and grain yield in both seasons. Most genotypes produced shrived seed (Figure 9). The range of 0 g to 300 g and 0 $kgha^{-1}$ to 2,531 $kgha^{-1}$ were recorded for thousand seed weight and grain yield for 2018/2019 cropping season respectively, while for 2019/2020 cropping season the range was, 0 to 297 g and 0 $kgha^{-1}$ to 2,604 $kgha^{-1}$, respectively.

4.4.1.5. Seed Color

The majority of the cold susceptible genotypes showed a faded seed color. Some of the genotypes had shown plumped seed with faded seed color.

Table 11. Mean square and mean for the tested traits of 673 (562 EBI genotypes, 83exotic and 28 improved chickpea) genotypes grown at Bakelo, Debre Birhan, Ethiopia grown in 2018/2019 (I) and2019/2020(II)

Sources of Variation	**Degree of freedom**	**Type III Mean Squares**									
		PLH	NPB	NSB	FPPP	IPPP	DTF	DTP	DTM	TSW	GY
(I)											
Block	9	0.6^{ns}	0.3^{ns}	1.6^{ns}	168.3^{ns}	35.9^{ns}	4.55^{ns}	14.9^{ns}	3.51^{ns}	5.0^{ns}	7224^{ns}
Treatment	612	23.5^{***}	2.2^{***}	4.9^{***}	429.4^{***}	47.6^{*}	8.15^{*}	17.4^{*}	28.57^{*}	814.8^{***}	154550^{***}
Treatment: check	2	0.99^{ns}	0.3^{ns}	0.6^{ns}	35.3^{ns}	277^{***}	41.6^{**}	61.9^{**}	21.43^{ns}	13.1^{ns}	174411^{***}
Treatment: test and test vs. Check	610	24***	2.2^{***}	4.9^{*}	430.7^{***}	46.8^{*}	4.04^{*}	17.2^{*}	28.59^{*}	817.4^{***}	154485^{***}
Residuals	18	0.78	0.43	0.53	84.8	21.6	4.82	7.8	11.59	6.8	8976
CV		2.32	13.32	20.06	22.88	41.34	4.16	4.57	2.56	3.07	8.72
Mean		38.35	4.89	3.65	40.31	10.9	52.72	61.22	133.0	87.11	1118.9
(II)											
Block	9	8.74	2.29^{ns}	0.9^{ns}	295^{ns}	59.6^{ns}	2.1^{ns}	34.4^{ns}	50^{ns}	10^{ns}	7435^{ns}
Treatment	612	38.4^{***}	2.2^{*}	3.6^{*}	513^{**}	180^{***}	22.5^{***}	29.45^{*}	49.13^{*}	1177^{***}	253659^{***}
Treatment: check	2	8.59^{ns}	0.8^{ns}	1.8^{ns}	211^{ns}	27^{ns}	7.03^{ns}	$103.^{**}$	65.1^{ns}	75.8^{*}	65909^{**}
Treatment: test and test vs. Check	610	38.5^{***}	2.2^{*}	3.6^{*}	514^{**}	180^{***}	22.6^{***}	29.21^{*}	49.08^{*}	1180^{***}	254275^{***}
Residuals	18	2.57	1.38	2.56	184	26.3	5	14.49	49.36	16.7	9573
CV (%)		3.89	21.47	41.33	25.47	16.0	4.09	5.29	5.36	6.78	14.0

Table 11: Continued…

Mean	41.39	5.49	3.87	53.76	31.75	54.72	72.19	130.66	61.39	720.0

Symbols for level of significance:‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05, ns is none significant, PLH=Plant Canopy Height (cm), NPB=Number of primary branches, NSB=Number secondary branches, FPPP=Fertile pods per plant, IPPP=Infertile pods per plant, DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, TSW=Thousand seed weight, and GY =Grain yield in kg ha^{-1}

Figure 8. Cold response in chickpea at different growing stages: chickpea genotypes seedling coverage (A), plant death during pre-flowering stage (B), reduced pod setting (C and D) and better pod setting (E and F)

4.4.2. Plant Survival Rate (SR)

Cold tolerance was assessed using plant survival rate (SR) for 673 diverse chickpea germplasms for two growing seasons under field condition (Table 12). It was observed that the SR values ranged from 0.0 (60 genotypes) to 0.86 (genotypes 16341-A, 24159-C and 30290-A) and 0.0 (60 genotypes) to 0.87 (genotype 41167-C) for 2018/2019 and 2019/2020 growing seasons, respectively. One hundred fifty seven and 87 chickpea genotypes had shown above 0.8 SR score, while the remaining 516 and 586 genotypes were below 0.8 SR score for 2018/2019 and 2019/2020 growing seasons, respectively. Eighty five genotypes consistently showed SR score value above 0.8 in both growing seasons.

Table 12. Frost survival rate (SR) of 562 Ethiopian chickpea, 83 exotic and 28 improved chickpea genotypes tested at Bakelo, Debre Birhan, Ethiopia, 2018 to 2020 growing seasons

No	SR Rating	2018/2019		2019/2020		Common genotypes for both years	
		No of Genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	>0.8	157	23.3	87	12.9	85	19.6
2	>0.6 to <0.8	273	40.6	199	29.6	155	35.8
3	>0.4- <0.6	136	20.2	213	31.7	96	22.2
4	>0.2- <0.4	33	4.9	60	8.9	23	5.3
5	<0.2	74	11.0	114	16.9	74	17.1
Total	673		673		433		

4.4.3 Freezing Tolerance Rate (FTR)

Based on FTR, 169 (1-3 score) and 84 (2-3 score) genotypes were described as tolerant to highly tolerant, while 504 (4-9) and 590 (4-9) were described as moderately tolerant to highly susceptible genotypes during 2018/2019 and 2019/2020 growing seasons, respectively (Table 13). Eighty three genotypes were rated within the score of 1-3 consistently in both growing season.

Table 13. Freezing tolerance rate (FTR) of 673 (562 Ethiopian chickpea, 83 exotic and 28 improved) chickpea genotypes tested at Bakelo, Debre Birhan, Ethiopia from 2018 to 2020 growing seasons

No	FTR Rating	2018/2019		2019/2020		Common genotypes for both years	
		No of Genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	1	27	4.0	0	0		
2	2	32	4.8	27	4.0		
3	3	110	16.3	57	8.5		
Sub Total		169	25.1	84	12.5	83	15.5
4	4	261	38.8	154	22.9		
5	5	82	12.2	131	19.5		
6	6	50	7.4	118	17.5		
Sub Total		393	58.4	403	59.9	341	63.9
7	7	29	4.3	57	8.5		
8	8	20	3.0	25	3.7		
9	9	62	9.2	104	15.5		
Sub Total		111	16.5	186	27.6		
Grand Total		673		673		424	

4.4.4. Seed Shriveling Score (SSS)

The visual shriveling seed score of the genotypes were indicated in Table 14. Two hundred seventy four (40.7%) and 389 (57.8%) genotypes developed shriveled seeds (Figure 9A, 9B and 9C), while 399 (59.3%) and 2840 (42.2%) genotypes produced medium to high plumped seeds (Score of 1-3: Figure 9D, 9E and 9F) in 2018/2019 and 2019/2020 cropping seasons, respectively. About 108 (16%) genotypes gave plumped seeds consistently in both seasons.

Table 14. Seed shriveling score (1-5) of 673 (562 Ethiopian chickpea, 83 exotic and 28 improved chickpea) genotypes tested at Bakelo, Debre Birhan, Ethiopia from 2018 to 2020 growing seasons

No	**SSS Rate**	**2018/2019**		**2019/2020**		**Common genotypes for both years**	
		No of genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	1	145	21.6	47	7.0	33	12.6
2	2	128	19.0	83	12.3	33	12.6
3	3	126	18.7	154	22.9	42	16.0
Sub Total	-	**399**	**59.3**	**284**	**42.2**	**108**	**16**
4	4	177	26.3	194	28.8	78	29.8
5	5	97	14.4	195	29.0	76	29.0
Sub Total	-	**274**	**40.7**	**389**	**57.8**	**154**	**22.9**
Total		**673**	**-**	**673**	**-**	-	-

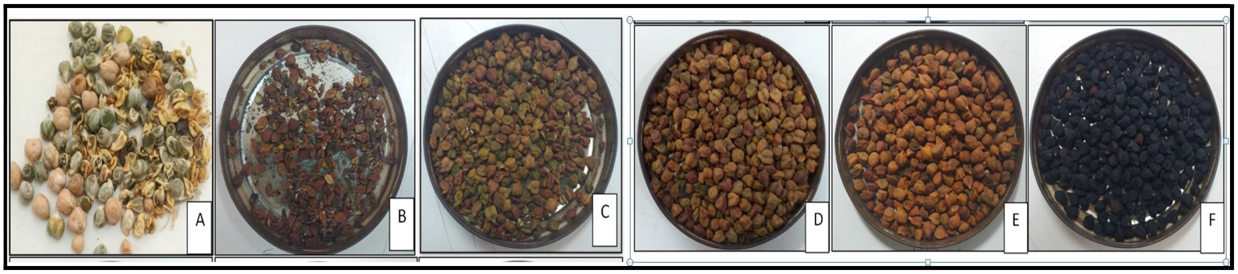

Figure 9. Seeds of chickpea genotypes showing different reaction to cold stress (A and B are very shriveled (Score of 5), C is Shriveled (Score of 4), D is intermediate (Score of 3), E is medium plumped (Score of 2) and F is plumped (Score of 1)

4.4.5 Phenotypic Correlation Coefficient

The phenotypic association of agronomic and cold tolerance related traits was analyzed for each genotype and results were obtained as shown in Table 15. Most of the cold tolerance related traits have shown a strong significant relationship with agronomic traits. Grain yield was positively and significantly correlated (p<0.01) with fertile pod per plant (0.33 and 0.21), thousand seed weight (0.69 and 0.72), SR (0.86 and 0.73), and stem/leaf pigmentation (0.59 and 0.48), while a strong negative correlation was seen for infertile pod per plant (-0.7 and -0.6), FTR (-0.70 and -0.6), SSS (-0.8 and -0.8), seed color (-0.52 and -0.30), and flower color (-0.43 and -0.21) for 2018/2019 and 2019/2020 growing seasons, respectively.

Table 15. Phenotypic Pearson's correlation matrix for 9 traits in chickpea 673 (562 Ethiopian chickpea, 83 exotic and 28 improved chickpea) genotypes tested at Bakelo, Debre Birhan, Ethiopia from 2018/2019 (above diagonal) to 2019/2020 (below diagonal) growing seasons

	PLH	NPB	NSB	FPPP	IPPP	DTF	DTM	TSW	GY	SR	FTR	SSS	FC	SLP	SC
PLH	0	0.65^{**}	0.43^{**}	0.51^{**}	-0.3^{**}	0.13^{**}	0.13^{**}	0.64^{**}	0.59^{**}	0.68^{**}	-0.66^{**}	-0.48^{**}	-0.52^{**}	0.36^{**}	-044^{**}
NPB	0.64^{**}	0	0.71^{**}	0.68^{**}	0.01^{**}	0.12^{**}	0.12^{**}	0.35^{**}	0.39^{**}	0.43^{**}	-0.40**	-0.31^{**}	0.41^{**}	0.28^{**}	-0.33^{**}
NSB	0.47^{**}	0.68^{**}	0	0.69^{**}	0.04^{ns}	0.13^{**}	0.19^{**}	0.18^{**}	0.23^{**}	0.25^{**}	-0.25^{**}	-0.18^{**}	0.20^{**}	0.16^{**}	-0.17^{**}
FPPP	0.52^{**}	0.69^{**}	0.69^{**}	0	-0.0^{ns}	-0.1^{ns}	0.12^{**}	0.28^{**}	0.33^{**}	0.37^{**}	-0.36^{**}	-0.3^{**}	0.28^{ns}	0.22^{**}	-0.26^{**}
IPPP	-0.2^{**}	0.17^{**}	0.19^{**}	0.22^{**}	0	0.08^{*}	0.06^{ns}	-0.4^{**}	-0.7^{**}	-0.6^{**}	0.56^{**}	0.70^{**}	0.1^{ns}	-0.3^{**}	0.11^{**}
DTF	0.01^{ns}	0.06^{ns}	0.15^{**}	-0.2^{**}	-0.1^{ns}		0.28^{**}	-0.0^{ns}	-0.2^{**}	-0.2^{**}	0.16^{**}	0.15^{**}	-0.4^{**}	-0.2^{**}	0.30^{**}
DTM	0.02^{ns}	0.00^{ns}	0.06^{ns}	-0.2^{**}	-0.1^{ns}	0.30^{**}	0	-0.0^{ns}	-0.08^{*}	-0.08^{*}	0.03^{ns}	0.01^{ns}	-0.2^{**}	-0.1^{ns}	0.11^{**}
TSW	0.47^{**}	0.24^{**}	0.22^{**}	0.20^{**}	-0.5^{**}	0.14^{**}	0.13^{**}	0	0.69^{**}	0.77^{**}	-0.76^{**}	-0.60^{**}	-0.27^{**}	0.32^{**}	-0.27^{**}
GY	0.42^{**}	0.20^{**}	0.18^{**}	0.21^{**}	-0.6^{**}	0.0^{ns}	0.00^{ns}	0.72^{**}	0	0.86^{**}	-0.84^{**}	-0.8^{**}	0.43^{**}	0.59^{**}	-0.52^{**}
SR	0.65^{**}	0.4^{**}	0.3^{**}	0.38^{**}	-0.4^{**}	0.0^{ns}	-0.1^{**}	0.66^{**}	0.73^{*}	0	-0.90^{**}	-0.79^{**}	0.41^{**}	0.47^{**}	-0.47^{**}
FTR	-0.6^{**}	-0.36^{**}	-0.3^{**}	-0.3^{**}	0.44^{**}	-0.1^{ns}	0.09^{*}	-0.6^{**}	-0.7^{**}	-0.9^{**}	0	0.77^{**}	-0.37^{**}	-0.6^{**}	0.53^{**}
SSS	-0.5^{**}	-0.18^{**}	-0.2^{**}	-0.2^{**}	0.54^{**}	-0.1^{**}	-0.1^{ns}	-0.8^{**}	-0.8^{**}	-0.8^{**}	0.75^{**}	0	-0.32^{ns}	-0.5^{**}	0.44^{**}
FC	0.57^{**}	0.46^{ns}	0.25^{**}	0.41^{**}	0.22^{**}	-0.4^{**}	-0.2^{**}	0.11^{*}	-0.21^{*}	-0.3^{**}	-0.20^{**}	-0.11^{**}	0	0.57^{**}	-0.79^{**}
SLP	0.43^{**}	0.30^{**}	0.18^{**}	0.31^{**}	-0.4^{**}	-0.2^{**}	-0.1^{ns}	0.37^{**}	0.48^{**}	0.47^{**}	-0.49^{**}	-0.51^{**}	0.60^{**}	0	-0.80^{**}
SC	-0.51^{*}	-0.4^{**}	-0.2^{**}	-0.3^{**}	0.11^{**}	0.30^{**}	0.18^{**}	-0.2^{ns}	-0.3^{**}	-0.5^{**}	0.40^{**}	0.32^{**}	-0.79^{**}	-0.8^{**}	0

^{ns}= non significant; **=Correlation is significant at the 0.01 level (2-tailed); *=Correlation is significant at the 0.05 level (2-tailed), PLH=Plant Canopy Height (cm), NPB=Number of primary branches, NSB=Number secondary branches, FPPP=Fertile pods per plant, IPPP=Infertile pods per plant, DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, TSW=Thousand Seed weight, GY =Grain yield in kg ha^{-1} , SR= Frost survival rate, FTR=Frost tolerance rate, SSS= Seed shriveling score, FC= Flower Color, SLP=Stem/leaf pigmentation, and SC=Seed color

4.5. Discussions

Chickpea is one of the most important crops used for crop rotation in highland areas to bring sustainable production and serves as a source of income for rural Ethiopian farmers. However, cold stress is one of the major production constraints that reduces biological yield and affects quality of seeds in highland areas. To date, a chickpea variety suited for Ethiopian highland production that is resistant to cold stress has yet to be explored. To address this issue, it is crucial to screen chickpea germplasm for cold resistance to identify cold tolerant genotypes. Therefore, in the present study, 673 chickpea genotypes were evaluated in field screening trials. The performances of the chickpea genotypes in response to cold stress were assessed in natural condition and the results obtained are discussed in the following sections.

4.5.1. The Effect of Cold on Agronomic Traits

The individual ANOVA analysis indicated that there were significant differences among genotypes for the recorded traits for both seasons. These differences in performance indicate the existences of variability among genotypes for cold tolerance. Similar finding was reported by Mir *et al*. (2019). The LSD mean values significant differences among genotypes and the mean range value of the traits further confirmed the existence of variable responses to cold stress among genotypes. The responses of genotypes to the effect of cold stresses at each crop stage are discussed below because the genotypes responses to the cold damage were variable at each stage.

4.5.1.1. Seedling and Vegetative Stage

All genotypes performed well in terms of seed germination and seedling establishment. Moreover, the genotypes did not show cold stress symptoms or sustain damage in response to cold stress, even though, the lowest temperature recorded during this stage was -2.0^{o}C in Sept 2018 and -8.0^{o}C in Sept 2019 growing seasons. This means that these genotypes had shown good tolerance to cold stress at seed germination and seedling development stages. However, most authors agree that germination percentage and seedling development are sensitive to cold stress which results in poor crop stand establishment, and reduced seedling vigor with stunted growth (Croser *et al*., 2003; Maphosa *et al*., 2020; Srinivasan *et al*., 1998).

At the vegetative stage, 60 (8.9 %) genotypes were identified as highly susceptible to cold stress because they could not resist the cold stress when the minimum temperature of -6.0°C and -12°C were recorded in Oct 2018 and Oct 2019, respectively**.** These genotypes showed poor growth development, wilting, chlorosis, necrosis and finally death of the whole plant, which was the manifestation of cold stress injury. Similar observations were reported by Croser *et al*. (2003) and Mahajan and Tuteja (2005). The remaining genotypes had shown medium to good reactions to cold stress at vegetative stage because the impact of cold stress at this stage was minimal in both growing seasons.

4.5.1.2. Number of Branches and Plant Height

Cold stress significantly reduced the number of primary, secondary branches and plant heights. In the current study variable responses of genotypes for cold stress were examined. The cold susceptible genotypes produced less number of primary (<5) and secondary branches (5), as well as stunted plant height (< 20cm), while the cold tolerant genotypes gave high number of primary (<5) and secondary branches (5), and medium to high plant height were recorded (>20cm). In this experiment, most genotypes gave good positive reaction for plant height to the cold stress effect though cold stress significantly reduced plant height. This is probability because of the duration of time of cold stress occurrence was not sufficient to trigger a negative impact on plant development. Reduced number of primary and secondary branches, and plant height were observed due to cold stress in Nayyar *et al*. (2005a) and Kumar *et al*. (2010)

4.5.1.3. Reproductive Stages

At reproductive stage, cold stress damages were examined in all the genotype with variable degree of damage. Because, the minimum temperature reordered during reparative stage especially at flowering and podding stages was below 5°C in both seasons which caused flower abortion and pod dropping for genotypes having poor response to cold stress. These cold stress symptoms were observed in most cold susceptible genotypes and they produced either empty pods or pods containing small shriveled seeds. Similar observation was reported by Gogoi *et al.* (2018) stating that temperature falls below 15°C causes flower and pod abortions. Various authors agree that the reproductive stage is more susceptible to cold stress than seedling stages because cold stress negatively affects pollen fertility, pod set, number of aborted flowers, total number of pods per plant, seed number, size and shape, rate and duration of seed filling which consequently reduced biomass and grain yield (Berger *et al.*, 2012; Croser *et al.*, 2003; Gogoi *et al.*, 2018; Kumar *et al.*, 2010; Nayyar *et al.*, 2007; Srinivasan *et al.*, 1999). Low temperature stress during reproductive development is responsible for the induction of flower abscission, pollen sterility, pollen tube distortion, ovule abortion and reduced fruit set leading to reduction in seed yield (Sharma and Nayyar, 2014).

4.5.1.4. Thousand Seed Weight and Grain Yield

Seed development of all genotypes was severely affected by cold stress because minimum temperatures of -3.0°C and -4.5°C were recorded during seed development stage in Jan 2019 and Jan 2020, respectively. The majority of the genotypes produced shrived seeds. Even those genotypes that had better vegetative performances until seed development stage gave shrived seed. The current finding is in line with previous studies. Wu *et al.* (2014) indicated that the prolonged period of chilling range of temperatures (0°C to 12°C) at any phenological stage of development in chickpea has detrimental effects on final seed yield. Low temperature (0°C to 12°C) has negative impact on yield and 15-20% yield loss was estimated and temperature below 15% during flowering leads to flower and pod abortion leading to poor yield (Croser *et al.*, 2003). Cold stress affects the source-sink balance by markedly decreasing the source of assimilates for grain filling which, in turn, reduces potential yield (Maphosa *et al.*, 2020). Chaturvedi *et al.*

(2009) estimated a yield loss of 15-20% associated with low temperature. Low temperature during vegetative stage led to decreased vegetative growth, biomass production and yield in north India (Mir *et al.*, 2019; Singh *et al.*, 1993).

4.5.1.5. Seed Color

The effects of cold stress were examined in seed color development. The susceptible genotypes produced seed with a faded seed color. This indicates that cold stress causes seed size and seed discoloration in chickpea. Similar observation was made for faba bean (Sallam *et al.*, 2015). This happened because cold stress affects the mobilization of plant resources in to seed setting (Croser *et al.*, 2003).

4.5.2. Plant Survival Rate (SR)

A wide range of SR score were recorded for the tested genotypes which indicated that the variable repose of the genotypes to cold stress. The value of SR score for the two growing seasons showed variation because of the different duration and intensity of cold stress, which occurred in different seasons. The cold stress intensity and length of occurrence were more sever in 2019/2020 growing season than in 2018/2019. So, high value of SR was recorded in 2018/2019 than in 2019/2020 growing season. In the experimental site, cold stress occurred consistently throughout the life cycle of the crop's development, and hence, the frost survival score were taken at the end of each crop stages. The fluctuation of minimum temperature of two different growing seasons exhibited a similar pattern of SR value change for all genotypes. Minimum temperature of 2019/2020 growing seasons was lower than that of 2018/2019 growing season clearly indicating that the SR of chickpea is closely associated to the temperature changes. Similar patterns were observed also in field pea (Liu *et al.*, 2017). This approach has been employed to screen cold tolerance in rapeseed/canola (Fiebelkorn and Rahman, 2016) and field pea (Liu *et al.*, 2017).

4.5.3 Freezing Tolerance Rate (FTR)

Freezing tolerance rate with a rating scale of 1-9 has been used for measuring cold stress injury during early vegetative stage or seedling stage in earlier studies (Singh *et al.* 1989). Based on FTR, Eighty three genotypes were rated within the score of 1-3 consistently in both growing season. In this experiment, it is observed that the majority of the genotypes that were resistant at seedling stages failed to resist cold stress that occurred late at reproductive stage. From this result we can conclude that FTR score must be taken throughout crop's developmental stages. Generally, single FTR score may work for areas where cold stress occurs once in the life cycle of the crop stages, however, in areas where, cold stress occurs consistently throughout the life cycle of the crop, FTR should be scored frequently. In addition, genotypes that showed better FTR value gave either shriveled seeds or empty pods. So, FTR cannot be used to evaluate the capacity of cold resistance at reproductive stages and the susceptible genotypes will be overlooked by this approach. FTR is the most important index used for freezing temperature screening of different crops tested at seedling stage (Badeck and Rizza, 2015; Nezami *et al*., 2012; Srinivasan *et al*., 1998; Toker, 2005).

4.5.4. Seed Shriveling Score (SSS)

According to SSS result, genotypes had shown variable response to cold stress and about 108 (16%) genotypes produced acceptable seed size, while the remaining genotypes produced shrived seeds. This result also supported by the report from (Behboudian *et al*., 2001) which indicated that the cold stress that occur during seed development affect the quality and size of chickpea seeds because of the reduced rate and duration of grain filling period (Nayyar *et al*., 2007; Kaur *et al*., 2008).

Visual assessment of seed damage by cold stress was done for all the genotypes for both seasons Genotypes that were rated as cold resistant based on SR and FTR indices failed to produce plumped seeds, which means that all genotypes that had a better SR and FTR score did not produce plumped seed. However, all genotypes that produced plumped seed had a better SR and FTR value. From this result, it is plausible to conclude that SR and FTR indices can indicate cold resistances at seedling or vegetative stages alone. Therefore, SR and FTR indices will not be applicable to screen genotypes for cold resistance at reproductive stage. Visual assessment of cold stress damaged seed has been applicable also to screen faba bean genotypes for cold resistance variability (Henriquez *et al*., 2017).

In general, to select promising cold tolerant genotypes, it is advisable to consider cold tolerance related traits and agronomic traits together. Genotypes that are consistently selected by all the parameters are considered as a promising cold tolerant genotype which can be directly taken by farmers or serve as a breeding material for further crop improvement activities. The selected cold tolerant genotypes will help to stabilize yield and expand the chickpea production areas into Ethiopian highland where chickpea production is not a common practice because of cold stress damage. In this study, 94 (51 black, 29 brown and 14 white) genotypes were selected as cold tolerant, and the remaining genotypes from intermediate to susceptible. The promising cold resistant genotypes were selected with the following criteria i.e. Frost survival rate (>0.75), seed shriveling score (1-2), and freezing tolerance rate (1-4). The selected genotypes are listed in Table 16.

Table 16. List of ninety four cold resistant chickpea genotypes and varieties selected based on SR (>0.75), FTR (score of 1,2,3) and seed score (1 and 2)

No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source
1	16341-A	Black	EBI	33	208994-A	Brown	EBI	65	30293-A	Brown	EBI
2	207674	Black	EBI	34	235036-A	Brown	EBI	66	207739-B	Brown	EBI
3	30336-A	Black	EBI	35	209016-B	Black	EBI	67	71875	Brown	ICARDA

Table 16: Continued…

No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source
4	30336-B	Black	EBI	36	209022-A	Black	EBI	68	75095	Brown	ICARDA
5	41004-C	Black	EBI	37	209026-A	Black	EBI	69	140941	Brown	ICARDA
6	41036-B	Black	EBI	38	212589-B	Black	EBI	70	116451	Brown	ICARDA
7	41051-A	Black	EBI	39	212914-B	Black	EBI	71	126302	Brown	ICARDA
8	41081-A	Black	EBI	40	214731-B	Black	EBI	72	9427	Red	ICARDA
9	41107-B	Black	EBI	41	214734-A	Black	EBI	73	128699	White	ICARDA
10	41133-A	Black	EBI	42	215067-A	Black	EBI	74	9632	White	ICARDA
11	41167-C	Black	EBI	43	215190-A	Black	EBI	75	10163	White	ICARDA
12	41206-B	Black	EBI	44	215289-B	Black	EBI	76	140394	White	ICARDA
13	207608	Black	EBI	45	236196-B	Black	EBI	77	7339	White	ICARDA
14	207622	Black	EBI	46	236459-B	Black	EBI	78	70753	White	ICARDA
15	207638	Black	EBI	47	236479-C	Black	EBI	79	73395	White	ICARDA
16	207640	Black	EBI	48	237054-B	Black	EBI	80	69420	White	ICARDA
17	207648	Black	EBI	49	207686	Black	EBI	81	132663	White	ICARDA
18	207652	Black	EBI	50	207664-A	Black	EBI	82	9415	White	ICARDA
19	207668	Black	EBI	51	30349-B	Black	EBI	83	Yelebe	White	EARCs
20	207670	Black	EBI	52	30348-B	Black	EBI	84	Akaki	Red	EARCs
21	207684	Black	EBI	53	41127-B	Black	EBI	85	mariye	Red	EARCs
22	207688-A	Black	EBI	54	207746	Black	EBI	86	Natoli	Red	EARCs
23	207692	Black	EBI	55	207173-C	Black	EBI	87	Teketay	Red	EARCs
24	207712	Black	EBI	56	41075-C	Brown	EBI	88	Kutaye	Brown	EARCs
25	207714	Black	EBI	57	41093-B	Brown	EBI	89	Teji	White	EARCs
26	207728-A	Black	EBI	58	41255-B	Brown	EBI	90	Shola	White	EARCs
27	207730	Black	EBI	59	207175-A	Brown	EBI	91	Worku	Red	EARCs
28	207748	Black	EBI	60	207635-C	Brown	EBI	92	Harbu	White	EARCs
29	208988-A	Red	EBI	61	30350-B	Red	EBI	93	Dalota	Brown	EARCs
30	209026-B	Red	EBI	62	41301-B	Red	EBI	94	Mastewal	Brown	EARCs
31	227152-B	Red	EBI	63	207766	Black	EBI				
32	30334-C	Red	EBI	64	207770	Black	EBI				

EBI= Ethiopian Biodiversity Institute, ICARDA is International Center for Agricultural Research in the Dry Areas, EARCs=Ethiopian Agricultural Research Centers

4.5.5 Phenotypic Correlation Coefficient

All the cold tolerance related traits had shown a strong significant relationship with grain yield. Grain yield was found to be positively and significantly correlated with fertile pod per plant, thousand seed weight, SR, and stem/leaf pigmentation, implying that enhancing these traits will boost yield under cold stress. Grain yield, on the other hand, was negatively and strongly correlated with the number of infertile pods per plant, the FTR vale, the SSS, the seed color, and the flower. Selection for low value of these traits will be accompanied by an increase in yield under cold stress.

It was observed that genotypes having strong stem/leaf pigmentation had shown a good performance in all agronomic traits and had also a better SR and FTR scores. Similarly, flower and seed color had shown also a strong correlation with agronomic performances. Genotypes having pink flower and black seed color had better performances than the ones with white flower and white seed colored ones. From this result, the selection of genotypes having strong stem/leaf pigmentations and genotypes with black seeded chickpea types and pink flower would greatly assist plant breeders to develop cold resistant varieties to reduce the risk of cold stress damages. Similar findings were reported for chickpea by Mugabe *et al.* (2019).

4.6 Conclusion and Recommendations

This experiment has shown that the degree of cold stress damage varied at different crop stages. The effect of cold stress was not seen on seed germination and seedling establishment. However, considerable cold stress damage was observed at vegetative and reproductive stages for most genotypes. The capacities of genotypes for cold tolerance were estimated using freezing tolerance rate (FTR) and frost survival rate (SR) and their agronomic performances. Eighty three and 85 genotypes were selected based on FTR and SR, respectively. However, both indices are not able to evaluate cold resistance of the genotypes at reproductive stage, if the cold stress occurs consistently throughout the crop stages.

Genotypes having good SR and FTR values produced shriveled seed and empty pods due to cold stress that occurred later at flowering and seed development stages. Therefore, to select the cold tolerant potential genotypes, it is advisable to consider SR and FTR values, pod setting, seed shriveling score, and grain yield together. Genotypes that are consistently selected by all these parameters are considered as promising cold tolerant genotypes. In addition, in areas where cold stress occurs consistently during the seedling and vegetative stages of the crop only, the selection of cold resistance at these stages by considering less FTR and high SR values are enough to select promising cold resistant genotypes.

The effect of cold stress to chickpea genotypes are variable depending on seed color type, presence and absence of stem/leaf pigmentation and different level of stem/leaf pigmentation. Chickpea genotypes with black seeded and/or having strong stem/leaf pigmentation performed well for cold stress reaction. From these observations, it can be concluded that stem/leaf pigmentation and black seeded color might be linked to a gene that confers cold resistance in chickpea. From this experiment, 94 genotypes were identified to be cold tolerant genotypes which can be taken by plant breeders for cold tolerant chickpea variety development program attesting that Ethiopian chickpea genotypes have a potential source for cold tolerance trait. Identification of the mechanism of stem/leaf pigmentation and black seed color for cold resistance is required. Also, identification of quantitative trait loci (QTLs) associated with gene controlling cold tolerances in chickpea is equally important to aid development of cold tolerant varieties.

Chapter 5

5. Evaluation of Chickpea (*Cicer arietinum* L.) Genotypes for Tolerance to Frost under Controlled Environment

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5.1. Abstract

Chickpea screening for frost tolerance has been conducted by various breeders through both field screening protocols under natural conditions and using growth chambers under controlled environments. The aim of this study was thus to evaluate the frost tolerance variability of Ethiopian chickpea (*Cicer arietinum* L.) germplasm under controlled environment using growth chamber. A total of 72 genotypes were screened for frost tolerance using complete randomized design with two replications. The analysis of variance result indicated that there was a significant (P<0.01) difference amongst genotypes for seedling height, number of foliage, number of primary branch, growth rate, and fresh biomass weight. Based on plant survival rate (SR), 31 (43.1%) genotypes scored above 0.8 values. Based on Freezing tolerance rate (FTR), 37(51.4%) and

31(43.1%) genotypes were rated at a score of 1 to 3 in freezing test 1 (T1) and freezing test 2 (T2), respectively. There was a strong negative correlation between fresh biomass yields with SR (-0.75^{**} for T1 and -0.71^{**} for T2 at $p<0.01$), while a strong positive correlation was observed for FTR at a value of 0.74 ($p<0.01$). Based on the combined result of FTR and SR scores, 26 genotypes were found to be frost tolerant genotypes at temperature level as low as -5°C at seedling stage. Based on our findings, Ethiopian chickpea germplasm has a genetic potential for cold tolerance traits for use in breeding programs.

Key words/phrases: Chickpea, Freezing test, Frost screening, Frost tolerant

5.2. Introduction

Plant genetic resources play a significant role in the variety development program by serving as a reservoir for enormous genes that confer tolerance to abiotic and biotic stresses and potential sources of gene for most important agronomic traits (Rao, 2004). The maintenance of a wide array of genetic pool for different crops is the main target for gene bank manager. Cognizant of this, the Ethiopian Biodiversity Institute (EBI) has been collecting and maintaining plant genetic resources in its gene bank. And, extensive genetic characterization and evaluation of the germplasm for agronomic and quality traits are required to make them more useful to breeders and farmers (Castañeda-Álvarez *et al*., 2016).

Chickpea (*Cicer arietinum* L.) is currently the third largest food legume crop in Ethiopia in terms of area coverage and production volume next to faba bean and haricot bean, occupying roughly 239,786.13ha of land annually and producing nearly 0.5 million tone with an average productivity of 2,025 kgha^{-1} for desi and 1,682 kgha^{-1} for kabuli type chickpea (CSA, 2019). Currently, chickpea is introduced to lowland areas using irrigation and also to select areas of the Southern Nation and Nationality People Region (Nigusie Girma *et al*., 2017) contributing to the steady increase of chickpea production. Furthermore, chickpea production could be expanded to 'dega' agroecological zones (2,500 to 3,200 m.a.s.l.) where frost is a typical occurrence. The highland constitutes 2/3rd of the total cultivated land in Ethiopia (Mulugeta Assefa *et al*., 2014) and chickpea production can be extended to these areas if cold tolerant chickpea varieties made available to farmers.

Chickpea is a cold sensitive legume crop and cold stress is the second most important limiting factor in its production next to drought (Sassenrath *et al*., 1990). Cold stress is classified as Chilling injury (0°C to 15°C) and freezing/frost injury (below -1.5^{o}C) (Croser *et al*., 2003; Jha *et al*., 2014), both of which have an overlapping effects on chickpea growth and production (Croser *et al*., 2003; Jha *et al*., 2014). Low field temperature causes poor seed germination, poor crop stand establishment, chlorosis, wilting, necrosis of leaf tips, reduced plant height and branches, full leaf curling, and plant death (Croser *et al*., 2003; Kumar *et al*., 2010). Moreover, frost stress lowers leaf water status and chloroplast membrane stability, resulting in the loss of respiration and photosynthesis (Croser *et al*., 2003; Yadav, 2010).

Cold tolerance is one of the most important pre-requisites to grow cool season legumes in frost prone areas. The degree of frost damages varies among genotypes due to differences in their cold tolerance capacity. Evaluation of plant germplasm for cold tolerance variability is very crucial to identify resistant genotypes. For example, genetic variation of cold tolerance has been reported for field pea seedlings (Bourion *et al*., 2003), chickpea (Kanouni *et al*., 2009; Nezami *et al*., 2012; Mir *et al*., 2019).However, there was no documented information regarding the potential of Ethiopian chickpea genotypes for cold tolerance variability.

Two types of chickpea frost screening protocols; field screening under natural condition and under controlled environment using growth chamber have been used by various breeders. The natural field screening method is expensive and time consuming, there is unpredictable frost severity and irregular low temperature frequency (Maqbool *et al.*, 2010), the lowest temperature is not controlled and there are large temporal and spatial variations in the field (Nezami *et al.*, 2012). However, a controlled environment (using freezing chamber) screening method offers much more precise control of the timing and intensity of frost treatment (Wu *et al.*, 2014). It is also inexpensive, quick and highly reproducible (Nezami *et al.*, 2012). Therefore, the objective of the study was to screen Ethiopian chickpea genotypes for cold tolerance variability under controlled environment using growth chamber and identify cold tolerant genotypes potential to be used in chickpea breeding programs or released as cultivars.

5.3. Material and Methods

5.3.1. Plant Materials

The study was conducted at Ethiopian Biodiversity Institute's Seed Germination Laboratory from October to December, 2021. About 72 genotypes constituting 45 (62.5%) Ethiopian chickpea genotypes, 13 (18.1%) genotypes from the International Center for Agricultural Research in the Dry areas (ICARDA), and 14 (19.4%) improved chickpea varieties from Debre Zeit Agricultural Research Centers were used. The genotypes were selected based on the field performances of genotypes showing poor to better reaction to frost stresses at vegetative and grain filling period (Sintayehu Admas *et al.*, 2021).

5.3.2. Experimental Design

A complete randomized design with two replications was used. Ten seeds of each genotype were planted in pots with 20 cm internal diameter and 20 cm depth. The pots were filled with homogeneous soil mixture which was prepared by mixing the sub-surface (0-30cm) soil thoroughly. The soil was vertisol soil types and collected from the research main station farm of Debre Zeit Research Center. The seedlings were thinned to five plants per pot at four leaf stage. DAP (100 kgha^{-1}) and other management practices were applied. Each morphological data were collected from all the five individual plants.

A modified frost screening protocol using freezing chamber designed by Nezami *et al*. (2012); Zhu *et al*. (2014), and Mugabe *et al*. (2019) was used. The plants were grown in lath house for two weeks at Ethiopian Biodiversity Institute and moved to the controlled environment chamber (Snijders labs climate chambers) for five weeks. Seedlings were subjected to a gradual and progressive low temperature acclimation protocol for four weeks. Acclimation treatment started at seven days with 7^{o}C days/5^{o}C nights, 11-h a photoperiod (PP) followed by 5^{o}C days/2^{o}C nights, 10-h PP for 7 days and then at 5^{o}C days/0^{o}C nights, 9-h PP for 14 days with 250 mmol m^{2}s^{1} levels of irradiance. Subsequently, the frost treatment test took place for seven days under 5^{o}C days/-2°C night, 10-h PP for 3 days, 5^{o}C days/-3°C nights, 10-h PP for 2 days and finally 5^{o}C days/-5°C nights, 10-h PP for 2 days. Finally, the pots were allowed to thaw overnight at 4^{o}C and the plants were moved back to the in lath house for one week for scoring to conduct freezing test 1: to evaluate the re-growth potential of both the foliage and auxiliary buds of the genotypes and freezing test 2: to test the viability of the foliage using 1-9 scale as indicated below.

5.3.3. Data Collection

Seedling height (cm): Average height of five seedlings taken before and after frost treatment

Number of foliage leaf per plant: Average number of foliage leaf per plant taken from five plants before and after frost treatment

Number of primary branches per plant: Average number of primary branches per plant taken from five plants before and after frost treatment

Seedling height growth rate: The ratio of the difference between seedling height before and after frost treatment to seedling height of before frost treatment

Number of foliage leaf growth rate: The ratio of the difference between number foliage leaf per plant before and after frost treatment to number of foliage leaf per plant before frost treatment.

Number of primary branches plant growth rate: The ratio of the difference between number of primary branches per plant before and after frost treatment to number of primary branches per plant before frost treatment.

Fresh biomass yield (g/plant): Average fresh weight of five plants taken at one week after the end of frost treatment

Freezing tolerance rate (FTR):Visual score of viability of the foliage, and foliage and auxiliary buds re-growth scored on 1-9 scale (Fiebelkorn, 2013 cited by Mugabe *et al.*, 2019), where, 1=Plant completely green, 2= Plant with minimal freezing damage, 3= Plant at least 75% green, 4= Plant between 50 and 75% green tissue, 5= Plant 50% green, 6= Plant between 25 and 50% green tissue, 7= Plant 25% green, 8= Plant almost dead but has minimal green, and 9= Plant completely dead

Plant survival rate (SR): Calculated by dividing the number of surviving plants after the frost period by the number of emerged plants after sowing following Heidarvand *et al.*, (2011).

5.3.4. Statistical Analysis

The performances of genotypes were tested for variability of parameters by performing an analysis of variance (ANOVA) in a complete randomized design using R-4.1.0 statistical software (Thomas *et al.*, 2013). Treatment mean comparison was performed for significance, using a Fisher's least significant difference (LSD) test at 5% probability using R software. Pearson correlation coefficients between variables was estimated and tested for significance using MINITAB release 14 statistical software (MINITAB, 1998).

5.4. Results

Based on the analysis of variance, it was found that there were significant differences (*$p<0.01$*) for frost tolerance among genotypes for seedling height before and after frost treatment, growth rate of seedling height during frost treatment, number of foliage before and after frost treatment, growth rate of number of foliage during frost treatment, number of primary branches before and after frost treatment, growth rate of number of primary branch during frost treatment, and fresh weight (Table 17).

Fisher's least significant difference (LSD) result indicated that the differences among the means of the genotypes for a given trait were significant ($P<0.05$). Wide mean ranges were observed for all the collected traits in the genotypes (Table 18). The range for each measurement were as follows: 13.1cm to 23.7 cm for seedling height before frost treatment, 0.0 to 25.3 cm for seedling height after frost treatment, 0.0 to 0.7 for growth rate of seedling height during frost treatment, 1.7 to 8.0 for number of foliage before frost treatment, 0.0 to 20.2 for number of foliage after frost treatment, 0.0 to 0.8 for growth of number of foliage during frost treatment, 0.0 to 16 for number of primary branches after frost treatment, 0.0 to 1.0 for growth rate of number of primary branch during frost treatment, and 0.0 to 14.7 for fresh weight.

Table 17. Mean square and mean for the tested traits of 72 chickpea genotypes (45 Ethiopian genotypes, 13 elite frost resistant genotypes from ICARDA, and 14 improved varieties) grown under controlled environment at Ethiopian Biodiversity Institute, 2021

Sources of Variation	**Degree of freedom**	**Mean Squares**								
		SH BF	**SH AF**	**GR SH**	**NF BF**	**NF AF**	**GR NF**	**NPB AF**	**GR NPB**	**FW**
Genotypes	71	8.0^{**}	55.8^{**}	0.04^{**}	2.22^{**}	45.43^{**}	0.06^{**}	31.55^{**}	0.27^{**}	26.3^{**}
Error	72	2.12	7.48	0.01	1.45	3.95	0.01	2.58	0.02	2.94

SHBF=Seedling height before frost treatment in cm, SHAF= Seedling height after frost treatment in cm, GRSH= Growth rate of seedling height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight in gm

Table 18. Mean value of quantitative traits of 72 chickpea genotypes (45 Ethiopian genotypes, 13 elite frost resistant genotypes from ICARDA, and 14 improved varieties) grown under controlled environment at Ethiopian Biodiversity Institute, 2021

No	**Acc**	**Types**	**Source**	**PLH BF**	**PLH AF**	**GR PLH**	**NF BF**	**NF AF**	**GR NF**	**NPB AF**	**GRN PB**	**FW**	**SR**	**FT1**	**FT 2**
1	207674	Desi	EBI	11.3	22.6	0.50	5.9	18.7	0.7	12.2	1.0	14.7	0.9	2	3
2	30350-B	Desi	EBI	10.6	21.1	0.49	5.4	14.8	0.6	7.4	1.0	10.5	1.0	2	3
3	41133-A	Desi	EBI	9.6	21.1	0.55	5.3	18.5	0.7	4.7	1.0	10.6	0.8	2	3
4	207173-B	Desi	EBI	13.1	25.3	0.48	5.4	17.5	0.7	10.4	1.0	13.6	1.0	1	1
5	207175-A	Desi	EBI	11.2	20.5	0.46	7.3	19.0	0.6	7.4	1.0	7.5	0.8	1	2
6	207766	Desi	EBI	11.9	22.5	0.47	6.6	20.2	0.7	14.7	1.0	12.0	0.8	1	2
7	209026-B	Desi	EBI	8	17.2	0.53	5.8	11.1	0.5	3.0	1.0	13.2	1.0	1	1
8	227152-B	Desi	EBI	10.3	19.3	0.47	5.2	16.7	0.7	7.3	1.0	10.9	0.8	1	2
9	41301-B	Desi	EBI	8.1	16.4	0.50	5.2	13.7	0.6	6.8	1.0	11.1	1.0	1	2
10	207746	Desi	EBI	7.5	14.5	0.48	5.5	8.0	0.3	1.5	1.0	10.7	1.0	2	3
11	Teketay	Desi	DZARC	8.3	16.1	0.48	6.2	17.5	0.7	11.2	1.0	11.1	1.0	1	1
12	Natoli	Desi	DZARC	5.0	9.7	0.48	5	8.6	0.4	0.8	0.5	10.2	1.0	2	3
13	Akaki	Desi	DZARC	9	19.9	0.55	5.7	13.5	0.6	4	1.0	11.6	1.0	1	1
14	9427	Kabuli	ICARDA	9.1	16.6	0.46	6	12.3	0.5	5.8	1.0	9.2	1.0	1	1
15	69420	Kabuli	ICARDA	7.1	17.2	0.59	5.5	9.9	0.5	0	0.0	11.9	1.0	1	1
16	Worku	Desi	DZARC	8.6	20.2	0.58	5.1	10.7	0.5	2.8	1.0	8.8	0.9	3	3
17	Mariye	Desi	DZARC	6.2	12.2	0.50	6.0	8.05	0.3	0	0.0	9.0	1.0	1	1
18	DBB	Desi	EBI	8.8	17.9	0.51	6.2	13.0	0.5	4.3	1.0	10.0	0.8	2	3
19	ENR	Desi	EBI	11.2	21.4	0.48	6.2	19.1	0.7	8.3	1.0	9.1	1.0	2	2
20	TEGR	Desi	EBI	9.9	20.8	0.52	6.2	18.1	0.7	10.0	1.0	9.1	1.0	2	3
21	30334-C	Desi	EBI	12.8	22.5	0.44	7.7	18.9	0.6	10.9	1.0	13.6	0.8	1	2
22	207648	Desi	EBI	12.2	19.1	0.36	6.3	19.1	0.7	16.0	1.0	13	1.0	1	1
23	207728-A	Desi	EBI	10.6	18.0	0.41	5.6	14.0	0.6	5.2	1.0	12.1	1.0	2	2
24	208988-A	Desi	EBI	12.1	19.9	0.39	6.4	15.5	0.8	12.4	1.0	14.6	1.0	1	1
25	Kutaye	Desi	DZARC	8.3	15	0.45	5.4	14.5	0.6	8.9	1.0	9.3	1.0	1	1
26	Yelbie	Kabuli	DZARC	8.4	13.8	0.39	5.9	8.9	0.3	5.6	1.0	9.8	1.0	2	3
27	Teji	Kabuli	DZARC	5.7	12.8	0.56	4.2	8.9	0.5	0.5	0.5	10.8	0.7	1	2
28	Mastewal	Desi	DZARC	8.9	18.3	0.52	6.4	12.5	0.5	3.5	1.0	12.8	0.7	1	2
29	Shahso	Kabuli	DZARC	8.1	19.2	0.58	5.5	15.0	0.7	6.9	1.0	9.4	0.6	1	1
30	30300-A	Desi	EBI	12.3	24.2	0.49	6.5	13.5	0.5	7.3	1.0	13.5	0.9	4	5
31	30309-A	Desi	EBI	8.1	17.8	0.26	6.1	13.8	0.6	7.0	1.0	5.5	0.6	6	6
32	41075-C	Desi	EBI	11.9	20.1	0.41	6.1	12.0	0.5	11.4	1.0	9.4	1.0	4	4
33	41078-B	Desi	EBI	10.3	20.6	0.50	5.6	16.5	0.7	8.8	1.0	9.1	0.6	3	5
34	41094-C	Desi	EBI	7.3	14.0	0.47	5.1	15..	0.7	6.8	1.0	7.9	0.8	4	5
35	41153-A	Desi	EBI	11.2	22.4	0.49	5.1	16.6	0.7	5.9	1.0	12.0	0.7	3	4
36	41282-B	Desi	EBI	11.4	20.1	0.44	6.0	16.9	0.6	8.2	1.0	13.8	0.7	3	3
37	41323-A	Desi	EBI	10.0	19.9	0.50	5.5	17.0	0.7	9.1	1.0	11.2	0.7	3	5
38	207167-A	Desi	EBI	11.8	20.9	0.43	6.9	18.9	0.6	6.5	1.0	10.3	0.8	4	4
39	207640	Desi	EBI	10.4	14.2	0.27	5.1	12.7	0.6	3.8	1.0	10.3	0.7	5	6
40	207652	Desi	EBI	9.5	17.4	0.46	4.5	16.0	0.7	6.7	1.0	9.2	0.9	4	5
41	207670	Desi	EBI	11.4	21.4	0.42	8.0	18.9	0.6	13.5	1.0	12.1	0.7	5	6
42	209026-A	Desi	EBI	10.1	19	0.47	5.9	16.3	0.6	6.8	1.0	9.1	0.8	4	5
43	212477-A	Desi	EBI	11.0	17.7	0.38	5.8	12.4	0.5	3.9	1.0	12.5	0.6	1	2
44	241800-A	Desi	EBI	9.9	18.9	0.48	5.6	15.8	0.7	5.5	1.0	9.8	0.9	3	5
45	30339-A	Desi	EBI	10.1	19.3	0.48	6.3	13.1	0.5	5.8	1.0	10.1	1.0	4	5
46	Minjar	Desi	DZARC	8.8	17.5	0.50	4.3	12.4	0.7	4.3	1.0	11.2	0.6	3	4
47	140941	Kabuli	ICARDA	10.3	16.6	0.38	6.2	14.2	0.6	9.3	1.0	11.7	0.7	4	4

Table 18. Continued....

No	Acc	Types	Source	SH BF	SH AF	GR SH	NF BF	NF AF	GR NF	NPB AF	GRN PB	FW	SR	FT1	FT 2
48	141693	Kabuli	ICARDA	8.6	18.7	0.54	6.5	8.2	0.2	0	0.0	1.5	0.5	8	8
49	125187	Kabuli	ICARDA	8.3	14.4	0.71	5.1	10.4	0.5	2.9	1.0	3.7	0.5	8	8
50	Dubie	Desi	DZARC	8.1	15.7	0.48	3.9	17.3	0.9	4.2	1.0	10.8	0.6	6	6
51	140294	Kabuli	ICARDA	9.1	16.9	0.47	4.5	7.1	0.4	0	0.0	8.2	0.6	3	5
52	132663	Kabuli	ICARDA	6.6	14.1	0.53	5.3	11.0	0.5	3.6	1.0	5.8	0.8	4	4
53	Kasech	Kabuli	DZARC	10.5	0	0	2.2	0	0	0	0.0	0	0.0	9	9
54	16341-A	Desi	EBI	12.3	24.1	0.49	5.9	16.8	0.7	10.3	1.0	13.2	0.7	5	6
55	41081-A	Desi	EBI	9.2	16.9	0.46	5.1	11.7	0.6	6.1	1.0	5.2	0.5	7	7
56	207608	Desi	EBI	8.3	17.4	0.52	3.9	10.1	0.6	0	0.0	5.1	0.4	8	9
57	207638	Desi	EBI	9.3	19.8	0.53	4.4	14.4	0.8	6.8	1.0	10.4	0.4	7	7
58	207649-A	Desi	EBI	6.8	15	0.56	5.0	7.7	0.3	2.1	1.0	8.0	0.5	6	7
59	207668	Desi	EBI	9.7	21.8	0.56	6.1	13.5	0.6	3.7	1.0	10.1	0.7	5	5
60	209008-A	Desi	EBI	9.2	18	0.49	5.8	16.6	0.7	9.4	1.0	11.4	0.6	5	5
61	209016-B	Desi	EBI	11.3	20.7	0.46	6.0	15.6	0.6	5.9	1.0	9.5	0.6	5	7
62	212688-C	Desi	EBI	10	23.2	0.57	5.4	14.4	0.6	4.7	1.0	10.4	0.7	5	6
63	215190-A	Desi	EBI	9.2	19.1	0.53	5.1	12.0	0.6	5.8	1.0	12	0.6	6	7
64	237054-B	Desi	EBI	6.4	16.9	0.62	4.9	11.5	0.6	2.8	1.0	6.9	0.4	7	7
65	Dimtu	Desi	DZARC	6.5	16.6	0.61	5.1	11.5	0.6	0.7	0.5	9.6	0.5	6	6
66	141720	Kabuli	ICARDA	11.5	21.4	0.46	6.2	19.0	0.7	9.1	1.0	3.2	0.3	8	8
67	75095	Kabuli	ICARDA	5.6	13.4	0.58	4.3	6.8	0.4	0.0	0.0	6.2	0.0	9	9
68	209026-A	Desi	EBI	10	18.9	0.47	5.1	13.0	0.6	4.0	1.0	4.8	0.4	7	8
69	10163	Kabuli	ICARDA	3.7	10.4	0.65	3.0	5.6	0.5	0.0	0.0	1.5	0.4	8	8
70	8191	Kabuli	ICARDA	8.2	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	9	9
71	139930	Kabuli	ICARDA	6.1	0.0	0.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0	9	9
72	73221	Kabuli	ICARDA	8.2	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	9	9
	Mean			9.3	17.3	0.5	5.4	13.1	0.5	5.6	0.8	9.2	-	-	-
	±SE			1.5	2.8	0.1	1.2	2.0	0.1	1.6	0.2	1.9	-	-	-
	LSD (5%)			2.9	5.5	0.2	2.4	3.9	0.2	3.0	0.3	3.8	-	-	-

SHBF=Seedling height before frost treatment, SHAF= Seedling height after frost treatment, GRSH= Growth rate of seedling height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight, SR=Survival rate, FT1 =Freezing test 1, and FT2=Freezing test 2. SE=pooled standard deviation, LSD=Least square difference, EBI=Ethiopian Biodiversity Institute, DZAR=Debre Zeit Agricultural Research Centers, ICARDA=International Center for Agricultural Research in the Dry areas

The reaction of genotypes to frost as indicated by plant survival rate (SR) is given in Table 19. Thirty one genotypes (43.1%) rated more than 0.8 score, while the remaining genotypes rated less than 0.8 score (56.9%). Five genotypes scored 0 which means that they were killed by frost stress since it had poor tolerance to frost stress.

Table 19. Plant survival rate (SR) of 72 chickpea genotypes (45 Ethiopian genotypes, 13 elite frost resistant genotypes from ICARDA, and 14 improved varieties) grown under controlled environment at Ethiopian Biodiversity Institute, 2021

No	SR Rating	No of genotypes
1	> 0.8	31 (43.1%)
2	>0.6 to <0.8	22 (30.6%)
3	>0.4 to <0.6	9 (12.5%)

Table 19. Continued....

4	>0.2 to <0.4	5 (6.9%)
5	<0.2	5 (6.9%)
Total		72

The result of foliage and auxiliary buds re-growth (Freezing test 1), and foliage viability (Freezing test 2) is displayed in Table 20. The majority of the genotypes showed recovery from frost damage with variable degrees. Four genotypes did not recover because they were killed by the frost. The remaining genotypes recovered with low to high rate (Figure 10). The records of leaf damage of the frost susceptible genotypes were severely damaged and all plant leaves died, while in tolerant genotypes the leaf damage were nil to medium level (Figure 10). Although the foliage of these genotypes had injured foliage following a frost, re-growth occurred from auxiliary buds at the stem (Figure 11).The scores were done visually using freezing tolerance rate (FTR). The FTR scores were taken at one week after the end of the frost treatments. Thirty seven (51.4%) and 31 (43.1%) genotypes showed no or little leaf damage due to frost injury for freezing test 1 and freezing test 2 respectively. The remaining genotypes, 35 (48.6%) and 41 (56.9%) scored from 4 to 9 at freezing test 1 and freezing test 2, respectively. These genotypes were moderately cold tolerant to highly frost susceptible genotypes.

Table 20. Freezing tolerance rate (FTR) of 72 chickpea genotypes (45 Ethiopian genotypes, 13 elite frost resistant genotypes from ICARDA, and 14 improved varieties) grown under controlled environment at Ethiopian Biodiversity Institute, 2021

No	FTR Rating	No. of genotypes (Freezing Test 1)	No. of genotypes (Freezing Test 2)
1	1	19 (26.4%)	11 (15.3%)
2	2	10 (13.9%)	10 (13.9%)
3	3	8 (11.1%)	10 (13.9%)
Subtotal		**37 (51.4%)**	**31 (43.1%)**
4	4	9 (12.5%)	6 (8.3%)
5	5	7 (9.7%)	11 (15.3%)
6	6	5 (6.9%)	7 (9.7%)
7	7	4 (5.6%)	6 (8.3%)
8	8	5 (6.9%)	5 (6.9%)
9	9	5 (6.9%)	6 (8.3%)
Subtotal		**35 (48.6%)**	**41 (56.9%)**
Total		**72**	**72**

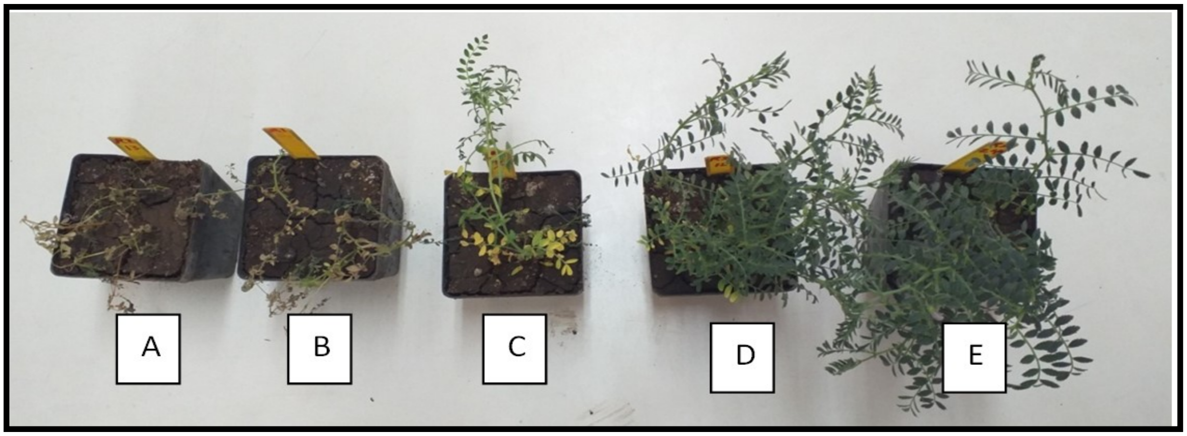

Figure 10. The reactions of genotypes to frost injury [A (139930) and B (73221) highly frost-susceptible genotypes, C (30339-A) moderately cold tolerant genotypes, D (209026-A) and E (Teketay) cold tolerant genotypes]

Figure 11. The variable potential of genotypes to the re-growth of the foliage and auxiliary buds one week after the end frost treatments [A (973221), B (141693), and C (207649-A) genotypes did not show foliage and auxiliary buds re-growth, D (Minjar) genotypes had shown foliage re-growth, E (Akaki) and F (41282-B) genotypes had shown foliage and auxiliary buds re-growth. The arrow indicates the growing of primary branches

The phenotypic association of agronomic and frost tolerance related traits were analyzed based on the mean values of the recorded traits of all genotypes and the result is given in Table 21. SR and FTR scores were strongly correlated with seedling height before and after frost, growth rate of seedling height during frost, number of foliage before and after frost, growth of number of foliage during frost, and number of primary branch after frost at $p<0.01$.

Table 21. Phenotypic Pearson's correlation matrix for 11 traits of 72 chickpea genotypes (45 Ethiopian genotypes, 13 elite frost resistant genotypes from ICARDA, and 14 improved varieties) grown under controlled environment at Ethiopian Biodiversity Institute, 2021

Traits	**SH AF**	**GR SH**	**NF BF**	**NF AF**	**GR NF**	**NPB AF**	**GR NPB**	**FW**	**SR**	**Test 1**	**Test 2**
SHBF	0.59^{**}	-0.12^{*}	0.51^{**}	0.62^{**}	0.41^{**}	0.71^{**}	0.50^{**}	0.44^{**}	0.29^{**}	-0.26^{**}	-0.22^{**}
SHAF		0.65^{**}	0.68^{**}	0.84^{**}	0.79^{**}	0.60^{**}	0.66^{**}	0.69^{**}	0.56^{**}	-0.45^{**}	-0.42^{**}
GRSH			0.31^{**}	0.46^{**}	0.59^{**}	0.08^{ns}	0.34^{**}	0.41^{**}	0.41^{**}	-0.31^{**}	-0.29^{**}
NFBF				0.68^{**}	0.42^{**}	0.61^{**}	0.55^{**}	0.56^{**}	0.61^{**}	-0.52^{**}	-0.51^{**}
NFAF					0.89^{**}	0.80^{**}	0.75^{**}	0.66^{**}	0.57^{**}	-0.51^{**}	-0.48^{**}
GRNF						0.65^{**}	0.70^{**}	0.62^{**}	0.47^{**}	-0.4^{**}	-0.40^{**}
NPBAF							0.67^{**}	0.55^{**}	0.47^{**}	-0.41^{**}	-0.39^{**}
GRNPB								0.60^{**}	0.54^{**}	-0.45^{**}	-0.43^{**}
FW									0.74^{**}	-0.75^{**}	-0.71^{**}
SR										-0.87^{**}	-0.85^{**}
Test 1											0.98^{**}

SHBF=Seedling height before frost treatment, SHAF= Seedling height after frost treatment, GRSH= Growth rate of seedling height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight, SR=Survival rate, Test 2=Freezing test 2, and Test 1=Freezing test 1

5.5. Discussions

In Ethiopia, chickpea is commonly grown in areas having vertisols with an altitude range of 1,400 to 2,300 m.a.s.l.(Geletu Bejiga *et al.* 1996).There is still an immense potential to introduce chickpea into 'dega' agroecological zones (2,500 to 3,200 m.a.s.l.) areas as crop diversification scheme. In highland area, however, the existence of frost stress limits crop production. So, to bring chickpea as an alternative crop in this area; it requires the improvement of chickpea for frost stress tolerance. This requires an extensive germplasm screening to identify frost tolerant genotypes. Studying frost tolerance and breeding for cold tolerant chickpea varieties play a fundamental role in increasing chickpea production in frost prone areas. To this end, in the present study, chickpea genotypes were evaluated under controlled environment using growth chamber. The experiment has revealed the response variability of 72 chickpea genotypes with respect to frost tolerance stress in two weeks old chickpea seedlings under controlled conditions.

The ANOVA result indicated that there was differences among the genotypes in their reaction to frost stress was variable, which is an indication of the existence of variability among genotypes for frost tolerances. LSD (5%) and mean range value further confirms the presence of a variable responses among chickpea genotypes for frost stress similar to results obtained by Mir *et al.* (2019).

SR and FTR results showed that the levels of frost tolerance among different genotypes were highly variable. Based on FTR and SR scores most genotypes were found to exhibit moderate frost tolerance tested at a temperature of -5°C. A plant survival score was used as an index to describe genotypes tolerance to low temperature (Heidarvand *et al.*, 2011). The susceptible four genotypes were killed at -2°C, while the remaining genotypes had shown variable number of plant deaths which indicates the different capacity of genotypes for frost reactions.

A rating scale of 1-9 has been used for measuring frost stress injury during early vegetative stage or seedling stage in earlier studies (Singh *et al.*, 1989). The score was done by visual observation of the viability of the foliage, and re-growth of foliage and auxiliary buds. The susceptible genotypes did not show foliage and auxiliary bud re-growth at all, in addition the percent of damage on foliage and auxiliary buds were severe. However, the frost tolerant genotypes displayed better reaction to frost stress and moderate to high foliage and auxiliary bud re-growth rate were observed. Freezing and/or chilling range temperatures cause poor establishment, reduced vigor resulting in stunted seedlings and retarding plant growth and, in extreme cases, may lead to plant death (Croser *et al* 2003; Maphosa *et al.*, 2020).

Previous studies have reported that, at the seedling stage, long periods of chilling range temperatures can retard the growth of the plant and, in severe cases, cause plant death (Croser *et al.*, 2003; Maphosa *et al.*, 2020). However, an expected result was observed for 19 genotypes

(Table 18, genotype listed from no 54 to 72) in which they showed better growth rate in plant height, foliage leaf and primary branch, while their reaction to frost stress were poor with FTR score of 5 and above. This happened because these genotypes had performed well during a frost treatment of -2°C and -3°C, however when frost treatment temperature dropped to a level of -5°C, then, these genotypes could not withstand the frost stress and whole plant death started which were manifested a week after the end of frost treatment.

The correlation coefficient result of the agronomic and frost indices showed that there was a strong correlation among the characters recorded. FTR score showed a negative strong correlation between the traits considered and SR score, while SR score showed a positive strong correlation between the recorded traits and FTR value. A strong positive correlation between two traits means that increasing one trait would be accompanied by an increasing in the other trait also. But, if the correlation is negative, increasing one trait would result in the reduction of the other. Such types of traits are governed by a pleiotropic effect of genes or linkage of genes controlling the inheritance of two or more characters (Dabholkor, 1992). Similar findings were reported by Mugabe *et al.* (2019).

5.6. Conclusions and Recommendations

Frost tolerant screening of chickpea seedlings in controlled environment using growth chamber has enabled the identification of frost tolerant genotypes at an early growth stage. The cold tolerant genotypes were selected based on SR and FTR values (Freezing test 1 and freezing test 2). Genotypes that were consistently rated as cold tolerant genotypes in both indices (SR value of $\geq$ 0.8 and FTR score of 1 to 3) were selected. Twenty six chickpea genotypes (Table 18, genotypes listed from no. 1 to 26) were identified as cold tolerant genotypes tested at seedling stage which can withstand a temperature as low as -5°C. Based on these findings, it can be concluded that Ethiopian chickpea landraces have genetic potential for frost resistance traits. We recommend that these genotypes be used for future cold tolerant cultivar development program through implementing multi-locations and multi-year field trials to test the frost tolerance adaptation to a more wide range of chickpea growing environments.

Chapter 6

6. Phenotypic Variability of Chickpea (*Cicer arietinum* L) Germplasm with Temporally Varied Collection from the Amhara Regional State, Ethiopia

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6.1. Abstract

Information on diversity changes occurring in farmers' field overtime is very important for effective genetic resource conservation and use. Thus, this study was initiated to investigate the phenotypic diversity changes between the current (2017) and previous chickpea (*Cicer arietinum* L.) collections (1979-1983) of the Amhara Regional State, Ethiopia. An experiment was conducted using simple lattice design with two replications at Debra Zeit Agricultural Research Center for two consecutive years (2018/2019 to 2019/2020). Based on qualitative traits analysis, almost all genotypes of current and previous collections exhibited low anthocyanin stem pigmentation, angular seed shape, seed rough texture, and semi-erect growth habits. Genetic erosion of 30.4% to 100% was recorded in chickpea for the past 35years in the study areas. Shannon-Weaver diversity index estimates of black seeded and ivory white seeded chickpea types decreased from 1.99 to 0.69 and 1.33 to 0.0 in the past 35 years, respectively. In previous collections, 25.8% of black and 6.5% of white coated genotypes were observed, while these chickpea types were rare (black 6.5%) or unobtainable (white 0.0%) in current collections. There was a significant difference ($p<0.05$) between mean of current and previous collections for plant height, days to 50% flowering and podding, days to 90% maturity, number of primary and secondary branches, number of pods per plant, thousand seed weight, and grain yield tested at the individual site. T-test results indicated there was a significant difference ($p<0.05$) between mean of current collections and previous collections for most traits tested at the individual site. Cluster analysis showed that genotypes were clustered relatively with respect to the time of collections and irrespective of their source of origins. Generally, chickpea genotypes of current and previous collections were distinct from one another. The current collections lost diversity as compared to previous collections. Black and white seeded chickpea landraces were vulnerable to genetic erosion and it is recommended to implement immediate restoration of chickpea landraces to recover and maintain the lost chickpea landraces in the studied region.

Key words: Chickpea, Chickpea landrace, Genetic erosion, Landraces, Phenotypic diversity

6.2. Introduction

Exploration and identification of plant genetic resources' hot spot areas across the word has led to the identification of the twelve Vavilovian centers of origin/diversity for several domesticated crops and their wild and weedy relatives (Vavilov, 1951). In order to safeguard food and nutrition security for both the present and the future, genetic resources are conserved in more than 1,700 gene banks established around the world (Singh *et al*., 2012), with the Ethiopian Biodiversity Institute (EBI) representing one of the most important gene banks for sub-Saharan Africa. These gene banks, including EBI, conserve more than 7.5 million accessions of various plant species collected from different parts of the world in *ex-situ* gene banks (FAO, 2010). The plant genetic recourses have been promoting human health for centuries by delivering food, fiber, medicine, shelter etc. In addition, conserved plant genetic resources have the ability to contain genes that confer resistance to disease, insect pests, and improved agronomic traits which can give an opportunity to plant breeders for a variety of crop improvement options.

Ethiopia possesses diverse soil characteristics, climate, and cultures which provides a suitable region to develop a rich genetic resource variation within crop species (Melaku Worede, 1992). Because of this, Ethiopia is considered as one of the major Vavilovian centers of origin/diversity for several domesticated crops and their wild and weedy relatives (Vavilov, 1951). Chickpea (*Cicer aritenium* L) is among the crops, where Ethiopia is proposed as the secondary center of diversity (Van der Maesen, 1987). In the EBI gene bank, 1,223 chickpea accessions have been conserved until 2020, with 69 percent collected between 1977 and 1991 and 14 percent collected between 1992 and 2015. The remaining 17% genotypes lack information about year of collection. Regular chickpea diversity monitoring at farmers' field is equally important to indicate the detrimental evolutionary patterns, set conservation priorities and assesses the impact of conservation policies (Barry *et al*., 2008). Though the genetic diversity created in the farmers' fields over millennia provides the raw material for crop variety improvement (Upadhyaya *et al*., 2008), the conservation strategies that have been employed to date are not sufficient resulting in a significant loss of plant genetic resources among major crop landraces (i.e. Genetic erosion) (van de Wouw *et al*., 2009).

Mazhar (1997) estimated that three-fourths of the genetic diversity of major food crops have been exposed to genetic erosion with an annual rate of reduction by one or two percent. In Ethiopia, Bayush Tsegaye and Berg (2007) estimated a 77% and 95% loss of tetraploid wheat landraces diversity in Ejeri and Akaki districts, respectively. Yifru Teklu and Hammer (2006) reported reduction in the use of wheat landraces by farmers in Eastern Ethiopia. Firew Mekbib (2008) also reported that there was a genetic erosion of sorghum germplasm amongst individual farmers in Eastern Ethiopia. Hailemichael Shewayrga *et al*. (2008) indicated that some important sorghum landraces have disappeared either locally or regionally in the past 30 years. The major factors that cause genetic erosion in Ethiopia include: replacement of landraces by other crops, introduction and expansion of improved varieties, expansion of economical crops, lack of a mechanism to re-supply seeds of landraces, decline in size of land holdings, changes in land use and cropping patterns, and lack of policy support (Yifru Teklu and Hammer, 2006; Bayush Tsegaye and Berg, 2007; Hailemichael Shewayrga *et al*., 2008; Firew Mekbib, 2008).

A significant amount of changes in genetic diversity has occurred in major crops in Ethiopia. In chickpea, though a considerable effort has been practiced for *ex-situ* conservation work, information regarding the diversity changes that occur overtime at farmers' field is minimal. Genetic diversity change can be assessed by comparing the level of diversity occurring at time zero and a subsequent time at species, varietal, agromorphological and molecular diversity level (Barry *et al*., 2008). Therefore, the objectives of the present study were to (1) To assess changes in chickpea on-farm genetic diversity and its agronomic performances in the Amhara Regional State during a 35-year period, from 1979 (when the first collections were made) to 2017 (when the current collections were carried out) and (2) estimate the level and extent of chickpea genetic erosion by evaluating the temporal changes of population genetic structure and to provide some suggestions for conservation actions in the Amhara Regional State. The finding of this study will help to design appropriate conservation strategies to conserve and maintain chickpea germplasm for sustainable use of chickpea.

6.3. Material and Methods

6.3.1. Plant Materials

Twenty-seven chickpea accessions from EBI's collection were selected based on the year of collection from three zones (East Gojjam, North and South Gonder) of Amhara Regional state and their subsequent districts representing the major chickpea growing areas of the region. These germplasms were collected from 1979 to 1983 and conserved *ex-situ* for over 35 years. These germplasms were not subjected to multiplication or regeneration. Another collection program was executed in 2017 in these three zones to recollect chickpea germplasm and 29 chickpea accessions were collected in the locations that match the previous collection conducted between 1979 and 1983. To ensure the matching of locations, reference to the gene bank passport data containing information on administrative region, district name, altitude, latitude, and longitude of collection sites, including the distance from the nearby town was used. The geographical distribution of the germplasm is indicated in Figure 12. Two improved medium sized varieties (Mastewal and Worku) of chickpea were also included in the study (Table 22).

The chickpea accessions of current and previous collections naturally exist as a mixture of different lines. Before going for characterization, each accession was sorted into nearly homogenous genotypes based on seed color, texture, size, and shape. Forty one homogenous genotypes were developed from the 29 accessions of current collections, while 93 genotypes were developed from 27 accessions of previous collections. These homogenous genotypes (134) were used for qualitative trait analysis (For further details see Appendix 4). Eighty one (32 from current, 47 previous collections and two varieties) selected from 134 homogenous genotypes were used for quantitative traits analyses.

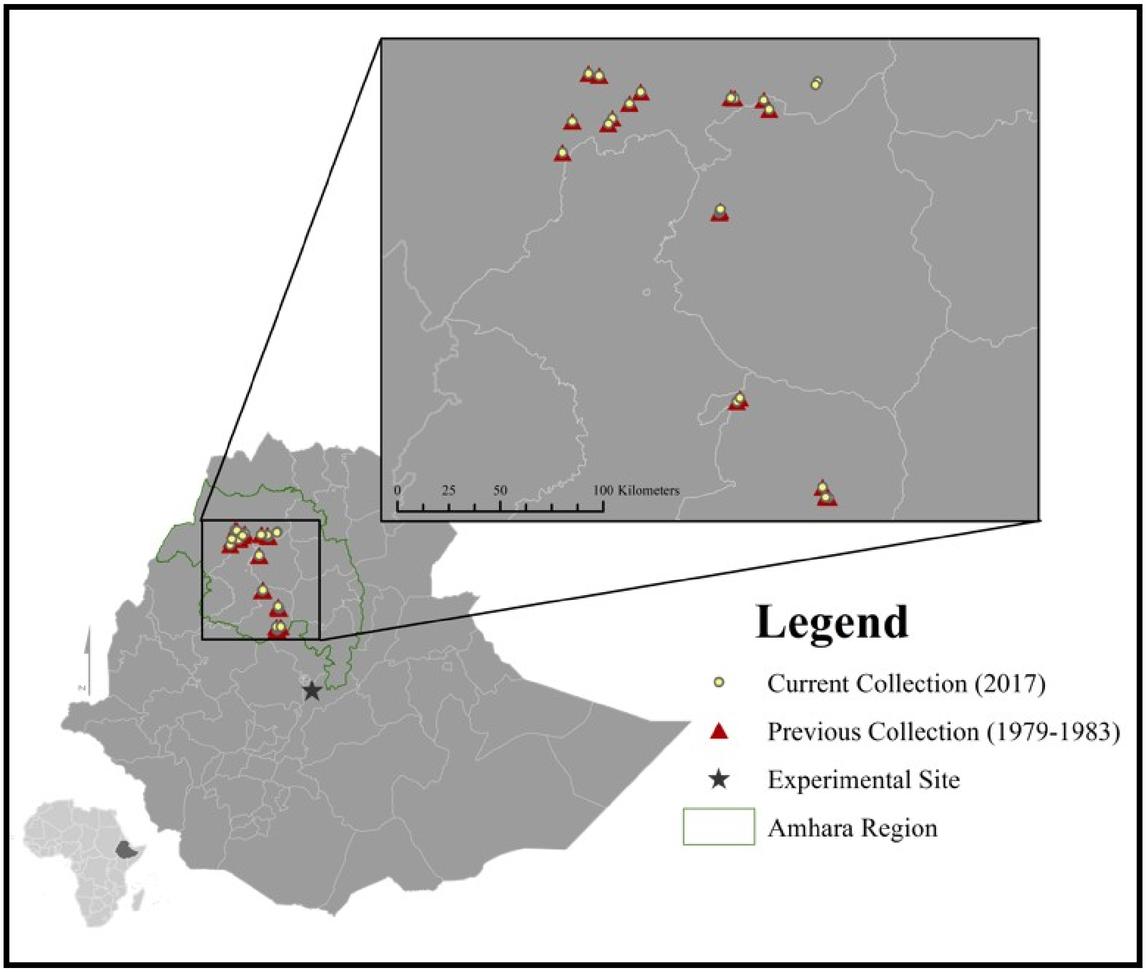

Figure 12. Map showing the geographical distribution of chickpea germplasm of current (2017 and previous (1979-1983) collections from Amhara Regional State, Ethiopia and the Experimental site

Table 22. List of Ethiopia chickpea accessions and its passport data for current (2017) and previous collections (1979 to 1983) of eight districts of Amhara Regional State, Ethiopia

No	Accession Number	Zone	District	Latitude	Longitude	Collection Date
1	207170	North Gonder	Belesa	12-23-25.85-N	037-55-37.55-E	1983
2	207166	North Gonder	Belesa	12-25-54.4-N	037-54-09.3-E	1983
3	241800	North Gonder	Belesa	12-26-23.3-N	037-46-29.1-E	1983
4	241801	North Gonder	Belesa	12-26-29.9-N	037-45-34.2-E	1983
5	30319	North Gonder	Belesa	12-23-25.85-N	037-55-37.55-E	2017
6	30320	North Gonder	Belesa	12-25-54.4-N	037-54-09.3-E	2017
7	30321	North Gonder	Belesa	12-26-23.3-N	037-46-29.1-E	2017
8	30322	North Gonder	Belesa	12-26-29.9-N	037-45-34.2-E	2017

Table 22: Continued....

No	**Accession Number**	**Zone**	**District**	**Latitude**	**Longitude**	**Collection Date**
9	30317	North Gonder	Belesa	12-30-52.2-N	038-08-19.6-E	2017
10	30318	North Gonder	Belesa	12-29-52.3-N	038-07-46.4-E	2017
11	41046	North Gonder	Chilga	12-32-56.4-N	037-08-14.4-E	1979
12	207143	North Gonder	Chilga	12-32-23.4-N	037-11-02.3-E	1983
13	30331	North Gonder	Chilga	12-32-56.4-N	037-08-14.4-E	2017
14	30332	North Gonder	Chilga	12-32-23.4-N	037-11-02.3-E	2017
15	41310	North Gonder	Dembia	12-21-08.3-N	037-14-28.3-E	1982
16	41311	North Gonder	Dembia	12-19-38.1-N	037-13-25.8-E	1982
17	41306	North Gonder	Dembia	12-28-05.1-N	037-21-56.3-E	1984
18	227158	North Gonder	Dembia	12-25-00.8-N	037-18-56.1-E	1983
19	225887	North Gonder	Dembia	12-25-00.8-N	037-18-56.1-E	1983
20	30325	North Gonder	Dembia	12-21-08.3-N	037-14-28.3-E	2017
21	30326	North Gonder	Dembia	12-19-38.1-N	037-13-25.8-E	2017
22	30323	North Gonder	Dembia	12-28-05.1-N	037-21-56.3-E	2017
23	30324	North Gonder	Dembia	12-25-00.8-N	037-18-56.1-E	2017
24	227161	North Gonder	Takusa	12-12-04.2-N	037-01-25.2-E	1983
25	227160	North Gonder	Takusa	12-20-15.8-N	037-03-59.3-E	1983
26	30327	North Gonder	Takusa	12-12-04.2-N	037-01-25.2-E	2017
27	30329	North Gonder	Takusa	12-20-15.8-N	037-03-59.3-E	2017
28	41322	Eastern Gojjam	Dejen	10-10-06.4-N	038-08-00.6-E	1982
29	41324	Eastern Gojjam	Dejen	10-11-49.6-N	038-08-26.0-E	1982
30	41222	Eastern Gojjam	Dejen	10-14-34.8-N	038-06-55.9-E	1982
31	41223	Eastern Gojjam	Dejen	10-14-25.5-N	038-13-18.6-E	1982
32	30287	Eastern Gojjam	Dejen	10-10-06.4-N	038-08-00.6-E	2017
33	30288	Eastern Gojjam	Dejen	10-11-49.6-N	038-08-26.0-E	2017
34	30289	Eastern Gojjam	Dejen	10-14-34.8-N	038-06-55.9-E	2017
35	30293	Eastern Gojjam	Dejen	10-14-25.5-N	038-13-18.6-E	2017
36	41229	Eastern Gojjam	Enarji Enawiga	10-41-05.7-N	038-11-10.6-E	1982
37	236493	Eastern Gojjam	Enarji Enawiga	10-41-10.4-N	038-10-28.5-E	1982
38	41231	Eastern Gojjam	Enarji Enawiga	10-43-49.1-N	038-09-36.7-E	1982
39	30301	Eastern Gojjam	Enarji Enawiga	10-41-05.7-N	038-11-10.6-E	2017
40	30302	Eastern Gojjam	Enarji Enawiga	10-41-10.4-N	038-10-28.5-E	2017
41	30304	Eastern Gojjam	Enarji Enawiga	10-43-49.1-N	038-09-36.7-E	2017
42	41026	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	1979
43	41078	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	1980
44	41267	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	1982
45	41268	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	1982
46	41269	Eastern Gojjam	Hulet ej enese	11-07-18.9-N	037-47-55.3-E	1982
47	30308	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	2017
48	30309	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	2017
49	30310	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	2017
50	30311	Eastern Gojjam	Hulet ej enese	11-06-24.5-N	037-47-06.7-E	2017
51	30312	Eastern Gojjam	Hulet ej enese	11-07-18.9-N	037-47-55.3-E	2017
52	41295	South Gonder	Fogera	11-56-10.4-N	037-42-30.7-E	1982
53	207145	South Gonder	Fogera	11-56-44.4-N	037-42-43.6-E	1983
54	30339	South Gonder	Fogera	11-56-10.4-N	037-42-30.7-E	2017
55	30340	South Gonder	Fogera	11-56-44.4-N	037-42-43.6-E	2017
56	30341	South Gonder	Fogera	11-57-07.5-N	037-42-51.4-E	2017

6.3.2. Experimental Site

The experiment was conducted at Debre Zeit Agricultural Research Center (DZARC) for two consecutive cropping seasons (2018/19 to 2019/20). The experimental site is located 47 km South East of Addis Ababa (8^{o} 44'N, 38^{o} 58' E) at an altitude of 1,860 meter above sea level (m.a.s.l.) (Figure 12). It receives an annual rainfall ranging from 412.9 to 926.9 mm with an annual mean precipitation of 867 mm. The temperature ranges from $9^{o}C$ to $26^{o}C$ with mean annual temperature of $17.7^{o}C$ (https://en.climate-data.org/). The dominant soil type is vertisol.

6.3.3. Experimental Design and Data Collection

Simple lattice design with two replications was used. Genotypes of current and previous collection were grown together and assigned to each plot randomly. Each genotype was sown in two rows with 3 m row length and 0.2 m spacing between rows and 0.1 m between plants. DAP (100 $Kgha^{-1}$) and other management practices were applied. Five individual plants were tagged randomly from each genotype per plot and they were used for morphological data collection. Chickpea descriptor (IBPGR ICRISAT and ICARDA, 1993) was used to score five qualitative (stem/foliage pigmentation, seed shape, seed testa texture, seed color and growth habit) and nine quantitative (plant canopy height, days to 50% flowering, days to 50% podding, days to 90% maturity, number of primary branches, number secondary branches, number of pods per plant, thousand seed weight and grain yield) traits.

6.3.4. Data Analysis

The genetic erosion of chickpea genotypes was estimated using the formula suggested by Hammer and Laghetti (2005).

GE = 100% - GI, where GE is genetic erosion, GI is genetic integrity which is the ratio of the number of samples (landraces) collected at different times multiplied by 100.

Qualitative Traits Analyses: The phenotypic frequencies of the qualitative traits were analyzed by the Shannon-Weaver diversity Index (H) to assess the diversity changes of each trait over time at farmers' field by zones and region. H was computed using GenStat 16th edition versions 4.2 - 7.1 14 statistical software (Payne *et al*., 2013) using the following formula described by Shannon (1948).

$H' = \sum_{i=1}^{n} p_i \, log_e^{p_i}$, where n is the number of phenotypic classes for a character and pi is the proportion of total number of entries in the i^{th} class, $\sum$ is summation of entries for the i^{th} "n" phenotypic class trait. The index was standardized to keep its value in the range of 0 to 1, by dividing H' by log_e^n (Hutcheson, 1970).

Quantitative Traits Analyses: Mean comparison test between current and previous collections were conducted for each of the quantitative traits for each collection site (eight sites in total) separately using *t*-test with 95% confidence interval by using MINTAB 14 statistical package (MINITAB, 1998). The significance of the difference between the population mean of current and previous collection for an individual site was tested using t table, i.e., when the calculated value of *t*-test was more than the tabulated t value, the difference was considered to be significant (Armitage *et al*., 2002).

The standard error of the difference between the two means is estimated using this formula:

$$SE(\bar{X}_1 - \bar{X}_2) = \sqrt{(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2})}$$

Degree of freedom (Df) is calculated as;

$$Df = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 \quad 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 \quad 1}}$$

A significance test of the null hypothesis is tested using the statistic

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

The approximate100 (1-α)% confidence limits are given by :

$$\bar{X}_1 - \bar{X}_2 \pm Z_\alpha SE(\bar{X}_1 - \bar{X}_2),$$

where; Z_a is the appropriate standardized normal deviate corresponding to the two-sided probability α, $\bar{X}_1\ \mathit{mean\ of\ sample}\ 1$is mean of sample 1, $\bar{X}_2\ \mathit{mean\ of\ sample}\ 2$ is mean of sample 2 $S_1^2\ \mathit{variance\ of\ sample}\ 1$ is variance of sample 1, $S_2^2\ \mathit{variance\ of\ sample}\ 2$ is variance of sample 2.

The performances of genotypes were tested for significance by performing an analysis of variance (ANOVA), using a General Linear Model (GLM) in a simple lattice design, using R statistical package (META-R, 2016). Mean separation at 1% or 5% probability level was conducted using Dunkan's Multiple Range Test (DMRT) following Gomez and Gomez (1984).

The statistical parameters such as mean, range, phenotypic, genotypic variance, and coefficient of variations were used to quantify the variability of each quantitative morphological trait. The phenotypic and genotypic variation and coefficient of variations was calculated following Singh and Chaundhary (1985) as follows:

$$Phenotypic\ Variance\ (V_p) = \frac{Genotypic\ MS}{r}$$

$$Error\ Variance\ (V_e) - \frac{Error\ MS}{r * s}$$

$$Genotypic\ Variance\ (V_g) = V_p - V_e$$

$$Phenotypic\ Coefficient\ of\ Variation\ (PCV) = 100 * (\frac{\sqrt{V_p}}{m})$$

$Genotypic\ Coefficient\ of\ Variation\ (GCV) = 100 * (\frac{\sqrt{V_g}}{m})$, where r is the number of replications, m is the mean value, and s is the seasons

Broad sense heritability (H) expressed as the percentage of the ratio of the genotypic variance to the phenotypic variance (Allard, 1960).

$H = \frac{V_g}{V_p}$, where Vg=genotypic variance; Vp=phenotypic variance

Genetic advance (GA) is the difference between the mean values of the generation obtained from the selected population over the mean value of the base population and it was measured using genetic advance (GA) (Allard, 1960).

$$Genetic\ Advance\ (GA) = i * h^2 * \sqrt{V_p}$$

$GA\ (\%\ of\ mean) = \left(\frac{GA}{m}\right) * 100$, where, m represents the mean value, i represents selection differential (varies depending upon the selection intensity and stands at 2.06 for selecting 5% of the genotypes (Singh and Chaudhary, 1985)

The mean values of all quantitative traits were standardized to the mean of zero and variance of unity to avoid bias that comes from the different scale of measurements before cluster and principal component analyses (PCA). A dendrogram was constructed using Ward's agglomerative hierarchical minimum variance method (Ward, 1963), employing the MINITAB 14 statistical package. Contribution of individual characters towards divergence was estimated according to the method described by Singh and Choudhary (1985). Grouping of variety into various clusters was completed and average intra and inter cluster distance were estimated. Genetic distance between clusters was determined using standardized Mahalanobis D^2statistics.

Mahalanobis D^2 were calculated as:

$$D_{ij}^2 = (X_i - X_j)' COV^{-1}(X_i - X_j)$$

Where;$D_{ij}^2 = the\ distance\ between\ cases\ i\ and\ j;$ is the distance between cases i and j; $X_j\ and\ X_j vectors\ of\ the\ values\ of\ the\ variables\ for\ cases\ i\ and\ j$ are vectors of the value of the variables for cases I and j

Principal component analyses (PCA) was computed following the formula described by Noirot *et al*. (1996) by using MINITAB 14 software.

The first PCA value (Y_1) is given by the linear combination of the variables X_1, X_2,…X_p

$$Y_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1p}X_p$$

The second principal component is calculated in the same way,

$$Y_2 = a_{21}X_1 + a_{22}X_2 + \cdots + a_{2p}X_p ,$$

This continues until a total of p principal components have been calculated, equal to the original number of variables.

6.4. Results

Qualitative Traits Diversity Analyses: The distribution of seed color classes was assessed in three districts from East Gojjam, four districts from North Gonder and one district from South Gonder. East Gojjam and North Gonder were represented by nearly equal number of genotypes. South Gonder was represented by a smaller number of genotypes (five genotypes for current and six genotypes for previous) and because of this South Gonder was included in North Gonder.

Qualitative trait diversity was assessed on the 134 homogenous genotypes. Result from the score of leaf/stem pigmentation, seed shape and texture, and growth habit, showed that the majority of the genotypes exhibited similar score for the respective traits. One hundred twenty-eight genotypes (95.5%: 41 from the current, 87 from the previous collections) had a pigmented leaf or stem (score 5), angular seed shape (Score 1), rough texture (score 1) and semi-erect growth habit (score 4). Hence, these qualitative traits were uniform for most genotypes and were abundant in both zones in both collection times. Six genotypes (4.5%: 41324-B, 41026-A, 41267-A, 207170-A, 41306-B and 41311-A) showed no leaf/stem pigmentation had white smooth coat and showed pea-shaped seeds (Figure 13). These characteristics were not observed in current collections and occurred at low frequencies in previous collections of the two zones (East Gojjam and North Gonder).

The distribution and frequencies of seed color classes and its genetic erosion by zones over years were indicated in Table 23. A range of 30.4% to 100% genetic erosion of chickpea genotypes was recorded at regional level. Highest genetic erosion was recorded for genotypes with white seed coat (100%) followed by with black seed coat (91.7%) from 1979 to 2017. Brown, light brown and dark brown seed color classes were dominant and distributed with more or less similar frequency at regional level. Black and ivory white seed class types occurred in low/nil frequencies in both zones. The pattern in the distribution of black (25.8% to 4.9%) and ivory white seed (6.5% to 0.0%) class type decreased overtime, while the remaining phenotypic classes increased and occurred in more or less similar frequency at regional level overtime.

The Shannon-Weaver diversity index estimates (H) for individual traits, and zones are presented in Table 24. The estimates of H were variable for individual traits and zones for current and previous collections. In East Gojjam zone, the H' estimates ranged from 0.64 for ivory white to 1.08 for light brown in previous collection and 0.0 for ivory white to 1.06 for light brown in the current collections. In North Gonder zone, the H' estimates ranged from 0.64 for ivory white to 1.52 light brown in previous collection and 0.0 for ivory white to 1.56 for brown in current collection. The estimate of diversity was higher in North Gonder (0.95) than East Gojjam (0.63).

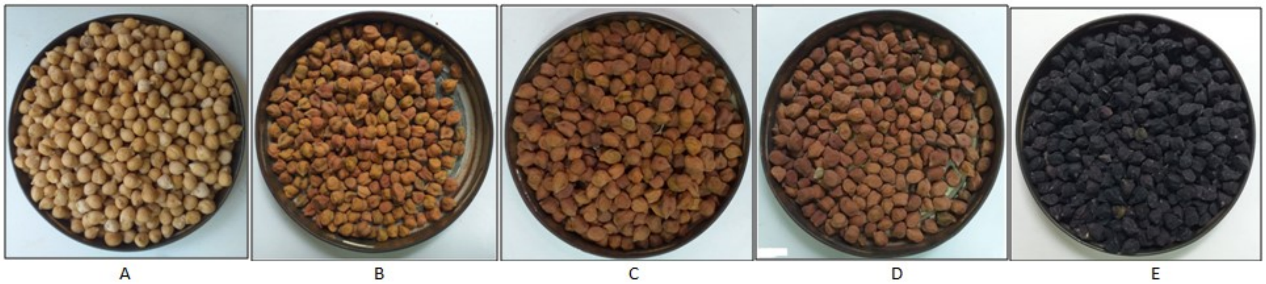

Figure 13. Diversity of seed characteristics in Ethiopian chickpea accessions: color (A-Ivory white, B-Brown, C-Light Brown, D-Dark Brown, and E-Black), texture (A-smooth, and B to E-rough), shape (A-Pea shape, B to E-Angular) and size (Desi-A to E)

Table 23. Seed color classes, weighted mean percentage, genetic integrity (GI), and genetic erosion (GE) of chickpea genotypes collections in two zones of the Amhara Regional State, Ethiopia of 1979-1983 and 2017

Zones	**Seed color types**	**Number of genotypes collected in**		**GI**	**GE**	**Percentage of seed color class in**	
		1979 to 1983	2017			1979 to 1983	2017
East Gojjam	Black	11	0	0.0	100.0	26.2	0.0
	Brown	9	8	88.9	11.1	21.4	47.1
	Light brown	12	5	41.7	58.3	28.6	29.4
	Dark	7	4	57.1	42.9	16.7	23.5
	Ivory white	3	0	0.0	100.0	7.1	0.0
	Total	42	17				
North Gonder	Black	13	2	15.4	84.6	25.5	8.3
	Brown	14	8	57.1	42.9	27.5	33.3
	Light brown	12	8	66.7	33.3	23.5	33.3
	Dark	9	6	66.7	33.3	17.6	25.0
	Ivory white	3	0	0.0	100.0	5.9	0.0
	Total	51	24				
Region	Black	24	2	8.3	91.7	25.8	4.9
	Brown	23	16	69.6	30.4	24.7	39.0
	Light brown	24	13	54.2	45.8	25.8	31.7
	Dark	16	10	62.5	37.5	17.2	24.4
	Ivory white	6	0	0.0	100.0	6.5	0.0
	Total	93	41				

Table 24. Shannon diversity index (H) and mean Shannon diversity with standard errors of mean for two zones, region and seed color character of 1979-1983 (P) and 2017 (C) collected chickpea genotypes

Zones	Black		Brown		Light brown		Dark brown		Ivory white		H mean ±SE	H mean ± SE
	P	C	P	C	P	C	P	C	P	C	P	C
East Gojjam	1.07	0.00	1.00	1.04	1.08	1.06	0.96	1.04	0.64	0.0	0.95±0.08	0.63±0.26
North Gonder	1.50	0.69	1.47	1.56	1.52	1.49	1.22	1.01	0.64	0.0	1.27±0.17	0.95±0.29
Region	1.99	0.69	1.95	1.99	1.99	1.99	1.79	1.70	1.33	0.0	1.80±0.13	1.27±0.4

Quantitative Traits Diversity Analyses: The group means values of each quantitative trait were tested for the current and previous collections of each individual collection site at 95% confidence interval (Appendix 5 for further details). There was a significant difference between current and previous collections for the mean value of plant canopy height, days to 50% flowering, days to 50% podding, days to 90% maturity, number of primary branches, number of secondary branches, number of pods per plant, thousand seed weight, and grain yield.

The result of ANOVA indicated that there were significant differences among genotypes for plant height, number of primary branch, number of secondary branch, number of pod per plant, days to flowering, days to podding, days to maturity, thousand seed weight, and grain yield at *p<0.01* probability level (Table 25). The effect of genotype by season interaction on the performances of genotypes was significant at *p<0.01* probability level.

Table 25. Variance for combined analyses over seasons for quantitative traits of 79 chickpea genotypes and two improved varieties grown at Debre Zeit in 2018/219 to 2019/2020 cropping seasons

Statistic	**PLH**	**NPB**	**NSB**	**NPPP**	**DTF**	**DTP**	**DTM**	**TSW**	**GY**
Genotype Variance	12.5	3.8	5.3	187.1	7.2	7.8	22.9	269.5	379058.5
GenxSeason Variance	2.9	0.4	1.0	16.4	5.9	9.0	3.2	55.6	72618.0
Seasonal Variance	0.1	0.2	0.7	0.7	1.4	1.3	0.1	58.7	132638.2
Residual Variance	2.7	0.8	0.8	31.7	3.3	4.6	8.5	65.5	56078.4
Grand Mean	34.5	6.4	8.0	51.7	44.8	56.3	97.2	155.3	1946.6
CV	4.8	13.7	11.3	10.9	4.0	3.8	3.0	5.2	12.2
No Replicates	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
No Seasons	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Genotype significance	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
GenxSeaon significance	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Season significance	**0.3**	**0.1**	**0.0**	**0.5**	**0.1**	**0.0**	**0.7**	**0.0**	**0.0**

PLH=Plant canopy height (cm), DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, NPB=Number of primary branches, NSB=Number secondary branches, NPPP=Number of pods per plant, TSW=Thousand Seed weight, GY in kg ha^{-1}=Grain yield

The mean values indicated that the differences among most genotypes for a given traits were significant (*P<0.05*). Wide mean ranges were observed for all quantitative traits in the genotypes (see Appendix 6 for further details). The range of the record of each measurement were as follows: 27.07 to 42.87cm for plant height, 2.97 to 10.14 for number of primary branch, 3.14 to 11.09 for number of secondary branch, 25.29 to 79.88 for number of pod per plant, 41.82 to 51.46 for days to flowering, 52.21 to 62.18 for days to podding, 86.29 to 103.87 for days to maturity, 126.58 to 166.66 gm for thousand seed weight and 1071.13 to 3264.4k g/ha for grain yield.

Mean separation test was done among means of quantitative traits for current and previous collections. Three genotypes 30324-A, 30319-A, and 30310-A, all from current collections, produced grain yield above the mean value of the best standard checks (Mastewal, 2641 $kgha^{-1}$) and, 22 (15 from current and 7 from previous collections) genotypes the majority from current collections, gave comparable grain yield with the best standard check . None of the genotypes mature later than the standard checks. Regarding thousand seed weight, since the standard checks are medium sized chickpea, they showed better thousand seed weight than the other which is desi chickpea type, however among the desi type, the majority of current collections produced better thousand seed weight than the previous chickpea collections (see Appendix 6 for further details). In all districts, most current collected genotypes had shown a significant difference in mean value ($p<0.05$) among previous collected genotypes for plant canopy height, days to flowering, days to 50% podding, days to maturity, number of primary branches, number secondary branches, number of pods per plant, and thousand seed weight and grain yield This further confirms that the existence of quantitative traits change over time.

Phenotypic and genotypic coefficients of variation results in the present study indicated that there were low to high level of phenotypic coefficient of variations and genotypic coefficient of variations. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values were recorded for days to flowering (7.2 and 5.97), days to podding (6.25 and 4.95), days to maturity (5.76 and 4.92), plant height (11.33 and 10.28), thousand seed weight (11.79 and 10.57), number of primary branch (33.56 and 30.64), number of secondary branch (31.02 and 28.9), number of pod per plant (28.6 and 26.45), grain yield (33.89 and 31.63). GCV and PCV ranges of 4.92 (days to maturity) to 31.63 (grain yield) and 5.76 (days to maturity) to 33.89 (grain yield) were estimated, respectively (Table 26).

The heritability of all traits considered for this study was high ranging from 63% to 87%. Regarding GA (as % of mean) low to high genetic advance % mean were obtained which ranged from 8.08% to 60.81 (Table 26).

Table 26. Mean genotypic (GVC) and phenotypic (PCV) coefficients of variation, genotypic (V_g), phenotypic (V_p) and environmental (V_e) variances, heritability (H), genetic advance (GA), genetic advance as percent of mean (GA % mean) of chickpea genotypes for quantitative traits for 2018/19 to 2019/2020 seasons

Traits	Mean	Vp	Vg	Ve	PCV	GCV	H	GA	GA % mean
PLH	34.46	15.23	12.54	2.69	11.33	10.28	0.82	6.62	19.21
NPB	6.38	4.59	3.82	0.76	33.56	30.64	0.83	3.68	57.62
NSB	7.99	6.13	5.33	0.81	31.02	28.90	0.87	4.43	55.47
NPPP	51.71	218.76	187.06	31.70	28.60	26.45	0.86	26.05	50.38
DTF	44.78	10.40	7.15	3.25	7.20	5.97	0.69	4.57	10.20
DTP	56.25	12.37	7.76	4.61	6.25	4.95	0.63	4.54	8.08
DTM	97.24	31.35	22.89	8.46	5.76	4.92	0.73	8.42	8.66
TSW	155.31	335.04	269.53	65.52	11.79	10.57	0.80	30.33	19.53
GY	1946.56	435136.96	379058.54	56078.43	33.89	31.63	0.87	1183.75	60.81

PLH=Plant canopy height (cm), DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, NPB=Number of primary branches, NSB=Number secondary branches, NPPP=Number of pods per plant, TSW=Thousand Seed weight, GY in kg ha^{-1}=Grain yield

The result of hierarchical cluster analyses indicated that the 81 genotypes were grouped into six clusters (Figure 14) with variable number of genotypes per cluster (Table 27). The highest number of genotypes grouped in Cluster IV (24 genotypes) and the least is in cluster VI (2 genotypes). Cluster I and III contained 19 and 9 genotypes respectively, and all of them were from the previous collections. Cluster II and V were composed of 7 and 20 genotypes respectively, and all were from current collections except 207143-B, 241801-C, 41026-B from cluster II and 41222-B from cluster V which were from previous collections. Cluster IV contained 15 genotypes from previous collections and 9 genotypes from current collections. Cluster VI was entirely consisted of improved genotypes. Cluster I and VI had biggest squared distance (10.366) from each other, while Cluster II and III were closer (2.216) (Table28) to each other.

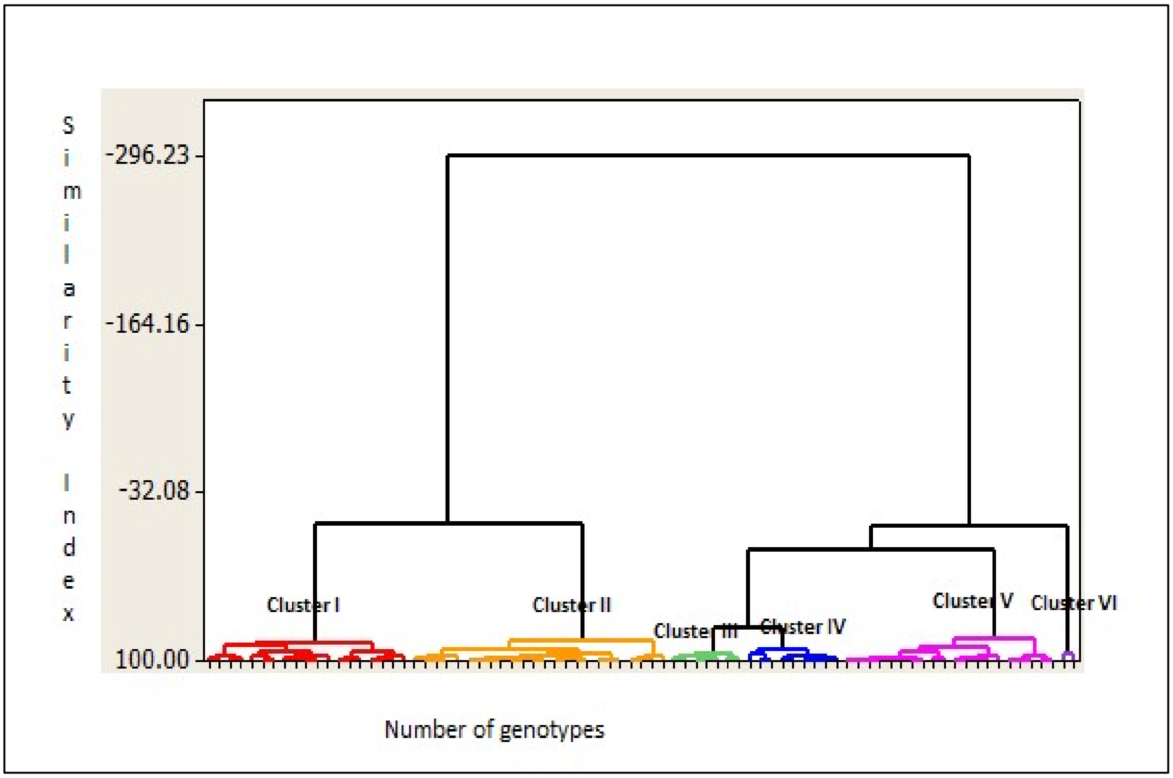

Figure 14. Dendrogram constructed using Ward's method based on quantitative morphological traits of 79 chickpea genotypes and two improved chickpea varieties

Table 27. Clustering of 79 chickpea genotypes and two improved chickpea varieties into six clusters using means of nine quantitative traits

Cluster Name	No of genotypes	Genotypes
Cluster I	19	207143-A, 207170-A, 227158-C, 41026-C, 41267-A, 41310-A, 41324-B, 207145-A, 225887-A, 227161-B, 41078-A, 41295-A, 41310-B, 207145-B, 227158-A, 227161-C, 41229-A, 41306-B, and 41322-B
Cluster II	7	207143-B, 241801 -C, 30301-C, 30311-A, 30322-B, 30341-A, and 41026-B
Cluster III	9	207166-A, 241801-A, 41223-A, 41231-A, 41231-B, 41268-B, 41269-B, 227160-B and 41046-A
Cluster IV	24	225887-B, 30301-A, 30312-A, 30331-B, 41078-B, 41268-A, 41311-B, 236493-A, 30302-A, 30323-A, 41026-A, 41222-A, 41295-B, 41322-A, 241800-A, 30304-A, 30323-B, 41046-B, 41223-B, 41311-A, 41324-C, 241800-B, 30309-A, and 30331-A
Cluster V	20	30287-A, 30293-A, 30317-A, 30320-A, 30325-A, 30329-A, 30340-A, 30288-A, 30308-A, 30318-A, 30321-A, 30326-A, 30332-A, 41222-B, 30289-A, 30310-A, 30319-A, 30324-A, 30327-A, and 30339-A
Cluster VI	2	Mastewal and Worku

Table 28. Pair-wise generalized squared distance among five clusters constituting 79 chickpea genotypes and two improved chickpea varieties (Distances)

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	0	4.264	6.103	2.806	5.412	**10.360**
Cluster II	4.264	0	**2.216**	2.231	2.314	7.016
Cluster III	6.103	**2.216**	0	4.125	3.383	6.050
Cluster IV	2.806	2.231	4.125	0	2.726	8.362
Cluster V	5.412	2.314	3.383	2.726	0	6.957
Cluster VI	**10.366**	7.0156	6.050	8.362	6.957	0

Principal Component Analyses (PCA) result indicated that the first two principal components (PCs), with eigen values greater than unity, explained about 81.9% of the total variation among accessions for all traits (Table 29). All the quantitative traits considered had contributed a comparable relative magnitude of eigenvectors for the first principal component, while number of pod per plant, days to flowering, days to podding, and grain yield had major contribution of eigenvectors in the second principal component.

Table 29. Eigen value, percentage and cumulative variances and eigenvectors on the first eight principal components for quantitative traits in 79 chickpea genotypes and two improved chickpea varieties

Parameters	**Principal Components (PCs)**							
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Eigenvalue	5.7824	1.5894	0.6302	0.3372	0.2394	0.2062	0.0975	0.073
Proportion	0.642	0.177	0.07	0.037	0.027	0.023	0.011	0.008
Cumulative	0.642	0.819	0.889	0.927	0.953	0.976	0.987	0.995
Variable	**Eigenvectors**							
PLH	-0.367	0.016	0.346	0.088	0.382	-0.703	-0.008	-0.281
NPB	-0.385	-0.119	-0.179	-0.286	-0.119	0.144	0.764	-0.277
NSB	-0.401	-0.139	-0.067	0.007	-0.014	-0.021	-0.026	0.035
NPPP	-0.328	-0.302	0.34	-0.555	-0.403	-0.002	-0.367	0.213
DTF	-0.254	0.605	0.053	-0.05	0.047	-0.087	0.232	0.707
DTP	-0.216	0.642	0.073	-0.22	0.059	0.366	-0.31	-0.503
DTM	-0.358	0.048	0.095	0.71	-0.565	0.077	-0.048	-0.072
TSW	-0.305	-0.032	-0.832	-0.034	0.064	-0.195	-0.348	0.052
GY	-0.341	-0.307	0.141	0.215	0.59	0.547	-0.087	0.191

PLH=Plant Canopy Height (cm), DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, NPB=Number of primary branches, NSB=Number secondary branches, NPPP=Number of pods per plant, TSW=Thousand Seed weight, GY in kg ha^{-1}=Grain yield

6.5. Discussion

The presence of wide genetic variability in cultivated crop species and its wild relatives is essential for plant breeders as it provides an opportunity to obtain a diverse form of genes which are associated with different types of desirable agronomic and quality traits. Genetic variability has reduced due to different manmade and natural calamities leading to genetic loss. The loss and gain of genetic variability will have a direct effect on the success of crop variety development. Loss of genetic diversity is a major concern especially for countries like Ethiopia which are considered as crop centers of origin or diversity. Previously, the amount and extent of genetic loss has been assessed for tetraploid wheat and sorghum and a significant amount of genetic erosion has occurred in Ethiopia (Yifru Teklu and Hammer, 2006; Bayush Tsegaye and Berg, 2007: Frew Mekbib, 2008; Hailemichael Shewayrga, *et al.,* 2008). However, assessment of the amount and the extent of phenotypic diversity change of chickpea landraces that occur over time have not been estimated until the current study. The aim of the present study was to assess the genetic variability of current (collected in 2017) and previous collections (collected from 1979 to 1983) of chickpea germplasm and to assess the existence of genetic loss in selected chickpea growing areas of the Amhara Regional State, Ethiopia.

Based on qualitative traits characterization, all genotypes did not show variation in stem/leaf pigmentation, seed shape, seed texture, and growth habit in both current and previous collections, except very few genotypes. These traits are not sufficient enough to see the level of diversity within Ethiopian desi type chickpea because the majority of the collections are uniform for these traits. Vishnyakova *et al*. (2017) also indicated that Ethiopian collections have a narrow genetic base amongst their qualitative traits, though the Ethiopian collections are unique, primitive type and endemic to Ethiopia.

Genetic erosion has occurred in all chickpea genotypes with different magnitude. The loss is more alarming for black and ivory white coated seed genotypes in all study sites. The frequency and Shannon-Weaver diversity index estimates (H) has decreased over time. The current collections are more or less uniform in seed color diversity in comparison to the previous collections. In the recent collections, no entry with black and ivory white seeds was obtained in most studied areas. However, black chickpea seeds are frequently observed in the previous collections. Vavilov (1927) also reported the occurrence of black seeded chickpea type in Ethiopia in 1927. White seed desi type chickpea existed in previous collection in very rare frequency. Vishnyakova *et al.* (2017) also reported their observation on the presence of white seeded desi chickpea type (pea types) from Ethiopian collections though it was rare. The black and ivory white chickpea seeds are likely nearing extinction because these types of seeds are either absent or have rare occurrence at farmers' field in the studied districts.

Desi ivory white chickpea seeds are categorized as pea-shaped chickpea type that are thought to have developed from a hybrid between desi and kabuli types chickpea (Muehlbauer and Abebe Tullu, 1997). Even in 1927, these chickpea types existed in Ethiopia (Vishnyakova *et al.*, 2017). To accept the above idea the origin of pea-shaped chickpea, both parents must be found in Ethiopia. In the EBI old collections, however, there was no accession or specimens containing kabuli types and also there was no report regarding the existence of kabuli chickpea types in Ethiopia in 1927. From this observation, two hypotheses can be derived. The first hypothesis is that these chickpeas types are exotic to Ethiopian, implying that they were introduced to Ethiopia some time before 1927, albeit there is no proof for this.Kabuli chickpea varieties have been introduced to Ethiopia since the 1970s, when the chickpea breeding program began (Asnake Fikre and Dagnachew Bekele, 2020). The second hypothesis is that kabuli chickpea varieties were introduced to Ethiopia some time before 1927, and pea-shaped chickpeas were formed as a result of a cross between kabuli and desi chickpea varieties. Then the kabuli chickpea types disappeared, which is really unusual. As a result, more research is needed to gain a better understanding of the origin of pea-shaped chickpea.

Generally, it is an unfortunate senario to witness the loss of black seed chickpea from farmers within the studied areas. Loss of the black seed means a loss of the health benefits of black chickpea. It is well known that desi type chickpea with black seed coats had the highest total phenolic, flavonoid, monomeric anthocyanin and proanthocyanidin contents than desi chickpea with brown and green seed coats and Kabuli chickpea (Segev *et al*., 2010; Egan and Wood, 2016; Ghosh *et al*., 2019). Because of the chemical composition of black chickpea seeds having numerous health benefits which include reduction of cholesterol levels. They are rich in iron, a powerhouse of phytochemicals which serve as antioxidants potentially preventing cancers, aids in digestion, regularizes blood sugar, stimulates weight loss, improves heart health, reduces inflammation, cures some skin diseases, reduces white spots in Leukoderma, treats fungal infections, arrests hair fall, clears dandruff and prevent grey hair (Nestel *et al*., 2004; Yang *et al.,* 2007; Abete *et al*., 2010; Jukanti *et al*., 2012; Mollard *et al*., 2012; Wallace *et al*., 2016).

Though the black seeded chickpea has a paramount importance to human health benefits, it receives less attention among plant breeders in chickpea development programs of Ethiopia and, moreover, there is no improved variety released with black seed coat to date. Additionally, the EBI gene bank has not implemented the restoration of black seeded chickpea genotypes in its original place of collection. It is clear that black seed chickpea faced a major threat of genetic erosion at farmers' level because of the substitution of a diverse set of genetically variable crop landraces with few genetically uniform improved varieties and partly because of a negative selection. Similar view was also observed during the collection time (in 2017) from traditional healers. Traditional healers claim that the local farmers' do not keep black seed chickpea. The local farmers sorted black seeds from chickpea populations to sell it at a higher price to traditional healers. This practice gradually affects the genetic composition of chickpea in the areas resulting in reduction of black seed from time to time which can be considered as a negative selection.

Based on *t-test*, the mean values of each quantitative trait of the current and previous collections were significant for individual sites. It is possible to draw information from this result that the populations mean values for quantitative traits of chickpea germplasm collected at different times are different. This indicates that the current and previous chickpea populations are quite different for the mean values for most agronomic triats and the level of diversity between the current collection and previous collection are also different.

Results from ANOVA and mean and range values of the analysed traits revealed the presence of significant difference among genotypes for the considered traits. The results have shown the existence of sufficient variability of agronomic traits among chickpea genotypes. Similar observation was reported in prior studies for chickpea (Parameshwarappa *et al*., 2012; Archak *et al*., 2016).

Result from LSD, from current collections, three genotypes out yielded and 22 genotypes gave comparable yield to the best standard check. The majority of current chickpea collections had better thousand seed weight than previous chickpea collections. The mean value of quantitative traits of current chickpea collections for each individual location are non-significant for most locations, while for previous chickpea collections some genotypes showed a significant mean difference among the others in all quantitative traits considered which means that genotypes of current collection had shown similar performance while previous collection did not. Looking at the performance of genotypes collected in the same locality but collected at different times, the performances were different. For example, genotypes 41222-A (previous collection) and 30287-A (current collection) collected from the same site from Dejen district gave 1895.7 kg/ha and 2435.13 kg/ha grain yield, respectively (Appendix 2.2 for details). From these result it is possible to conclude that current and previous chickpea populations are quite different and in addition the genotypes of current chickpea collection are homogeneous, while the previous collections are heterogeneous.

Low yield performances were observed in the previous collections as compared to the current collections. These genotypes were grown for this study after 35-38 years of conservation, because the genotypes were not subjected to either multiplication or regeneration for 35-38 years. As a result of this, the climate might be new to these genotypes when grown for experiment during 2019/20 which affect their agronomic performances. This is, of course, the drawback of *ex-situ* conservation. *Ex-situ* conservation does not maintain an evolutionary process that creates new germplasms (Hamilton, 1994) which can withstand the changing climate or agricultural management practices.

Regarding the current collections, the genotypes have been grown by the farmers for decades so that the genotypes may not face adaptation problem. However, due to environmental and human selection a loss in heterogeneity has been observed as management and preferential treatment among farmers often results in greater uniformity which results in loss of germplasm. Because of these reasons gene bank managers recommend *in-situ* conservation be implemented side by side with *ex-situ* conservation. *In-situ* conservation will help the evolutionary process to continue and allow genetic resources adapt to the changing environments (Horovitz and Feldman, 1991;

Hawkes, 1995). Similar findings were observed in other crops (Yifru Teklu and Hammer, 2006; Bayush Tsegaye and Berg, 2007; Firew Mekibib, 2008).

Crop genetic population variability arises as a result of the differences in the genetic makeup of individuals in the population, the environment and the interaction between genes with the environments (Allard, 1960). The variability is estimated using variance of these three components. These three variance components can be used to estimate phenotypic (PCV) and genotypic (GCV) coefficient of variations. According to Khorgade *et al.* (1985) PCV and GCV values greater than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10% and 20% to be medium. Based on this delineation, among 79 chickpea germplasms and two improved varieties low level of PCV and GCV values were recorded for days to flowering, days to podding, and days to maturity and; medium level for plant height, and thousand seed weight; and high level for number of primary branch, number of secondary branch, number of pod per plant, and grain yield. The difference between GCV and PCV values for each respective quantitative trait are close which indicates that the variation observed in the genotypes have a strong genetic bases. The result agreed with reports of various authors (Gemechu Kenene *et al.,* 2011; Parameshwarappa *et al.,* 2012; Archak *et al*., 2016; Vishnyakova *et al*., 2017).

Heritability is an important parameter to see the relationship between genotypic and phenotypic variance and estimate the inheritance of quantitative traits by providing the genetic gains that may be possible through selection (Pondery and Tiwari, 1983). High heritability estimates alone are not sufficient to depend on for selection to improve quantitative traits, rather it must be accompanied by substantial amount of GA % mean (Johanson *et al*., 1955). In this study, quantitative traits like plant height, number of primary and secondary branch, number of pod per plant, thousand seed weight and grain yield can be improved through selection with medium to high success rates, while days to flowering, days to podding and days to maturity will have a little chance to improve through conventional selection from naturally existing genetic variation. High degree of heritability and expected genetic advancement of quantitative traits were demonstrated among chickpea kabuli types (Upadhyaya *et al*., 2002; Parameshwarappa *et al*., 2012; Archak *et al*., 2016).

Cluster analyses grouped all the chickpea genotypes into six distinct clusters having variable number of individuals in each cluster. Cluster II and V contained individuals from the majority of current collections, while cluster I and III contained individuals solely from previous collection. Cluster VI was made entirely from improved genotypes. Genotypes having similar characteristics were grouped into the same cluster, while genotypes with different characteristics were grouped at different cluster. This indicates that that genotypes grouped in the same cluster had little divergence from each other with respect to the traits considered than individuals from different clusters. Moreover, genotypes were clustered relatively in respect to time of collection and irrespective of their source of origin. Similar grouping of genotypes irrespective of their origin was also reported for EBI collections by Gemechu Keneni *et al*. (2012a).

Based on principal component analyses (PCA), the value of principal components further confirms the presence of variation among genotypes and also indicates the level of contribution of each studied traits to the total variation. The result will be helpful for breeders to focus on certain traits which can be improved through conventional selection. The contribution of each trait to the total variation leads to different level of differentiation among the genotypes into different clusters. Traits with higher contribution to the total variations are more effective for variety development through selection than that trait that contributes fewer amounts. These results were in line with reports from Gemechu Keneni *et al*. (2013).

6.6. Conclusion and Recommendation

Current collections (2017) and previous collections (1979-1983) are distinct from each other in terms of the degree of phenotypic variability and agronomic performances. The genotypes of the current collections were more or less homogeneous for the studied traits, while genotypes from the previous collection were heterogeneous. The ongoing human selections among the current collections made the chickpea germplasm uniform which resulted in the loss of germplasm. Black and white seeded chickpea have been lost from farmers' field within the study area. The majority of the current collections performed better in major agronomic performance than the previous collections. High heritability coupled with high genetic advance percentages were recorded for most quantitative traits which further confirms the existence of high phenotypic variability among chickpea germplasms.

The above conclusions were made based on data from eight districts of two Zones in one region. To provide a more holistic approach consideration of all major chickpea growing areas of the country are required to obtain information at the national level on genetic variability and/or genetic erosion. Molecular studies are necessary to complement the phenotypic studies to ascertain the genetic distinctness of currently and previously collected chickpea genotypes. Immediate implementation of a restoration program is required to restore the lost genotypes in the studied districts. Additionally, strengthening on-farm conservation by establishing community seed banks in representative chickpea growing areas are required to supply landrace seeds to the farmers locally through farmers-to-farmers seed exchange programs.

Chapter 7

7. Overall Summary, Conclusions and Recommendations

Chickpea (*Cicer arietinum* L.) is a major food legume farmed in more than 50 countries in the world and in about 50 districts in Ethiopia. It is Ethiopia's third most important legume crop in terms of area coverage and production volume, as well as the second most important export commodity crop among legume crops, accounting for roughly a quarter of total legume export income. Chickpeas are used as a rotation crop, a break crop, a source of protein for the community, and animal fodder. Ethiopia is regarded as one of the chickpea's secondary diversity hotspots. Recognizing the aforementioned reasons, so far over 1,223 chickpea germplasm accessions have been gathered and stored in the Ethiopian Biodiversity Institute gene bank to protect the country's chickpea genetic resources. To establish successful conservation and improvement program, characterization and evaluation of the genetic make-up of Ethiopian chickpea germplasm accessions as well as a regular chickpea diversity monitoring activity in farmers' fields are required.

Plant germplasm is an essential source of important agronomic features that could be utilized in varietal development. These genetic resources, on the other hand, are constantly threatened with natural and human-caused pressures. Given the critical importance of various plants genetic resources to humanity's survival, major conservation and sustainable use measures are essential. As a result, many countries have prioritized germplasm conservation by creating their own gene banks, resulting in the *ex-situ, in-situ, and* on-farm conservation of a vast number of accessions. Germplasm conservation through maintaining the genetic integrity of the germplasm, as well as the use of genetic resources, are two of the most important operations carried out in the gene bank. Germplasm conservation, diversity monitoring, and germplasm utilization efforts should all be prioritized equally by researchers, because conservation without utilization and diversity monitoring is less meaningful. However, conservation has received more attention than diversity monitoring and utilization activities, indicating a research vacuum in the area of germplasm monitoring and sustainable utilization.

Crop genetic diversity changes throughout time for a variety of causes, and most crop species, whether wild or cultivated, are always at risk of genetic erosion. To measure the genetic loss or gain in farmers' fields, these diversity changes must be systematically evaluated on regular basis. Intensive germplasm characterization and evaluation are also important activities for efficient germplasm conservation and utilization because they provide information such as the magnitude and pattern of genetic diversity, as well as identifying economically important traits or genes in germplasm that can be used in breeding. Therefore, in the present study, the similarity, relationship and structure of chickpea germplasm was assessed, the capacity of Ethiopian chickpea germplasm was evaluated against low temperature under control and field environment, as well as the level of chickpea diversity change over years was quantified at farmers' fields. The main results achieved are summarized below.

Result from SSR analysis indicated the presence of considerable allelic richness per locus, relatively moderate to high PIC, Ho and He values, the presence of private alleles, high level of genetic diversity score, and high percentage of polymorphism which were all evidence for the existence of molecular variation among the analyzed chickpea genotypes. The results of genetic distances, PCoA, and genetic relationship analysis revealed that Ethiopian genotypes differed from exotic genotypes. Most of the improved genotypes released in Ethiopia were developed from exotic genotypes. This suggests that exotic germplasm was used for variety development rather than local genotypes in the chickpea breeding effort. The examined genotypes evolved from two populations identified through population structure analysis, with varied degrees of introgression of the two types into respective genotypes.

Concerning the field screening of genotypes for cold tolerance showed that the response to the frost effect was variable amongst genotypes as well as each genotype reacted differently at different developmental stages. During germination and seedling establishment, most Ethiopian genotypes performed well under frost stress. Several genotypes performed well throughout the vegetative and reproductive stages, however the majority of genotypes developed frost damage symptoms with different degrees. There were a wide range of genotypes' responses to frost damage during seed development stages. In both seasons, some genotypes (12.6 percent of the genotypes studied) consistently produced seed with a higher thousand seed weight and normal seed color. The remaining genotypes produced medium to shriveled seeds with a damaged seed coat and a medium to low thousand seed weight. A total of 83 and 85 genotypes were chosen as cold tolerant genotypes based on freezing tolerance rate (FTR) and frost survival rate (SR), respectively.

Seventy-two genotypes selected from the field experiment were tested for cold tolerance at the seedling stage in a growing chamber under controlled conditions. According to the results of the SR and FTR values, 26 chickpea genotypes were chosen as cold tolerant genotypes tested at seedling stage and were able to survive temperatures as low as -5^oC in both indices. The remaining genotypes are rated as medium to low cold tolerant genotypes. This result also revealed that Ethiopian genotypes had the genetic potential to withstand frost at the seedling stage.

Result from on-farm diversity monitoring, the Shannon-Weaver diversity index estimations and the distribution and frequency of seed color classes have reduced over time in the study area. The black and white seeded chickpea types experienced severe genetic erosion, while the other seed color classes had varying degrees of genetic erosion. Furthermore, in terms of the degree and amount of variability as well as agronomic performance, chickpea collections from 2017 (current) differ from those from 179-1983 (prior). The current collections are more homogeneous than the former ones, which were more varied. The likely causes include continual human selections and adoption of better cultivars, which have resulted in chickpea germplasm uniformity and germplasm losses.

When we examine the response of the 81 chickpea genotypes for cold tolerance at natural environment and controlled condition, under natural conditions, all genotypes did not show frost symptoms at seedling stages, even though the lower temperature records during this stage were -2.0^{o}C in Sept 2018 and -8.0^{o}C in Sept 2019 growing seasons. However, four genotypes were killed by frost stress at controlled environment when the temperature became -2^{o}C for two consecutive days during night time. The possible reason could be due the variable duration of frost occurrence at both screening conditions and it was higher under controlled condition which was sufficient enough to cause frost damages to the genotypes. Of course, these genotypes were likewise killed at vegetative stage under natural environments and rated as frost susceptible. About 22 frost tolerant genotypes were selected under both experimental conditions, while three genotypes from controlled condition and 20 genotypes from natural condition were selected as frost resistant separately. The remaining 27 genotypes were not selected under both experimental conditions. From this, we can conclude that it is advisable to use both approaches simultaneously to screen the frost resistance potential of chickpea genotypes because both approaches complement each other.

As a conclusion, First, Ethiopian chickpea genotypes exhibited high molecular variation among each other and they are unique to the exotic genotypes. Second, Ethiopian genotypes have the genetic potential for cold tolerance traits for use in chickpea breeding programs. Third, chickpea seed color and leaf/steam pigmentation levels were found to have a strong relationship with frost tolerance. Fourth, chickpea genotypes with black seed color and/or strong leaf/stem pigmentation outperformed genotypes with brown and white seed color and little or no pigmentation. Lastly, on-farm diversity of chickpea has reduced over time and specially black and creamy white types chickpeas were rare/ absent in the farmers' field.

From this study it is recommended that

1. Based on SSR research, it is recommended to use this result for efficient and systematic conservation and sustainable utilization of germplasm because it helps to avoid conserving duplicate genotypes and can also assist chickpea breeders in selecting diverse parental materials for crossing activities to take the advantage of heterosis. Despite the fact that this research showed preliminary evidence for the presence of genetic diversity, more research into marker trait associations is needed. As a result, a comprehensive investigation is needed to map the relationships of the markers with economically important agronomic features.
2. To screen germplasm for cold tolerance, we have to consider the time and the pattern of frost occurrence. These details are crucial when choosing cold tolerant screening indices. If frost occurs during seedling or vegetative stages, for example, choosing genotypes with high freezing tolerance rates (FTR) and frost survival rates (SR) is sufficient to select cold tolerant genotypes. FTR and SR values aid in the selection of cold tolerances at the seedling or vegetative phases. However neither index can assess genotype cold tolerance at the reproductive or seed development stages. It was discovered in this experiment that

some genotypes that were chosen as cold tolerant genotypes based on high FTR and SR values failed to produce normal seed.

If frost occurs at all phenological stages, it is critical to examine the capacity of cold tolerance genotypes at the reproductive and seed development stages. It is also critical to consider the FTR value, SR value, number of fertile and infertile pods, seed shriveling score, seed color, and thousand seed weight simultaneously in this scenario. As a result, genotypes with high FTR and SR values, as well as a large number of fertile pods, a small number of infertile pods, a low degree of seed shriveling, and a high thousand seed weight, are considered to select promising cold tolerant genotypes. Based on these criteria, 94 genotypes were identified as cold tolerant genotypes, indicating that Ethiopian chickpea genotypes could be a source of frost tolerance. As a result, it is suggested that these genotypes be used in future cold tolerant cultivar development programs by implementing multi-locations and multi-year field trials to test frost tolerance adaptation to a wider range of chickpea growing environments, as well as, as crossing parent material for cold tolerance gene sources.

3. Genotypes with black seed coat color and strong leaf/stem pigmentation performed better in frost screening experiments than genotypes with white or brown seed coat color and weak or no leaf/stem pigmentation. Seed color and leaf/stem pigmentations are suggested as potential indicators for chickpea cold tolerance. Further research into the genetic relationship and mechanism between seed color and pigmentations and frost tolerances is also required. Furthermore, identification of quantitative trait loci (QTLs) associated with gene controlling frost tolerances in chickpea is equally important.
4. As a pilot study, 8 districts selected from three Zones from one region were studied to see how diversity changed over time. However, similar research is needed in other chickpea-

growing regions across Ethiopia to estimate genetic variability change and/or genetic erosion status at the national level. To determine the genetic distinctness of currently and previously collected chickpea genotypes, molecular research must be conducted in addition to phenotypic studies. To restore the lost genotypes in the examined districts, an immediate restoration program must be implemented. Furthermore, strengthening on-farm conservation by establishing community seed banks in representative chickpea-growing areas is required to provide local genotype seeds to local farmers through farmers-to-farmers seed exchange. It is advisable to learn lessons from Ethio Organic Seed Action (local NGOs) experiences and achievements and be implemented to the study areas.

References

Abete, I., Astrup, A., Martínez, J. A., Thorsdottir, I., and Zulet, M. A. (2010). Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr. Rev.***68**(4):214-231.

Agarwal, G., Sabbavarapu, M.M., Singh, V.K., Thudi, M., Sheelamary, S., Gaur, P.M. and Varshney, R.K. (2015). Identification of a non-redundant set of 202 in silico SSR markers and applicability of a select set in chickpea (*Cicer arietinum* L.). *Euphytica***205**:381-394.

Ahmad, F., Gaur, P. and Croser, J. (2005). Chickpea (*Cicer arietinum* L.). **In**: *Genetic Resources, Chromosome Engineering and Crop Improvement-Grain Legumes*, pp. 187-217, (Singh, R. and Jauhar, P.P., eds) Vol. 1. CRC Press.

Akimoto, M., Shimamoto, Y. and Morishima, H. (1999). The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: a case study in Thailand. Genet. *Resour. Crop Evol*. **46**:419-425.

Allard, R.W. (1960). *Principle of Plant Breeding*. JhonWiley and Sons, New York.

Amina, B., Rida, M.M,. Abdelkader, A.A.. Sripada, U. and Semir, G.S.B. (2020). Genetic Diversity Analysis in Chickpea (*Cicer arietinum* L) Genotypes Grown in Northwestern Algeria using Microsatellite Markers (SSR). *Indian Journal of Agricultural Research***54**:129-138.

Andrews, C.J. (1996). How do plants survive ice? *Ann. Bot*. **78**:529-536.

Aravind, J., Sankar, S. M., Wankhede, D. P. and Kaur, V. (2020). AugmentedRCBD: analysis of augmented randomised complete block designs.R package version 0.1. 2.

Arbaoui, M. and Link, W. (2008). Effect of hardening on frost tolerance and fatty acid composision of leaves and stems of a set of faba bean (*Vicia faba* L.) genotypes. *Euphytica***162**(2):211-219.

Archak, S., Tyagi, R.K., Harer, P.N., Mahase, L.B., Singh, N., Dahiya, O.P., Nizar, M.A., Singh, M., Tilekar, V., Kumar, V. and Dutta, M. (2016). Characterization of chickpea germplasm conserved in the Indian National Genebank and development of a core set using qualitative and quantitative trait data. *Crop J.* **4**(5):417-424.

Armitage, P., Berry, G. and Matthews, J.N.S. (2002). *Statistical Methods in Medical Research*, 4th edition, Blackwell Science.

Arumuganathan, K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.***9**:208-219.

Asnake Fikre and Dagnachew Bekele (2020). Chickpea Breeding and Crop Improvement in Ethiopia: Past, Present and the Future. *Universal Journal of Agricultural Research* **8**(2):33-40.

Asnake Fikre, Assefa Funga, Lijalem Korbu, Million Eshete, Niguse Girma, Asrat Zewdie, Dagnachew Bekele, Redwan Muhamed, Ketema Daba and Ojiewo, C. (2018). Stability analysis in chickpea genotype sets as tool for breeding germplasm structuring strategy and adaptability scoping. *Ethiop J Crop Sci***6**(2):19-37.

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

Asnake Fikre; Tulu Degefu; Tesfaye Geleta; Thudi, M., Gaur, P. Ojiewo, C., Hickey. L. and Varshney, R.K. (2021). Rapid Generation Advance in Chickpea for Accelerated Breeding Gain in Ethiopia: What Speed Breeding Imply? *Ethiop. J. Agric. Sci*. **31**(1):1-10.

Awol Mohammed and Bulti Tesso (2018). Characterization and Assessment of Genetic Diversity for AgroMorphological Traits of Ethiopian Chickpea (*Cicer arietinum* L.) Landraces. *Uganda J. Agri. Sci.* **18**:1-13.

Badeck, F.W. and Rizza, F. (2015). A combined field/laboratory method for assessment of frost tolerance with freezing tests and chlorophyll fluorescence. *Agronomy***5**(1):71-88.

Bakht, J., Bano, A. and Dominy, P. (2006). The role of abscisic acid and low temperature in chickpea (*Cicer arietinum*) cold tolerance. II. Effects on plasma membrane structure and function*. J. Exp. Bot*. **57**:3707-3715.

Bakht, J., Bano, A., Shafi, M. and Dominy, P. (2013). Effect of abscisic acid applications on cold tolerance in chickpea (*Cicer arietinum* L.). *Eur. J. Agron.* **44**:10-21.

Baloch, F.S., Derya, M., Andeden, E.E., Alsaleh, A., Cömertpay, G., Kilian, B. and Özkan, H. (2015). Interprimer binding site retrotransposon and intersimple sequence repeat diversity among wild Lens species. *Biochem. Syst. Ecol*. **58**:162-168.

Bansal, K.C., Lenka, S.K. and Mondal, T.K. (2014). Review: Genomic resources for breeding crops with enhanced abiotic stress tolerance. *Plant Breed*. **133**(1):1-11.

Barry, M. B., Pham, J. L., Be´avogui, S., Ghesquie`re, A. and Ahmadi, N. (2008). Diachronic (1979-2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion. *Genet Resour Crop Evol***55**:723-733.

Basandrai, A.S., Basandrai, D., Duraimurugan, P. and Srinivasan, T. (2011). Breeding for biotic stress. **In**: *Biology and Breeding of Food Legumes*, pp. 220-240, (Pratap, A. and Kumar, J., eds). CAB International.

Bayush Tsegaye and Berg, T. (2007). Genetic erosion of Ethiopian tetraploid wheat landraces in Eastern Shewa, Central Ethiopia. *Genet Resour Crop Evol* **54**(4):715-726.

Behboudian, M.H., Ma, Q., Turner, N.C. and Palta, J.A. (2001). Reactions of chickpea to water stress: yield and seed composition. *J Sci Food Agric***81**:1288-1291.

Bekele Shiferaw and Hailemariam Teklewold (2007). Structure and functioning of chickpea markets in Ethiopia: evidence based on analyses of value chains linking smallholders and markets. IPMS Working Paper 6. 55pp. Nairobi (Kenya): ILRI.

Berger, J. D., Kumar, S., Nayyar, H., Street, K. A., Sandhu, J. S., Henzell, J. M., Kaur, J. and Clarke, H. C. (2012). Temperature-stratified screening of chickpea (*Cicer arietinum* L.)

genetic resource collections reveals very limited reproductive chilling tolerance compared to its annual wild relatives. *Field Crops Res* **126**:119-129.

Bevan, M.W. and Uauy, C. (2013). Genomics reveals new landscapes for crop improvement. *Genome Biol*. **14**:206-215.

Bharadwaj, C., Chauhan, S. K., Rajguru, G., Srivastava, R., Satyavathi, C. T., Yadav, S., Rizvi, A.H., Kumar, J. and Solank, K.R. (2010). Diversity analysis of chickpea (*Cicer arietinum*) cultivars using STMS markers. *Indian J. Agri. Sci.* **80**(11): 947-951.

Bharadwaj, C., Srivastava, R., Chauhan, S. K., Satyavathi, C. T., Kumar, J., Faruqui, A., Yadav, S., Rizvi, A.H. and Kumar, T. (2011). Molecular diversity and phylogeny in geographical collection of chickpea (*Cicer sp*.) accessions. *J. Genet*. **90**:e94-e100.

Bhasker, P., Nandwal, A. S., Kumar, N., Chand, G., Yadav, S. P., Devi, S. and Singh, S. (2017). High temperature significance of anthocyanins accumulation stress responses in chickpea (*Cicer arientinum* L.). *Int J Agric Innov Res* **6**:2319-2473.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism, *Am J Hum Genet*. **32**:314-331.

Bourion, V., Lejeune-Henaut, I., Munier-Jolain, N. and Salon, C. (2003). Cold acclimation of winter and spring peas: carbon partitioning as affected by light intensity. *Eur. J. Agron*. **19**:535-548.

Boyer, J. S., (1982). Plant productivity and environment. *Science* 218:443-448.

Brush, S., Kesseli, R., Ortega, R., Cisneros, P., Zimmerer, K. and Quiros, C. (1995). Potato diversity in the Andean center of crop domestication. *Conservation Biology***9**:1189-1198.

Buddenhagen, I.W. and 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, (Summerfield, R.J., ed). Dordrecht, Kluwer Academic.

Burstin, J., Deniot, G., Potier, J., Weinachter, C., Aubert, G. and Barranger, A. (2001). Microsatellite polymorphism in *Pisum sativum*. *Plant Breed***120**(4):311-317.

Cal, J.P. and Obendorf, R.L. (1972). Imbibitional chilling injury in *Zea mays* L. altered by initial kernel moisture and maternal parent. *Crop Sci*. **12**:369-373.

Castañeda-Álvarez, N.P., Khoury, C.K., Achicanoy, H.A., Bernau, V., Dempewolf, H., Eastwood, R.J., Guarino, L., Harker, R.H., Jarvis, A., Maxted, N., Müller, J.V., Ramirez-Villegas, J., Sosa, C.C., Struik, P.C., H. Vincent, H., and Toll, J. (2016). Global conservation priorities for crop wild relatives. *Nat. Plants*. **2**: 16022.

Castro, P., Millan, T., Gil, J., Merida, J., Garcia, M.L., Rubio, J. and Fernádez-Romero, M.D. (2011). Identification of chickpea cultivars by microsatellite markers. *J. Agric. Sci.***149**: 451-460.

Chaturvedi, S. K., Mishra, D. K., Vyas, P. and Mishra, N. (2009). Breeding for cold tolerance in chickpea. *Trends Biosci* **2**(2):1-4.

Chen, A., Gusta, L.V., Brûlé-Babel, A., Leach, R., Baumann, U., Fincher, G.B. and Collins, N.C. (2009). Varietal and chromosome 2H locus-specific frost tolerance in reproductive tissues of barley (*Hordeum vulgare* L.) detected using a frost simulation chamber. *Theor. Appl. Genet*. 119:685-694.

Chen, L.J., Xiang, H.Z., Miao, Y., Zhang, L., Guo, Z.F., Zhao, X.H., Lin, J.W. and Li, T.L. (2014). An overview of cold resistance in plants. *J. Agron. Crop Sci.***200:**237-245.

Chen, T.H.H., Yamamoto, S.D.K., Gusta, L.V., and Slinkard, A.E. (1983). Imbibitional chilling injury during chickpea germination. *J. Am. Soc. Horti. Sci*.**108**(6):944-948.

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

Choudhary, S., Sethy, N.K., Shokeen, B. and Bhatia, S. (2009). Development of chickpea EST-SSR markers and analysis of allelic variation across related species. *Theor Appl Genet.* **118**(3):591-608.

Clarke, H. J., Khan, T. N. and Siddique, K. H. M. (2004). Pollen selection for chilling tolerance at hybridization leads to improved chickpea cultivars. *Euphytica***139**:65-74.

Clarke, H.J. and Siddique, K.H.M. (2004). Response of chickpea genotypes to low temperature stress during reproductive development. *Field Crops Res* **90**(2):323-334.

Collard, B.C.Y., Jahufer, M.Z.Z., BrouwerJ.B. and Pang, E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and markerassisted selection for crop improvement: The basic concepts. *Euphytica***142**(1-2):169-196.

Croser, J,S,, Clarke, H.J., Siddique, K.H.M. and Khan, T.N. (2003). Low-temperature stress: implications for chickpea (*Cicer arietinum* L.) improvement. *Crit Rev Plant Sci.***22**(2):185-219.

CSA (Central Statistical Agency) (2019). Agricultural Sample Survey Report on Area and Production of Crops Private Peasant Holdings, Meher Season. Central Statistical Agency, statistical bulletin 589, Addis Ababa.

Cubero, J.I. (1975). The research on chickpea (*Cicer arietinum*) in Spain in Proceedings of the International Workshop on Grain Legumes, 13-16 Jan 1975, pp 117-122, International Crops Research Institute for the Semi-Arid Tropics., Hyderabad, India.

Cubero, J.I., Pieterse, A., Saghir, A. R. and Borg, S. (1986). Parasitic weeds on cool season food legumes. **In**: *World Crops: Cool Season Food Legumes*, pp. 549-561, (Summerfield, R.J., ed). Kluwer Academic, Dordrecht, the Netherlands.

Dabholkor, A.R. (1992). *Elements of Biometrical Genetics*. Concept Publishing Company. New Delhi.

Datta, S., Kaashyap, M. and Gupta, P. (2015). Development of EST derived microsatellite markers in chickpea and their validation in diversity analysis. *Indian J Biotechnol.* **14**:55-58.

de Oliveira, L.O. and Martins, E. R. (2002). A quantitative assessment of genetic erosion in ipecac (*Psychotria ipecacuanha*). *Genet. Resour. Crop Evol*. **49**:607-617.

Dereje Gorfu and Eshetu Ahmed (2003). Crops and Agro-ecological Zones of Ethiopia. Ethiopian Institute of Agricultural Research, P.O.Box 2003, Addis Ababa.

Doupis, G., Chartzoulakis, K., Beis, A. and Patakas, A. (2011). Allometric and biochemical responses of grapevines subjected to drought and enhanced ultraviolet-B radiation. *Aust J Grape Wine Res***17**:36-42.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull.***19**:11-15.

Earl, D.A. and von Holdt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genet. Resour.* **4**:359-361.

Edwards, D., Henry, R.J. and Edwards, K.J. (2012). Advances in DNA sequencing accelerating plant biotechnology. *Plant Biotechnol. J.***10**:621- 622.

Egan, N. and Wood, J. (2016). Investigating the diversity of chickpea seed coat phenolics.66th Australasian Grain Science Association (AGSA) Conference-"From the soil to the supermarket".Australasian Grain Science Association.

Ellis, R.H., Covell, S., Roberts E.H., and Summerfield, R.J. (1986). The influence of temperature on seed germination rate in grain legumes. XI. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. J. *Exp. Bot*. **37**(183):1503-1515.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol*. **14**:2611-2620.

FAO. (2010). 'Chapter 3 The state of ex situ conservation' The second report on the state of the world's plant genetic resources for food and agriculture, pp. 54-90

FAO. (2020). FAO in Ethiopia: Ethiopia at a glance. Retrieved from http://www.fao.org/ethiopia/fao-in-ethiopia/ ethiopia-at-a-glance/en/ on 07 Dec 2020

FAOSTAT. (2021). FAO (Food and Agriculture Organization of the United Nations), Rome. Available at: http://www.fao.org/statistics/en/. Accessed 3 May 2021.

Fiebelkorn, D. (2013). Characterization of selected winter hardiness traits in pea (*Pisum sativum* L.). MS thesis, North Dakota State Univ., Fargo.

Fiebelkorn, D. and Rahman, M. (2016). Development of a protocol for cold tolerance evaluation in rapeseed/canola (*Brassica napus* L.). *Crop J.* **4**(2):147-152.

Firew Mekbib (2008). Genetic erosion of sorghum (Sorghum bicolor (L.) Moench) in the centre of diversity, Ethiopia. *Genet Resour Crop Evol***55**(3): 351-364.

Flowers, T.J., Gaur, P.M., Gowda, C.L.L., Krishnamurthy, L., Samineni, S., Siddique, K.H.M., Turner, N.C., Vadez, V., Varshney, R.K. and Colmer, T.D. (2010). Salt sensitivity in chickpea. *Plant Cell Environ***33**:490-509.

Franzluebbers, A.J. (2005). Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. *Soil Tillage Res.* **83**:120-147.

Fridovich, I. (1986). Super oxide dismutases. **In**: *Advances in Enzymology and Related Areas of Molecular Biology,* pp 61-97, (Merister, A., ed). John Wiley & Sons, New York.

Gecit, H.H. (1991). Chickpea utilization in Turkey. **In**: *Proceedings of a Consultants Meeting*, pp. 69-74. ICRISAT, Andhra Pradesh, India.

Geletu Bejiga and van der Maesen L.J.G. (2006). *Cicer arietinum* L. **In**: *PROTA 1: Cereals and pulses*, (Brink, M. and Getachew Belay, eds). PROTA, Wageningen, Netherlands.

Geletu Bejiga, Million Eshete and Yadeta Anbessa (1996). Improved cultivars and production technology of chickpea in Ethiopia. Research Bulletin No. 2. DebreZeit Agricultural Research Center, Alemaya University of Agriculture, DebreZeit , Ethiopia.

Geletu Bejiga, Tadesse, N., Solh, M.B., Suliman, W., Abu-Zied, N. and Halila, H. (1988). Resistance breeding for wilt and root rot disease in chickpea. **In**: *World Crops: Cool Season Food Legume*, pp 267, (Summerfield, R.J., ed). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Gemechu Keneni, Endashaw Bekele, Emana Getu, Muhammad Imtiaz, Kifle Dagne and Fassil Asefa (2011). Characterization of Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Accessions for Response to Infestation by Adzuki Bean Beetle (*Callosobruchus chinensis* L.) II. Phenotypic Diversity. *Ethiop. J. Agric. Sci.***21**:66-83.

Gemechu Keneni, Endashaw Bekele, Fassil Assefa, Imtiaz, M., Tolessa Debele, Kifle Dagne and Emana Getu (2012a). Evaluation of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions for symbio-agronomic performance. *Renewable Agriculture and Food Systems***28**:338-349.

Gemechu Keneni, Endashaw Bekele, Fassil Assefa, M. Imtaz, Tolessa Debele, Kifle Dagne and Emana Getu (2013). Genetic variation and gains from selection for symbio-agronomic performance in Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions. *Int. J. Plant Breed* **7**:22-35.

Gemechu Keneni, Endashaw Bekele, Imtiaz, M., Kifle Dagne, Emana Getu and Fassil Assefa (2012b). 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.

Ghaffari, P., Talebi, R. and Keshavarzi, F. (2014). Genetic diversity and geographical differentiation of Iranian landrace, cultivars, and exotic chickpea lines as revealed by morphological and microsatellite markers. *Physiol Mol Biol Plants*. **20**:225-233.

Ghosh, A., Dadhich, A., Bhardwaj, P., Babu, J. N. and Kumar, V. (2019). Comparative analysis of metabolites in contrasting chickpea cultivars. *J. Plant Biochem. Biotechnol* **29**:253-265.

Gilbert, J.E., Lewis, R.V., Wilkinson, M.J. and Caligari, P.D.S. (1999). Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theor Appl Genet*. **98**:1125-1131.

Gilmour, S.J., Hajela, R.K. and Thomashow, M.F. (1988). Cold acclimation in Arabidopsis thaliana. *Plant Physiol*. **87**(3):745-750.

Gogoi, N., Farooq, M., Barthakur, S., Baroowa, B., Paul, S., Bharadwaj, N. and Ramanjulu, S. (2018). Thermal stress impacts on reproductive development and grain yield in grain legumes. *J. Plant Biol.* **61**(5):265-291.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & Sons.

Govindaraj, M., Vetriventhan, M. and Srinivasan, M. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int.* **2015**:1-15.

Graham, D. and Patterson, B.D. (1982). Response of plant to low, nonfreezing temperatures: protein, metabolism and acclimation. *Annu. Rev. Plant Physiol*. **33**:347-372.

Graner, A. and Kilian, B. (2012). NGS technologies for analyzing germplasm diversity in genebanks. *Brief. Funct. Genom.* **11**(1):38-50.

GRDC (Grains Research and Development Corporation), 2009. Frost risk fact sheet, Canberra, Australia.

Guarino, L. (1999). Approaches to measuring genetic erosion. In: Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, pp. 26-28, (Serwinski, J. and Faberova´, I., eds). Research Institute of Crop Production, Prague, Czech Republic, 21-23 June 1999.

Guler, M., Adak, MS. and Ulukan, H. (2001). Determining relationships among yield and some yield components using path coefficient analysis in chickpea (*Cicer arietinum* L.). *Eur. J. Agron.***14**:161-166.

Gupta, S., Nawaz, K. Parween, S., Roy, R., Sahu, K., Pole, A.K., Khandal, H., Srivastava, R., Parida, S.K. and Chattopadhyay, D. (2016). Draft genome sequence of Cicer reticulatum L., the wild progenitor of chickpea provides a resource for agronomic trait improvement. *Dna Research* 0(0):1–10.

Guy, C.L. (1990). Molecular mechanisms of cold acclimation **In:** *Environmental Injury to Plants*, pp. 35-61, (Katterman, F., ed). London, Academic Press Inc.

Hailemichael Shewayrga, Jordan, D. R. and Godwin, I. D. (2008). Genetic erosion and changes in distribution of sorghum (*Sorghum bicolor* L.(Moench)) landraces in north-eastern Ethiopia. *Plant Genet Resour* **6**(1):1-10.

Hajibarat, Z., Saidi, A., Hajibarat, Z. and Talebi, R. (2015). Characterization of genetic diversity in chickpea using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). *Physiol. Mol. Biol. Plants*. **21**:365-373.

Hamilton, M. B. (1994). Ex situ conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. *Conserv. Biol*. **8**:39-49.

Hammer, K. and Laghetti, G. (2005). Genetic erosion – examples from Italy[1,2]. *Genet Resour Crop Evol* **52:**629-634.

Hammer, K. and Yifru Teklu (2008). Plant genetic resources: selected issues from genetic erosion to genetic engineering. *J Agri Rural Dev Tropics Subtropics***109**(1):15-50.

Hammer, K., Knupffer, H., Xhuveli, L. and Perrino, P. (1996). Estimating genetic erosion in landraces-two case studies. *Genet. Resour. Crop Evol.***43**:329-336.

Harlan, J. (1992). *Crops and man. American society of agronomy*, crop science society of America, Madison, Wisconsin, pp. 63-262.

Hawkes, J.G. (1995). International workshop on dynamic in situ conservation of wild relatives of major cultivated plants: summary of final discussion and recommendations. *Israel J. of Bot*. **40**:529-536.

Hayward, H.D. and Breese, E.L. (1993). Population structure and variability. In: *Plant Breeding: Principles and Prospects*, pp. 16-29, (Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds). Chapman and Hall, Great Britain.

Heerden, P,D,R,V. and Krüger, G.H.J. (2000). Photosynthetic limitation in soybean during cold stress. *South African Journal of Science***96**(4):201-206.

Heidarvand, L., Amri, R.M., Naghavi, M.R., Farayedi, Y., Sadeghzadeh, B. and Alizadeh, K.H. (2011). Physiological and morphological characteristics of chickpea accessions under low temperature. *Russian J Plant Physi.***58:**157-163.

Henriquez, B., Olson, M., Hoy, C., Jackson, M. and Wouda, T. (2017). Frost tolerance of faba bean cultivars (*Vicia faba* L.) in central Alberta. *Canadian Journal of Plant Science* **98**(2):509-514.

Henry, R.J. (2014). Genomics strategies for germplasm characterization and the development of climate resilient crops. *Frontiers in plant science*. **5**(68):1-4.

Horovitz, A., and Feldman, M. (1991). Evaluation of the wild-wheat study at Ammiad: Population dynamics of the wheat progenitor, *Triticum turgidum var. dicoccoides,* in a natural habitat in Eastern Galilee. *Israel Journal of Botany***40**(5-6):501-508.

Huang, L., Deng, X., Li, R., Xia, Y., Bai, G., Siddique, K.H.M. and Guo, P. (2018). A fast silver staining protocol enabling simple and efficient detection of SSR markers using a non-denaturing polyacrylamide gel. *Journal of visualized experiments***134**:e57192.

Hughes, M.A. and Dunn, M.A. (1990). The effect of temperature on plant growth and development. *Biotech. Genet. Engin. Rev.***8**:161-188.

Hulse, J.H. (1991). Nature, composition and utilization of pulses. **In:***Uses of Tropical Grain Legumes, Proceedings of a Consultants Meeting*, pp.11-27. ICRISAT, Andhra Pradesh, India.

Hutcheson, K. (1970). A test for comparing diversities based on the Shannon formula. *J. Theor. Biol*. **29**:151-154.

Hüttel, B., Winter, P. and Weising, K. (1999). Sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Genome* **42**:210-217.

IBPGR, ICRISAT and ICARDA. (1993). Descriptors for Chickpea (*Cicer arietinum* L.). International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India and International Center for Agriculture Research in the Dry Areas. Aleppo, Syria.

ICB. (2012). *International Crop Biodiversity*; ICB: Addis Ababa, Ethiopia.

Inci, N. E. and Toker, C. (2011). Screening and selection of faba beans (*Vicia faba* L.) for cold tolerance and comparison to wild relatives. *Genet Resour Crop Evol* **58**(8):1169-1175.

Jacked, D., Peng, K., Feinstein, D., and Kilian, A. (2001). Diversity array: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res* 29:4e25.

Jarvis, D.I., Myer, L., Klemick, H., Guarino, L., Smale, M., and Brown, A.H.D. (2000). A training guide for in situ conservation on-farm. Version 1. International Plant Genetic Resources Institute, Rome, Italy.

Jha, U.C., Chaturvedi, S.K. Bohra, A., Basu, P.S., Khan, M.S., and Barh, D. (2014). Abiotic stresses, constraints and improvement strategies in chickpea. *Plant Breed.***133**:163-178.

Johanson, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimates of genetic and environmental variability in Soybean. *Agron.J.***47**:314-318.

Joshi, P.K., Rao, P., Gowda, C.L.L., Jones, R.B., Silim, S.N., Saxena, K.B. and 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.

Jukanti, A.K., Gaur, P.M., Gowda, C.L.L. and Chibbar, R.N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. *Brit J Nutr.***108**:11-26.

Kahraman, A., Kusmenoglu, I. N., Aydin, Aydogan, A., Erskine, W. and Muehlbauer, F.J. (2004). QTL mapping of winter hardiness genes in lentil. *Crop Sci.***44**:13-22.

Kaloki, P., Devasirvatham, V., and Tan, D.K. (2019). Chickpea Abiotic Stresses: Combating Drought, Heat and Cold. **In**: *Abiotic and Biotic Stress in Plants*. Intech Open.

Kanouni, H., Khalily, M. and Malhotra, R. S. (2009). Assessment of cold tolerance of chickpea at rainfed highlands of Iran. *Am-Eurasian J Agric Environ Sci* **5**:250-254.

Kaur, G., Kumar, S., Nayyar, H., and Upadhyaya, H.D. (2008). Cold stress injury during the pod-filling phase in chickpea (Cicer arietinum L.): Effects on quantitative and qualitative components of seeds. *J Agron Crop Sci***194**:457-464.

Kaur, S., Gupta, A. K., Kaur, N., Sandhu, J. S. and Gupta, S. K. (2009). Antioxidative enzymes and sucrose synthase contribute to cold stress tolerance in chickpea. *J. Agronomy Crop Sci.***195**:393-397.

Kebede Teshome. 2012. Genetic Diversity, Population Structure, Association Mapping and Development of Core Collection in Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm. Ph.D. Dissertation. Haromaya University, Ethiopia.

Khorgade, P.W., Narkhede, M.N. and Raut, S.K. (1985). Genetic variability studies in chickpea. *Int. Chickpea Newsl*.**12**:12-13.

Khoury, C., Laliberté, B. and Guarino, L. (2010). Trends in ex situ conservation of plant genetic resources: a review of global crop and regional conservation strategies. *Genet.Resour. Crop Evol.***57**(4):625-639.

Knight, H., Trewavas, A.J., and Knight, M.R. (1996). Cold calcium signaling in Arabidopsis involves two cellular pools and a change in calcium signature after acclimation. *The Plant Cell*. **8**(3):489-503.

Kujur, A., Bajaj, D., Upadhyaya, H.D., Das, S., Ranjan, R., Shree, T., Saxena, M.S., Badoni, S., Kumar, V., Tripathi, S., Gowda, C.L.L., Sharma, S., Singh, S., Tyagi, A.K, Swarup K. and Parida, S.K. (2015). A genome-wide SNP scan accelerates trait-regulatory genomic loci identification in chickpea. *Sci. Rep.* **5**:11166.

Kumar, K. Sardana, S., Singh, M. and Gautam, N.K. (2016). Management of Germplasm Collections in Chickpea. *International Journal of Environment, Agriculture and Biotechnology* **1**(3):565-576.

Kumar, S., Kaur, G. and Nayyar, H. (2007). Exogeneous application of abscisic acid improves cold tolerance in chickpea (*Cicer arietinum* L.). *J. Agron. Crop Sci*. **194**:449-456.

Kumar, S., Nayyar, H., Bhanwara, R.K. and Upadhyaya, H.D. (2010). Chilling stress effects on reproductive biology of chickpea. *J SAT Agric Res*. **8**:1-14.

Ladizinsky, G. (1975). A new cicer from Turkey. *Notes of the Royal Botanic Garden Edinburgh***34**:201-202.

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

Lang, V., Mantyla, E., Welin, B., Sundberg, B., Palva, E.T. (1994). Alterations in water status, endogenous abscisic acid content, and expression of rab18 gene during the development of freezing tolerance in Arabidopsis thaliana. *Plant Physiology***104**(4):1341-1349.

Lawson, D.**J.,** van Dorp, L. and Falush, D. (2018). "A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots". *Nature Communications*. 9 (1): 3258.

Lee, T.M., Lur, H.S., and Chu, C. (1997). Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings: II. Modulation of free polyamine levels. *Plant Science***126**(1):1-10.

Levitt, J. (1980). *Responses of plants to environmental stress: chilling, freezing and high temperature stresses*, Academic Press, New York, 1980.

Lev-Yadun, S., Gopher, A. and Abbo, S. (2000). The cradle of agriculture. *Science***288**:1602-1603.

Liu, K.J. and Muse, S.V. (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics***21**: 2128-2129.

Liu, R., Fang, L., Yang, T., Zhang, X., Hu, J., Zhang, H., Han, W., Hua, Z., Hao, J. and Zong, X. (2017). Marker-trait association analysis of frost tolerance of 672 worldwide pea (*Pisum sativum* L.) collections. *Sci. Rep.* **7**(1):1-10.

Lulseged Tamene Tilahun Amede, Kihara, J. Degefie Tibebe, Schulz, S. (2017). A review of soil fertility management and crop response to fertilizer application in Ethiopia: towards development of site- and context specific fertilizer recommendation. CIAT publication no. 443. Addis Ababa. pp. 86.

Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys.* **444**(2):139-158.

Malhotra, R.S. and Singh, K.B. (1990). The inheritance of cold tolerance in chickpea. *J. Genet. Breed.* **44**:227-230.

Malhotra, R.S., Pundir, R.P.S. and Slinkard, A.E. (1987). Genetic resources of chickpea. **In**: *The Chickpea*, pp. 67-81, (Saxena, M.C., Singh, K.B., eds). CAB International: Wallingford, UK.

Maphosa, L., Richards, M.F., Norton, S.L. and Nguyen, G.N. (2020). Breeding for Abiotic Stress Adaptation in Chickpea (*Cicer arietinum* L.): A Comprehensive Review. *Crop Breed Genet Genom.***2(4):**e200015.

Maqbool, A., Shafiq, S. and Lake, L. (2010). Radiant frost tolerance in pulse crops-a review. *Euphytica***172**:1-12.

Matthews, S., Beltrami, E., El-Khadem, R., Khajeh-Hosseini, M., Nasehzadeh, M. and Urso, G. (2011). Evidence that time for repair during early germination leads to vigour differences in maize. *Seed Sci. Tech*. **39**:501-509.

Matus, I.A. and Hayes, P.M. (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome***45**:1095-1106.

Maya, M. and Maphosa, M. (2020). Current status of chickpea production: Opportunities for promoting, adoption and adapting the crop in Zimbabwe: A review. *J. Dryland Agric.***6**(1):1-9.

Mazhar, F. (1997). Nayakrishi Andoland: an initiative of the Bangladesh peasants for a better living. **In**: *Using diversity: enhancing and maintaining genetic resources on-farm*, (Sperling L, and Loevinsohn M., eds) International Development Research Centre, Ottawa.

McCouch, S., Baute, G.J., Bradeen, J., Bramel, P., Bretting, P.K., Buckler, E., Burke, J.M., Charest, D., Cloutier, S., Cole, G. and Dempewolf, H. (2013). Agriculture: Feeding the future. *Nature* **499** (7456):23-24.

McKersie, B. D. and Bowley, S. R. (1997). Active oxygen and freezing tolerance in transgenic plants. **In**: *Plant Cold Hardiness, Molecular Biology, Biochemistry and Physiology*, pp. 203-214, (Li, P.H. and Chen, T.H.H., eds). Plenum, New York.

Meilleur, B.A. and Hodgkin, T. (2004). *In situ* conservation of crop wild relatives: status and trends. *Biodiv. Conser*. **13**(4):663-684.

Meirmans, P.G. and Hedrick, P.W. (2010). Assessing population structure: FST and related measure. *Molecular Ecology Resources* 11(1): 5-18.

Meki Shehabu, Seid Ahmed and 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 J Pest Manage* **54**(2):143-149.

Menon, M., Barnes, W. J. and Olson, M. S. (2015). Population genetics of freeze tolerance among natural populations of *Populus balsamifera* across the growing season. *New Phytol.* **207**:710-722.

META-R (Multi Environment Trial Analyses with R for Windows) (2016). META-R users guide, Version 6.0 Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

Millan, T., Clarke, H.J., Siddique, K.H.M., Buhariwalla, H.K., Gaur, P.M., Kumar, J., Gil J., Kahl G. and Winter, P. (2006). Chickpea molecular breeding: new tools and concepts. *Euphytica***147**:81-103.

MINITAB. (1998). MINITAB users guide, release 14 .MINITAB Inc

Mir, A.H., Bhat, M.A., Fayaz, H., Dar, S.A., Maqbool, S., Bhat, N.A., Thudi, M. and Mir, R.R . (2019). Assessment of cold tolerance in chickpea accessions in North-Western Himalayas of Jammu and Kashmir, India. *J Pharm Phytochem* **8(4)**:2268-2274.

MoA. (2018). Crop variety register. Issue No. 21. Ministry of Agriculture (MoA), Addis Ababa, Ethiopia.

MoFED (Ministry of Finance and Economic Cooperation). (2016). Growth and Transformation Plan II (GTP II) (2015/16-2019/20) Volume I: Main Text. National Planning Commission, Addis Ababa.

Mollard, R. C., Luhovyy, B. L., Panahi, S., Nunez, M., Hanley, A., & Anderson, G. H. (2012). Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults. *Br J Nutr.***108**(S1):S111-S122.

Moore, K., Jenkins, L., Hertel, K., and Callaghan, G. (2010). Chickpea in 2010: Low temperature effects in 2009. GRDC update paper. Available at: www.grdc.com.au

Morgan, P.W. and Drew, M.C. (1997). Ethylene and plant response to stress. *Physoil. Plant.***100**:620-630.

Muehlbauer F.J. and Abebe Tullu (1997). New crop fact sheet: *chickpea Cicer arietinum* L. New York Times. http://hort.purdue.edu/new crop/crops/ crop fact sheets/chickpea.html

Muehlbauer, F.J. (1993). Food and grain legumes. **In**: *New Crops*, pp. 256–265 (Janick J. and Simon, J.E., eds). John Wiley & Sons, Inc., New York.

Mugabe, D., Coyne, C.J., Piaskowski, J., Zheng, P., Ma, Y., Landry, E., McGee, R., Main, D., Vandemark, G., Zhang, H. and Abbo, S. (2019). Quantitative trait loci for cold tolerance in chickpea. *Crop Sci* **59(2)**:573-582.

Mulugeta Assefa, Beneberu Shimelis, Punnuri, S., Sripathi, R., Whitehead, W. and Singh, B. (2014). Common Bean Germplasm Diversity Study for Cold Tolerance in Ehtiopia. *Am. J. Plant Sci.***5:**1842-1850.

Mulugeta Atnaf, Yao, N., Martina, K., Kifle Dagne , Dagne Wegary, and Kassahun Tesfaye (2017). Molecular genetic diversity and population structure of Ethiopian white lupin landraces: Implications for breeding and conservation. *PLoS ONE* **12**:e0188696.

Muoni, T., Barnes, A. P., Öborn, I., Watson, C. A., Bergkvist, G., Shiluli, M. and Duncan, A. J. (2019). Farmer perceptions of legumes and their functions in smallholder farming systems in east Africa. *Int. J. Agric. Sustain.* **17**(3):205-218.

Nayak, S.N., Zhu, H. and Varghese, N. (2010). Integration of novel SSR and gene base SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. *Theor Appl Genet.* **120**:1415-1441.

Nayyar, H. and Walia, D.P. (2004). Genotypic variation in wheat in response to water stress and abscisic acidinduced accumulation of osmolytes in developing grains. *J. Agron. Crop Sci*. **190**:39-45.

Nayyar, H., Bains, T., and Kumar, S. (2005a). Low temperature induced flower abortion in chickpea: relationship to abscisic acid and cryoprotectents in reproductive organs. *Environ Exp Bot***53**:39-47.

Nayyar, H., Bains, T.S. and Kumar, S. (2005b). Chilling effects during seed filling on accumulation of seed reserves and grain yield. *J. Sci Food Agric.***85**:1925-1930.

Nayyar, H., Kaur, G., Kumar, S. and Upadhyaya, H. D. (2007). Low temperature effects during seed filling on chickpea genotypes (*Cicer arietinum* L.): probing mechanisms affecting seed reserves and yield. *J. Agron. Crop Sci.* **193**(5):336-344.

Nei, M., Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. J *Mol Evol*. **19**:153-170.

Nestel, P., Cehun, M. and Chronopoulos, A. (2004). Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. *Am. J. Clin. Nutr.* **79**(3):390-395.

Nezami, A., Bandara, M. S. and Gusta, L. V. (2012). An evaluation of freezing tolerance of winter chickpea (*Cicer arietinum* L.) using controlled freeze tests. *Can. J. Plant. Sci.***92**(1):155-161.

Nigusie Girma, Asnake Fikre and Ojiewo, C.O. (2017). The Genotypic and Phenotypic Basis of Chickpea (*Cicer arietinum* L.) Cultivars for Irrigation-Based Production in Ethiopia. *J. Agric. Sci.* **9**:229-236.

Noirot, M., Hamon, S., and Anthony, F. (1996). The principal component scoring: a new method of constituting a core collection using quantitative data. *Genet. Reso. Crop Evol*. **43**(1):1-6.

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.***13**:1143-1155.

Ohri, D. and Pal, M. (1991). The origin of chickpea (*Cicer arietinum* L.): Karyotype and nuclear DNA amount. *Heredity* **66**:367-372.

Olien, C.R. and Smith, M.N. (1981). In: *Analysis and Improvement of Plant Cold Hardiness*, pp. 215. CRC Press, Boca Raton, Florida.

Parameshwarappa, S. G., Salimath, P. M., Upadhyaya, H. D., Patil, S. S. and Kajjidoni, S. T. (2012). Genetic variability studies in minicore collection of chickpea (*Cicer arietinum* L.) under different environments. *Karnataka J. Agric. Sci.* **25**(3):305-308.

Park, Yong-Jin, Lee, J. K. and Kim, Nam-Soo. (2009). Simple Sequence Repeat Polymorphisms (SSRPs) for Evaluation of Molecular Diversity and Germplasm Classification of Minor Crops. *Molecules***14**:4546-4569.

Parween, S., Kashif Nawaz, Riti Roy, Anil K. Pole, B. Venkata Suresh, Gopal Misra, Mukesh Jain, Gitanjali Yadav, Swarup K. Parida, Akhilesh K. Tyagi, Sabhyata Bhatia & Debasis Chattopadhyay (2015) An advanced draft genome assembly of a desi type chickpea (*Cicer arietinum* L.). *Sci. Rep.* **5**:12806.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. and Soutar, D. (2013). "GenStat for Windows (16th Edition) Introduction," VSN International.

Peakall, R. and Smouse, P.E. (2012). GenAlEx 6.502: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics***28**:2537-2539.

Pondey, R.L. and Tiwari, A.S. (1983). Heritability and genetic gain in chickpea. International chickpea Newsletter, no. 25, pp. 5-6.

Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J., and Jansen, M.A.K. (2007). Stress-induced morphogenic responses: growing out of trouble. *Trends Plant Sci***12**:98-105.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996).The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breed*. **2**:225-38.

Pritchard J.K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics***155**:945-959.

Provan, J., Russell, J.R., Booth, A. and Powell, W. (1999). Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus Hordeum. *Mol. Ecol*. **8**: 505-511.

Qadir,S. A., Datta, S., Singh, N. P. and Kumar, S. (2007). Development of highly polymorphic SSR markers for chickpea (*Cicer arietinum* L.) and their use in parental polymorphism. *Indian J. Genet*., **67**:329-333.

Rabara, R.C., Ferrer, M.C, Calayugan, M.I.C., Duldulao, M.D. and Jara-Rabara, J. (2015). Conservation of rice genetic resources for food security. *Adv. Food Technol. Nutr. Sci. Open J.***SE**(1):S51-S56.

Radhika, P., Gowda, S.J.M., Kadoo, N.Y., Mhase, L.B., Jamadagni, B.M., Sainani, M.N., Chandra, S. and Gupta, V.S. (2007). Development of an integrated map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. *Theor Appl Genet*. **115**:209–216.

Rao, N.K. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotechnol.***3** (2):136-145.

Rowland, I., Mason, M., Pritchard, I. and French, R. (1994). Effect of field peas and wheat on the yield and protein content of subsequent wheat crops grown at several rates of applied nitrogen. *Aust. J. Exp. Agric*. 34:641-646.

Ryan, J. (1997). A global perspective on pigeonpea and chickpea sustainable production system: present status and future potential. **In**: *Recent Advances in Pulses Research in India*, pp. 1-31, (Asthana, A. and Kanpur, A. M., eds). Indian Society for Pulses Research and Development, Kalyanpur, Kanpur.

Sajja, S.B., Samineni, S. and Gaur, P.M. (2017). Botany of Chickpea. **In**: *The Chickpea Genome*, pp 13-24, (Varshney R., Thudi M., and Muehlbauer F., eds). Compendium of Plant Genomes. Springer, Cham.

Sallam, A., Arbaoui, M., El-Esawi, M., Abshire, N. and Martsch, R. (2016). Identification and Verification of QTL Associated with Frost Tolerance Using Linkage Mapping and GWAS in Winter Faba Bean. *Front. Plant Sci.***7**:1098.

Sallam, A., Martsch, R. and Moursi, Y.S. (2015). Genetic variation in morpho-physiological traits associated with frost tolerance in faba bean (*Vicia faba* L.). *Euphytica* **205**(2):395-408.

Sassenrath, G. E., Ort, G. E. and Portis, A. R. (1990). Impaired reductive activation of stomatal biophosphatases in tomato leaves following low temperature exposure at high light. *Arch. Biochem. Biophy.***282**:302-308.

Savithri, K.S., Ganapathy, P.S., and Sinha, S.K. (1980). Sensitivity to low temperature in pollen germination and fruit set in *Cicer arietinum* L. *J Exp Bot***121**(31):475-81.

Saxena, M,C, (1990). Problems and potentials in chickpea production in the nineties. **In:** *Chickpea in the Nineties. Proceedings of the 2nd International Workshop on Chickpea*

Improvement, pp. 13-25, (Van Rheenen, H.A., Saxena, M.C., eds). ICRISAT, Patancheru, India.

Saxena, M.C., Saxena, N.P. and Mohammed, A.K. (1988). High temperate stress. **In**: *World Crops: Cool-Season Food Legumes,* pp. 845-856, (Summerfield, R.J., ed). Kluwer Academic Publisher, Dordrecht, the Netherlands.

Saxena, N.P, Johensen, C. Saxena, M.C. and Silim, S.N. (1993). Selection for drought and salinity tolerance in cool season food legumes. **In:***Breeding for Stress Tolerance in Cool Season Food Legumes*, pp. 245-255, (Singh, K.B. and. Saxena, M.C., eds). ICARDA, Willay Sayce Publication, UK.

Saxena, N.P. and Johansen, C. (1990). Realized yield potential in chickpea and physiological considerations for further genetic improvement. **In**: *Int Congress Plant Physiology*. New Delhi: Society for Plant Physiology and Biochemistry.

Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M. and Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *J. Food Sci.* **75**(2):S115-S119.

Selamawit Ketema, Bizuayehu Tesfaye, Gemechu Keneni, Berhanu Amsalu Fenta, Ermias Assefa, Greliche, N. Machuka, E. and Yao, N. (2020). DArTSeq SNP-based markers revealed high genetic diversity and structured population in Ethiopian cowpea [*Vigna unguiculata* (L.) Walp] germplasms. *PLoS ONE***15**:e0239122.

Sethy, N.K., Shokeen, B. and Bhatia, S. (2003). Isolation and characterization of sequence tagged-microsatellite markers in chickpea (*Cicer arietinum* L.). *Mol Eco Notes*. **3**:428-430.

Setotaw Ferede, Asnake Fikre and Seid Ahmed (2018). Assessing the competitiveness of smallholders chickpea production in the central highlands of Ethiopia. *Ethiop J Crop Sci***6**(2):51-65.

Shannon, C.E. (1948). A mathematical theory of communication. *Bell Syst. Tech. J.* **27**:379-423.

Sharma, K. D. and Nayyar, H. (2014). Cold stress alters transcription in meiotic anthers of cold tolerant chickpea (*Cicer arietinum* L.). *BMC res. notes* **7**(1):1-13.

Sheldrake, A.R. and Saxena, N.P. (1979). The growth and development of chickpea under progressive moisture stress. **In:***Stress Physiology in Crop Plants*, pp. 465-485, (Mussel, H. and Staples, R.C., eds). John Wiley & Sons, Ltd., Chichester.

Siddique, K.H., Johansen, C., Rao, K.C., Rao, J.V.D.K., and Ali, M. (2005). Role of legumes in sustainable cropping systems. **In**: *Abstracts, Fourth International Food Legumes Research Conference-Food legumes for Nutritional Security and Sustainable Agriculture*, 18-22 Oct, pp 31, New Delhi, India.

Siddique, K.H.M., and Sedgley, R.H. (1986). Chickpea (*Cicer arietinum* L.), a potential grain legume for southwestern Australia: seasonal growth and yield. *Crop Pasture Sci***37**:245-261.

Siddique, K.H.M., Marshall, C. and Sedgley, R.H. (1983). Temperature and leaf appearance in chickpea. *Int. Chickpea Newsl*. **8**:14-15.

Singh, A. K., Varaprasad, K. S. and Venkateswaran, K. (2012). Conservation costs of plant genetic resources for food and agriculture: seed genebanks. *Agricultural Research* **1(**3):223-239.

Singh, F. and Diwakar. B., (1995). Chickpea Botany and Production Practices: Skill Development Series no. 16. ICRISAT, Andhra Pradesh, India.

Singh, K,B., and Ocampo, B. (1997). Exploitation of wild Cicer species for yield improvement in chickpea. *Theor Appl Genet***95**:418-423.

Singh, K. B., Malhotra, R. S. and Saxena, M. C. (1989). Chickpea evaluation for cold tolerance under field conditions. *Crop sci.* **29**(2):282-285.

Singh, K. B., Malhotra, R. S. and Saxena, M. C. (1993). Relationship between cold severity and yield loss in chickpea (*Cicer arietinum* L.). *J.Agron.Crop Sci.* **170**(2):121-127.

Singh, K.B. (1987). Chickpea breeding. **In**: *The Chickpea*, (Saxena, M.C. and Singh, K.B., eds). pp. 127-162. CAB International, Wallingford, UK.

Singh, K.B. (1997). Chickpea (*Cicer arietinum* L.). *Field Crops Res*. **53**:161-170.

Singh, K.B. and M.C. Saxena. (1996). Winter Chickpea in Mediterranean–type Environments. A technical bulletin, ICARDA, Aleppo, Syria.

Singh, K.B., Malhotra, R.S., Halila, M.H., Knights, E.J., and Verma, M.M. (1994). Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica***73**:137-149.

Singh, K.B., Reddy, M.V., and Nene, Y.L. (1984). International testing of chickpeas for resistance to Ascochyta blight. *Plant Dis***68**:782-784.

Singh, R. K. and Chaudhary, B. D. (1977). Biometrical *Methods in Quantitative Genetices Analysis*. Kalyanin Puplishers, New Delhi.

Singh, R., Sharma, R., Varshney, R.K., Sharma S.K. and Singh N.K. (2008). Chickpea improvement: Role of wild species and genetic markers. *Biotechnol. Genet. Eng. Rev.***25**:267-314.

Sintayehu Admas and Gize Abeje (2017). Phenotypic diversity studies in chickpea (*Cicer arietinum* L.) germplasm of Ethiopian collections. *Int. J. Current Res.***9**(3):48506-48512.

Sintayehu Admas, Teklehaimanot Haileselassie, Kassahun Tesfaye, Eleni Shiferaw and Flynn, K.C. (2021). Evaluation of Ethiopian chickpea (*Cicer arietinum* L.) genotypes for frost tolerance. *Acta agriculturae Slovenica***117**(2):1-14.

Srinivasan, A., Johansen, C. and Saxena, N.P. (1998). Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): characterization of stress and genetic variation in pod set. *Field Crop Res***57**:181-193.

Srinivasan, A., Saxena, N.P., and Johansen, C. (1999). Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): genetic variation in gamete development and function. *Field Crop Res***60**(2):209-222.

Stagnari, F., Maggio, A., Galieni, A. and Pisante, M. (2017).Multiple benefits of legumes for agriculture sustainability: an overview. *Chem. Biol. Technol. Agric.***4**:2.

Summerfield, R.J., Hadley, P., Roberts, E.H., Minchin, F.R. and Rawsthrone, S. (1984). Sensitivity of chickpea (*Cicer arietinum* L.) to hot temperatures during the reproductive period. *Exp. Agric*. **20**:77-93.

Tadesse Sefera, Bekele Abebie, Gaur, P.M., Kebebew Assefa, and Varshney, R.K. (2011). Characterization and genetic diversity analysis of selected chickpea cultivars of nine countries using simple sequence repeat (SSR) markers. *Crop Pasture Sci*. **62**:177-187.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **30**:2725-2729.

Tanksley, S.D. and McCouch, S.R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science***227**(5329):1063-1066.

Tanno, K. and Willcox, G. (2006). The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: early finds from Tell el-Kerkh, north-west Syria, late 10th millennium B.P. *Veget. Hist. Archaeobot.* **15**:197-204.

Tesfashbamlak Mola, Shimelis Alemayehu, Asnake Fikre, Ojiewo, C., Ketema Alemu and Tulu Degefu (2018). Heat Tolerance Responses of Chickpea (*Cicer arietinum* L.) Genotypes in the Thermal Zone of Ethiopia, a Case of Werer Station. *Ethiop. J. Crop Sci*. **6** (2): 95-118.

Thakur, P., Kumar, S., Malik, J.A., Berger, J.D. and Nayyar. H. (2010). Cold stress effects on reproductive development in grain crops: An overview. *Environ. Exp. Bot.* **67**(3):429-443.

Thomas, R., Vaughan, I. and Lello, J. (2013). Data analysis with R statistical software. A guidebook for scientists. Eco-explore.

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. and Upadhyaya, H.D. (2011). Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS One*: *6*(11): p.e27275.

Tibayrenc, M. (2017). *Genetics and Evolution of Infectious Diseases,* Second Edition*,* Elsevier Inc.

Toker, C. (2005). Preliminary screening and selection for cold tolerance in annual wild *Cicer species*. *Genet Resour Crop Evol* **52**(1):1-5.

Toker, C., Berger, J., Eker, T., Sari, D., Sari, H., Gokturk, R.S., Kahraman, A., Aydin, B. and von Wettberg, E.J. (2021). *Cicer turcicum*: A New *Cicer* Species and Its Potential to Improve Chickpea. *Front Plant Sci. Frontiers* **12**: 662891.

Toker, C., Lluch, C., Tejera, N. A., Serraj, R. and Siddique, K. H. M. (2007). Abiotic stress. **In**: *Chickpea Breeding and Management*, pp, 474-496, (Yadav, S.S., Redden, R., Chen, W. and Sharma, B., eds). CAB International, UK.

Tóth, B., Francia, E., Rizza, F., Stanca, A.M., Galiba, G., and Pecchioni, N. (2004). Development of PCR-based markers on chromosome 5H for assisted selection of cold tolerant genotypes in barley. *Mol. Breeding* **14**:265-273.

Tsegaye Getahun, Kassahun Tesfaye, Asnake Fikre, Teklehaimanot Haileslassie, Annapurna Chitikineni, Thudi, M. and Varshney, R.K. (2021). Molecular Genetic Diversity and Population Structure in Ethiopian Chickpea Germplasm Accessions. *Diversity* **13**:247.

Tuberosa, R. and Salvi, S. (2006). Genomic approaches to improve drought tolerance in crops. *Trends Plant Sci.* **11**:405-412.

Upadhyaya H.D., Dwivedi, S.L., Baum, M., Varshney, R.K., Udupa, S.M., Gowda, C.L.L., Hoisington, D. and Singh, S. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biol*. **8**:1-12.

Upadhyaya H.D., Ortiz R., Bramel P.J. and Singh S. (2002). Phenotypic diversity for morphological and agronomic characteristics in chickpea core collection. *Euphytica***123**:333-342.

Upadhyaya, H., Thudi, M., Dronavalli, N., Gujaria, N., Singh, S., Sharma, S. and Varshney, R. (2011). Genomic tools and germplasm diversity for chickpea improvement. *Plant Genet. Resources* **9:**45-58.

Upadhyaya, H., Thudi, M., Dronavalli, N., Gujaria, N., Singh, S., Sharma, S. and Varshney, R.K. (2016). Genomic tools and germplasm diversity for chickpea improvement. *Plant Genetic Resour. Charact. Util.* **9**:45-58.

Valadez-Moctezuma, E., Cabrera-Hidalgo, A.d. and Arreguin-Espinosa, R. (2020). Genetic variability and population structure of Mexican chickpea (*Cicer arietinum* L.) germplasm accessions revealed by microsatellite markers. *J. Plant Biochem. Biotechnol.* **29:**357-367.

van de Wouw, M., Chris K.C., van Hintum, T., van Treuren, R. and Visser, B. (2009). Genetic erosion in crops: concept, research results and challenges. *Plant Genetic Resour. Charact. Util.* **8**(1):1-15.

van der Maesen L. G. J., Maxted Javadi N., Coles, F.S.and Davies A.R. M. (2007). Taxonomy of the Genus Cicer Revisited. **In**: *Chickpea breeding and management*, pp.14-47, (Yadav, S.S., ed). CAB International, Wallingford, Oxon, UK.

van der Maesen, L. J. G. (1972). *Cicer* L., a monograph of the genus, with special reference to the chickpea (*Cicerarietinum* L.), its ecology and cultivation. Agric. Univ. Wageningen, Comm. No. 72/10.

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, Wallingford, UK.

van Emden, H.F., Ball, H.F. and Rao, M.R. (1988). Pest disease and weed problems in pea, lentil, faba bean and chickpea. **In**: *World Crops: Cool Season Food Legumes,* pp 519-534, (Summerfield, R.J. ed.). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Varshney, R.K., Thudi, M., Nayak, S.N., Gaur, P.M., Kashiwagi, J., Krishnamurthy, L., Jaganathan, D., Koppolu, J., Bohra, A., Tripathi, S., Rathore, A., Jukanti, A.K., Jayalakshmi, V., Vemula, A., Singh, S.J., Yasin, M., Sheshshayee, M.S., Viswanatha, K.P. (2014). Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). *Theor Appl Genet* 127:445-462.

Varshney, R.V., Hoisington, D.A., Tyagi, A.K. (2006) Advances in cereal genomics and applications in crop breeding. *Trends Biotechnol***24**:490-499.

Vavilov, I. (1951). The Origin, Variation, Immunity and Breeding of Cultivated Plants Translated from the Russian by K.Starchester. Ronlard Press. New York.

Vavilov, N.I. (1927). Geographical regularities in the distribution of genes of cultivated plants. *Priroda* (Moscow) **10**:764-773.

Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C. de F. (2016). Microsatellite markers: what they mean and why they are so useful. *Genet. Mol. Biol.* **39**:312-328.

Vishnyakova, M. A., Burlyaeva, M. O., Bulyntsev, S. V., Seferova, I. V., Plekhanova, E. S. and Nuzhdin, S. V. (2017). Phenotypic diversity of chickpea (*Cicer arietinum* L.) landraces accumulated in the Vavilov collection from the centers of the crop's origin. *Russ. J. Genet. Appl. Res.***7**(7):763-772.

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M.R. (2007). Heat tolerance in plants: an overview. *Environ. Exp. Bot*. **61**:199-223.

Wallace, T. C., Murray, R. and Zelman, K. M. (2016). The nutritional value and health benefits of chickpeas and hummus. *Nutrients* **8**(12):766.

Ward, J.H. (1963). Hierarchical groupings to optimize an objective function. *J. Am. Stat. Assoc***58**:236-244.

Wery, J., Silim, S.N., Knights, E.J., Malhotra, R.S. and Cousin, R. (1994). Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes. *Euphytica* **73**:73-83.

Wery, J., Turc, O. and Lecoeur, J. (1993). Mechanisms of resistance to cold, heat and drought in cool-season legumes, with special reference to chickpea and pea. **In**: *Breeding for stress tolerance in cool-season food legumes*, pp. 271-291, (Singh, K.B. and Saxena, M.C., eds). John Wiley & Sons, Chichester.

Willemen, L., Scheldeman, X., Cabellos, V.S., Salazar, S.R. and Guarino, L. (2007). Spatial patterns of diversity and genetic erosion of traditional cassava (*Manihot esculenta* Crantz) in the Peruvian Amazon: An evaluation of socio-economic and environmental indicators. *Genet Resour Crop Evol* **54**: 1599-1612.

Winch T. (2006). *Growing Food, a Guide to Food Production*. Dordrecht, the Netherlands, Springer. pp. 155-158.

Winter, P. and Kahl, G. (1995). Molecular marker technologies for plant improvement. *World J. Microbiol.Biotechnol*. **11**(4):438-448.

Winter, P., Benko-Iseppon, A.M., Hüttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., Pfaf, T., Tekeoglu, M., Santra, D., Sant, V.J., Rajesh, P.N., Kahl, G. and Muehlbauer, F.J. (2000). A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for Fusarium wilt races 4 and 5. *Theor Appl Genet*. **101**:1155–1163.

Winter. P., Pfaf, T. and Udupa, S.M. (1999). Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mo.l Gen. Genet*. **262**:90-101.

Worede, M. (1992) Ethiopia: a genebank working with farmers. In *Growing Diversity: Genetic Resources and Local Food Security* (D. Cooper, R. Vellvé and H. Hobbelink, eds) pp. 78–94. London: Intermediate Technology Publications.

Wright, S. (1931). Evolution in Mendelian population. *Genetics***16**:97-159.

Wright, S. (1977). *Evolution and the Genetics of Populations*: *Experimental results and Evolutionary Deductions. London*, Volume 3, The University of Chicago Press. pp 622.

Wu, Y. F., Zhong, X. L., Hu, X., Ren, D. C., Lv, G. H., Wei, C. Y. and Song, J. Q. (2014). Frost affects grain yield components in winter wheat. *New Zealand Journal of Crop and Horticultural Science* **42**(3):194-204.

Yadav, S,K.(2010). Cold stress tolerance mechanisms in plants. A review. *Agron Sustain Dev.***30**(3):515-27.

Yadeta Anbessa and Geletu Bejiga (2002). Evaluation of Ethiopian chickpea landraces for tolerance to drought. *Genet. Resour. Crop Evol.* **49**:557-564.

Yang, J., Zhang, J., Wang, Z., Zhu, Q. and Liu, L. (2001). Water deficit-induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. *Agron. J.* 93:196-201.

Yang, Y., Zhou, L., Gu, Y., Zhang, Y., Tang, J., Li, F., Shang, W., Jiang, B., Yue, X. and Chen, M. (2007). Dietary chickpeas reverse visceral adiposity, dyslipidaemia and insulin resistance in rats induced by a chronic high-fat diet. *Br J Nutr.* **98**(4):720-726.

Yifru Teklu and Hammer, K. (2006). Farmers' perception and genetic erosion of tetraploid wheats landraces in Ethiopia. *Genet Resour Crop Evol* **53**(6):1099-1113.

Zeven, A.C. and de Wet, J.M.J. (1982). *Dictionary of Cultivated Plants and their Regions of Diversity*. Centre for Agricultural Publishing and Doeumentalion. Wageningen.

Zhu, C., Gore, M.A., Buckler, E.S., and Yu, J. (2008). Status and prospects of association mapping in plants. *Plant Genome***1**:5-20.

Zhu, J., Pearce, S., Burke,A., See, D.R., Skinner, D.Z., Dubcovsky, J. and Garland-Campbell, K. (2014). Copy number and haplotype variation at the VRN-A1 and central FR-A2 loci are associated with frost tolerance in hexaploid wheat. *Theor. Appl. Genet.***127:**1183-1197.

Zohary, D. and Hopf, M. (2000). *Domestication of Plants in the Old World*, 3rd edn. Clarendon Press, Oxford, Uk.

Appendices

Appendix 1. List of chickpea genotypes and its passport data for 673 chickpea genotypes (562 EBI collections, 83 ICARDA genotypes and 28 improved varieties) used for frost resistant screening at D/Brihan Ethiopia from 2018 to 2020 growing seasons

No	**EBI Collections**					
1	**Accession Number**	**Region**	**Zone**	**Woreda/District**	**Latitude**	**Longitude**
2	9643	Tigray	Mirabawi	Semema	14-11-83-N	38-20-38-E
3	9646-A	Amhara	Semen Gonder	Libbo	12-05-28-N	37-45-22-E
4	9646-C	Amhara	Semen Gonder	Libbo	12-05-28-N	37-45-22-E
5	**16341-A**	Oromiya	Misrak wellega	Nunu qumba	08-49-34-N	36-45-22-E
6	**16341-B**	Oromiya	Misrak wellega	Nunu qumba	08-49-34-N	36-45-22-E
7	16585-B	Tigray	Misrakawi	Hawzen	14-01-19-N	39-22-51-E
8	16586-A	Tigray	Mehakelegnaw	Axum	14-07-37-N	38-45-45-E
9	16588-A	Tigray	Mirabawi	Tahtay koraro	14-05-07-N	38-39-38-E
10	16589-A	Tigray	Mirabawi	Asgede tsimbela	13-54-31-N	38-10-42-E
11	16591-A	Amhara	Semen Gonder	Maksegnet	12-24-04-N	37-32-20-E
12	16591-B	Amhara	Semen Gonder	Maksegnet	12-24-04-N	37-32-20-E
13	207674	Oromiya	Misrak shewa	Ada'a chukala	08-43-00-N	38-52-00-E
14	24159-B	Amhara	Semen Gonder	Debark	13-07-02-N	37-52-35-E
15	24159-C	Amhara	Semen Gonder	Debark	13-07-02-N	37-52-35-E
16	26918-A	SNNP	Segen	Burji	05-36-61-N	38-24-14-E
17	30288-A	Amhara	East gojjam	Dejen	10-11-49.6-N	038-08-26.0-E
18	30288-B	Amhara	East gojjam	Dejen	10-11-49.6-N	038-08-26.0-E
19	30289-A	Amhara	East gojjam	Dejen	10-14-34.8-N	038-06-55.9-E
20	30289-B	Amhara	East gojjam	Dejen	10-14-34.8-N	038-06-55.9-E
21	30290-A	Amhara	East gojjam	Dejen	10-16-10.9-N	038-12-10.2-E
22	30290-C	Amhara	East gojjam	Dejen	10-16-10.9-N	038-12-10.2-E
23	30296-A	Amhara	East gojjam	Dejen	10-14-25.5-N	038-13-18.6-E
24	30300-A	Amhara	East gojjam	Enarji enawiga	10-40-56.5-N	038-11-13.8-E
25	30300-B	Amhara	East gojjam	Enarji enawiga	10-40-56.5-N	038-11-13.8-E
26	30301-A	Amhara	East gojjam	Enarji enawiga	10-41-05.7-N	038-11-10.6-E
27	30301-C	Amhara	East gojjam	Enarji enawiga	10-41-05.7-N	038-11-10.6-E
28	30302-A	Amhara	East gojjam	Enarji enawiga	10-41-10.4-N	038-10-28.5-E
29	30302-C	Amhara	East gojjam	Enarji enawiga	10-41-10.4-N	038-10-28.5-E
30	30307-A	Amhara	East gojjam	Debay tilatgin	10-29-52.8-N	038-00-11.1-E
31	30307-D	Amhara	East gojjam	Debay tilatgin	10-29-52.8-N	038-00-11.1-E
32	30309-A	Amhara	East gojjam	Mota	11-06-24.5-N	037-47-06.7-E
33	30309-C	Amhara	East gojjam	Mota	11-06-24.5-N	037-47-06.7-E
34	30310-A	Amhara	East gojjam	Mota	11-06-24.5-N	037-47-06.7-E
35	30314-A	Amhara	West gojjam	Yilmana densa	11-14-30.3-N	037-33-23.7-E
36	30314-B	Amhara	West gojjam	Yilmana densa	11-14-30.3-N	037-33-23.7-E
37	30315-C	Amhara	West gojjam	D/dare zuria	11-30-09.4-N	037-30-37.4-E
38	30316-A	Amhara	West gojjam	D/dare zuria	11-30-03.9-N	037-30-44.6-E
39	30316-B	Amhara	West gojjam	D/dare zuria	11-30-03.9-N	037-30-44.6-E
40	30318-B	Amhara	Central gonder	Misrak belesa	12-29-52.3-N	038-07-46.4-E
41	30319-A	Amhara	Central gonder	Mirab belesa	12-23-25.85-N	037-55-37.55-E
42	30319-B	Amhara	Central gonder	Mirab belesa	12-23-25.85-N	037-55-37.55-E
43	30320-C	Amhara	Central gonder	Mirab belesa	12-25-54.4-N	037-54-09.3-E
44	30324-A	Amhara	Central gonder	Misrak dendia	12-25-00.8-N	037-18-56.1-E
45	30325-A	Amhara	Central gonder	Mirab dendia	12-21-08.3-N	037-14-28.3-E
46	30325-C	Amhara	Central gonder	Mirab dendia	12-21-08.3-N	037-14-28.3-E
47	30326-A	Amhara	Central gonder	Mirab dendia	12-19-38.1-N	037-13-25.8-E
48	30327-A	Amhara	Central gonder	Takusa	12-12-04.2-N	037-01-25.2-E
49	30329-A	Amhara	Central gonder	Takusa	12-20-15.8-N	037-03-59.3-E
50	30329-B	Amhara	Central gonder	Takusa	12-20-15.8-N	037-03-59.3-E
51	30334-B	Amhara	Central gonder	Gonder zuria	12-22-02.3-N	037-33-35.7-E
52	30334-C	Amhara	Central gonder	Gonder zuria	12-22-02.3-N	037-33-35.7-E
53	30335-A	Amhara	Central gonder	Gonder zuria	12-21-21.4-N	037-34-00.4-E
54	30335-B	Amhara	Central gonder	Gonder zuria	12-21-21.4-N	037-34-00.4-E
55	30336-A	Amhara	Central gonder	Kemkem	12-06-09.0-N	037-45-43.8-E
56	30336-B	Amhara	Central gonder	Kemkem	12-06-09.0-N	037-45-43.8-E
57	30337-A	Amhara	Central gonder	Kemkem	12-05-45.7-N	037-45-30.7-E
58	30337-C	Amhara	Central gonder	Kemkem	12-05-45.7-N	037-45-30.7-E
59	30340-A	Amhara	South gonder	Fogera	11-56-44.4-N	037-42-43.6-E
60	30340-C	Amhara	South gonder	Fogera	11-56-44.4-N	037-42-43.6-E

Appendix 1: Continued....

Accession Number	Region	Zone	Woreda/District	Latitude	Longitude	Accession Number
61	30343-A	Amhara	South Wollo	Kobo	12-15-46.5-N	039-36-26.0-E
62	30345-B	Amhara	North Wollo	Habro	11-28-54.8-N	039-37-15.7-E
63	30345-C	Amhara	North Wollo	Habro	11-28-54.8-N	039-37-15.7-E
64	30346-A	Amhara	North Wollo	Habro	11-34-54.6-N	039-39-41.3-E
65	30346-C	Amhara	North Wollo	Habro	11-34-54.6-N	039-39-41.3-E
66	30347-A	Amhara	North Wollo	Habro	11-37-17.24-N	039-40-27.6-E
67	30347-B	Amhara	North Wollo	Habro	11-37-17.24-N	039-40-27.6-E
68	30348-C	Amhara	Semen shewa	Efratana gidim	10-21-06.9-N	039-56-06.0-E
69	30350-A	Amhara	Kemisie	Jile tumuga	10-21-06.9-N	039-58-06.0-E
70	30350-B	Amhara	Kemisie	Jile tumuga	10-21-06.9-N	039-58-06.0-E
71	30350-C	Amhara	Kemisie	Jile tumuga	10-21-06.9-N	039-58-06.0-E
72	41001-A	Oromiya	Misrak shewa	Lome	08-55-00-N	39-22-00-E
73	41003-C	Oromiya	Arssi	Tena	07-44-00-N	39-34-00-E
74	41004-C	Oromiya	Mirab shewa	Jeldu		
75	41018-B	Amhara	Semen Gonder	Wegera	11-51-00-N	37-42-00-E
76	41020-A	Amhara	Mirab gojam	Dega damot		
77	41020-B	Amhara	Mirab gojam	Dega damot		
78	41021-A	Amhara	Misrak gojam	Enarj enawga	10-38-00-N	38-10-00-E
79	41021-B	Amhara	Misrak gojam	Enarj enawga	10-38-00-N	38-10-00-E
80	41029-A	Amhara	Misrak gojam	Enarj enawga	10-37-00-N	38-11-00-E
81	41029-B	Amhara	Misrak gojam	Enarj enawga	10-37-00-N	38-11-00-E
82	41030-A	Oromiya	Bale	Sinanana dinsho		
83	41036-A	SNNP	Semen omo	Gofa zuria		
84	41036-B	SNNP	Semen omo	Gofa zuria		
85	41036-C	SNNP	Semen omo	Gofa zuria		
86	41043-B	Amhara	Semen Gonder	Gonder zuria	12-32-00-N	37-12-00-E
87	41044-A	Amhara	Semen Gonder	Gonder zuria	12-32-00-N	37-12-00-E
88	41045	Amhara	Semen Gonder	Chilga	12-33-00-N	37-04-00-E
89	41046-B	Amhara	Semen Gonder	Chilga	12-33-00-N	37-04-00-E
90	41035	Oromiya	Arssi	Chole		
91	216854	Oromiya	Arssi	Bekoji		
92	41057-B	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
93	41058-A	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
94	41058-B	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
95	41073-A	Amhara	Misrak gojam	Hulet ej enese		
96	41073-B	Amhara	Misrak gojam	Hulet ej enese		
97	41074-A	Amhara	Misrak gojam	Hulet ej enese		
98	41074-B	Amhara	Misrak gojam	Hulet ej enese		
99	**41075-A**	Amhara	Misrak gojam	Hulet ej enese		
100	**41075-C**	Amhara	Misrak gojam	Hulet ej enese		
101	**41076-A**	Amhara	Misrak gojam	Hulet ej enese		
102	**41076-C**	Amhara	Misrak gojam	Hulet ej enese		
103	**41077-B**	Amhara	Misrak gojam	Hulet ej enese		
104	41078-A	Amhara	Misrak gojam	Hulet ej enese		
105	41078-B	Amhara	Misrak gojam	Hulet ej enese		
106	41079-A	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
107	41080-A	Amhara	Misrak gojam	Enarj enawga		
108	41080-B	Amhara	Misrak gojam	Enarj enawga		
109	41081-A	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
110	41086-A	Amhara	Misrak gojam	Guzamn		
111	41086-B	Amhara	Misrak gojam	Guzamn		
112	41089-A	Amhara	Misrak gojam	Shebel berenta		
113	41089-B	Amhara	Misrak gojam	Shebel berenta		
114	41026-C	Amhara	Misrak gojam	Hulet ej enese	11-05-00-N	37-50-00-E
115	41090-A	Amhara	Misrak gojam	Shebel berenta		
116	41090-B	Amhara	Misrak gojam	Shebel berenta		
117	41091-A	Amhara	Misrak gojam	Shebel berenta		
118	41092-B	Amhara	Misrak gojam	Shebel berenta		
119	41093-B	Amhara	Semen shewa	Ankober	09-35-00-N	39-44-00-E
120	41093-C	Amhara	Semen shewa	Ankober	09-35-00-N	39-44-00-E
121	41094-B	Amhara	Semen shewa	Mafudmezezo mojana	09-57-00-N	39-51-00-E
122	41094-C	Amhara	Semen shewa	Mafudmezezo mojana	09-57-00-N	39-51-00-E
123	41097-B	Amhara	Debub Wollo	Debresina	10-39-00-N	38-48-00-E
124	41101-A	Amhara	Semen shewa	Mafudmezezo mojana	09-58-00-N	39-38-00-E
125	41104-B	Amhara	Misrak gojam	Enemay		
126	41107-A	Amhara	Semen shewa	Mafudmezezo mojana	09-53-00-N	39-51-00-E
127	41107-B	Amhara	Semen shewa	Mafudmezezo mojana	09-53-00-N	39-51-00-E
128	41116-A	Amhara	Debub Wollo	Dessie zuria		
129	41121-A	Oromiya	Misrak shewa	Ada'a chukala	08-52-00-N	38-49-00-E
130	41121-B	Oromiya	Misrak shewa	Ada'a chukala	08-52-00-N	38-49-00-E
131	41124-C	Oromiya	Misrak shewa	Akaki	08-52-00-N	38-47-00-E

Appendix 1: Continued....

	Accession Number	Region	Zone	Woreda/District	Latitude	Longitude
132	41127-A	Oromiya	Misrak shewa	Akaki	08-52-00-N	38-47-00-E
133	41133-A	Oromiya	Misrak shewa	Akaki	08-49-00-N	38-49-00-E
134	41133-B	Oromiya	Misrak shewa	Akaki	08-49-00-N	38-49-00-E
135	41136-A	Oromiya	Misrak shewa	Ada'a chukala	08-46-00-N	38-53-00-E
136	41136-B	Oromiya	Misrak shewa	Ada'a chukala	08-46-00-N	38-53-00-E
137	**41138-A**	Oromiya	Misrak shewa	Lome	08-34-00-N	39-19-00-E
138	41143-B	Amhara	Semen shewa	Minjarna shenkora	09-00-00-N	39-40-00-E
139	41145-A	Amhara	Semen shewa	Minjarna shenkora	09-00-00-N	39-04-00-E
140	41153-A	Oromiya	Misrak shewa	Ada'a chukala	08-45-00-N	38-51-00-E
141	41153-B	Oromiya	Misrak shewa	Ada'a chukala	08-45-00-N	38-51-00-E
142	41158-A	Oromiya	Misrak shewa	Ada'a chukala	08-45-00-N	38-33-00-E
143	41163-A	Oromiya	Misrak shewa	Ada'a chukala	08-48-00-N	38-37-00-E
144	41165-D	Oromiya	Misrak shewa	Ada'a chukala	08-51-00-N	38-56-00-E
145	41166-A	Oromiya	Misrak shewa	Ada'a chukala	08-46-00-N	38-50-00-E
146	41167-B	Oromiya	Misrak shewa	Ada'a chukala	08-47-00-N	38-53-00-E
147	41167-C	Oromiya	Misrak shewa	Ada'a chukala	08-47-00-N	38-53-00-E
148	**41169-A**	Oromiya	Mirab shewa	Ejerie(addis alem)	09-01-00-N	38-15-00-E
149	41173-A	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-50-00-E
150	41173-D	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-50-00-E
151	41180-A	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
152	41180-B	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
153	41198-A	Oromiya	Mirab shewa	Becho	08-43-00-N	38-19-00-E
154	41198-B	Oromiya	Mirab shewa	Becho	08-43-00-N	38-19-00-E
155	41200-B	Oromiya	Mirab shewa	Becho	08-45-00-N	38-19-00-E
156	41200-C	Oromiya	Mirab shewa	Becho	08-45-00-N	38-19-00-E
157	41203-B	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
158	41203-C	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
159	41206-A	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
160	41206-B	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
161	41206-C	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
162	41209-A	Amhara	Semen shewa	Siyadebrina wayu ens	09-46-00-N	39-11-00-E
163	41209-C	Amhara	Semen shewa	Siyadebrina wayu ens	09-46-00-N	39-11-00-E
164	41212-B	Amhara	Semen shewa	Moretna jiru	09-53-00-N	39-09-00-E
165	41221-A	Oromiya	Semen shewa	Wara jarso	10-01-00-N	38-15-00-E
166	41221-B	Oromiya	Semen shewa	Wara jarso	10-01-00-N	38-15-00-E
167	41222-A	Amhara	Misrak gojam	Dejen	10-11-00-N	38-09-00-E
168	41222-B	Amhara	Misrak gojam	Dejen	10-11-00-N	38-09-00-E
169	41223-A	Amhara	Misrak gojam	Dejen	10-14-00-N	38-08-00-E
170	41223-B	Amhara	Misrak gojam	Dejen	10-14-00-N	38-08-00-E
171	41225-B	Amhara	Misrak gojam	Enemay	10-32-00-N	38-09-00-E
172	41226-A	Amhara	Misrak gojam	Enemay	10-32-00-N	38-09-00-E
173	41226-B	Amhara	Misrak gojam	Enemay	10-32-00-N	38-09-00-E
174	**41228-A**	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
175	**41228-B**	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
176	**41228-C**	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
177	41229-A	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
178	41230-A	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
179	41231-A	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
180	41231-B	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
181	41233-B	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
182	41234-B	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
183	41234-C	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
184	41236-A	Amhara	Misrak gojam	Goncha siso enese	10-35-00-N	38-10-00-E
185	41241-A	Amhara	Misrak gojam	Shebel berenta	10-26-00-N	38-21-00-E
186	41241-B	Amhara	Misrak gojam	Shebel berenta	10-26-00-N	38-21-00-E
187	41242-A	Amhara	Misrak gojam	Shebel berenta	10-26-00-N	38-21-00-E
188	**41245-A**	Amhara	Bahir dar special	Bahir dar	11-24-00-N	37-06-00-E
189	**41245-B**	Amhara	Bahir dar special	Bahir dar	11-24-00-N	37-06-00-E
190	41247-A	Amhara	Misrak gojam	Enemay	10-24-00-N	38-16-00-E
191	41247-B	Amhara	Misrak gojam	Enemay	10-24-00-N	38-16-00-E
192	41250-A	Amhara	Misrak gojam	Shebel berenta	10-28-00-N	38-12-00-E
193	41251-B	Amhara	Misrak gojam	Shebel berenta	10-28-00-N	38-12-00-E
194	41255-B	Amhara	Misrak gojam	Debay telatgen	10-30-00-N	38-00-00-E
195	41256-A	Amhara	Misrak gojam	Debay telatgen	10-30-00-N	38-00-00-E
196	41257-A	Amhara	Misrak gojam	Debay telatgen	10-30-00-N	38-00-00-E
197	41258-A	Amhara	Misrak gojam	Debay telatgen	10-30-00-N	38-00-00-E
198	41258-B	Amhara	Misrak gojam	Debay telatgen	10-30-00-N	38-00-00-E
199	41261-B	Amhara	Misrak gojam	Enbise sar midir	10-42-00-N	38-08-00-E
200	41263-A	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
201	**41264-B**	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
202	**41265-C**	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
203	**41265-B**	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E

Appendix 1: Continued....

	Accession Number	Region	Zone	Woreda/District	Latitude	Longitude
204	**41266-C**	Amhara	Misrak gojam	Hulet ej enese	11-03-00-N	37-51-00-E
205	**41266-A**	Amhara	Misrak gojam	Hulet ej enese	11-03-00-N	37-51-00-E
206	41267-A	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
207	41268-A	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-43-00-E
208	41269-B	Amhara	Misrak gojam	Hulet ej enese	11-05-00-N	37-41-00-E
209	41270-A	Amhara	Mirab gojam	Adet	11-16-00-N	37-29-00-E
210	41270-B	Amhara	Mirab gojam	Adet	11-16-00-N	37-29-00-E
211	41278-A	Amhara	Bahir dar special	Bahir dar	11-28-00-N	37-29-00-E
212	41279-A	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
213	41282-A	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
214	41282-B	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
215	**41281-C**	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
216	41283	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
217	41285-B	Amhara	Debub Gonder	Dera	11-45-00-N	37-38-00-E
218	41292-A	Amhara	Debub Gonder	Kemekem	11-55-00-N	37-41-00-E
219	41292-B	Amhara	Debub Gonder	Kemekem	11-55-00-N	37-41-00-E
220	41294-B	Amhara	Debub Gonder	Fogera	12-00-00-N	37-45-00-E
221	**41295-A**	Amhara	Debub Gonder	Fogera	12-00-00-N	37-45-00-E
222	**41295-B**	Amhara	Debub Gonder	Fogera	12-00-00-N	37-45-00-E
223	41301-A	Amhara	Semen Gonder	Wegera	12-46-00-N	37-37-00-E
224	41306-A	Amhara	Semen Gonder	Dembia	12-28-00-N	37-22-00-E
225	41307-D	Amhara	Semen Gonder	Gonder zuria	12-14-00-N	37-16-00-E
226	**41308-A**	Amhara	Semen Gonder	Dembia	12-14-00-N	37-16-00-E
227	41309-B	Amhara	Semen Gonder	Dembia	12-14-00-N	37-16-00-E
228	41310-A	Amhara	Semen Gonder	Dembia	12-14-00-N	37-16-00-E
229	**41311-A**	Amhara	Semen Gonder	Dembia	12-25-00-N	37-18-00-E
230	**41311-B**	Amhara	Semen Gonder	Dembia	12-25-00-N	37-18-00-E
231	**41320-B**	Amhara	Misrak gojam	Debay telatgen	10-17-00-N	38-08-00-E
232	**41320-A**	Amhara	Misrak gojam	Debay telatgen	10-17-00-N	38-08-00-E
233	41322-A	Amhara	Misrak gojam	Dejen	10-07-00-N	38-09-00-E
234	**41323-A**	Amhara	Misrak gojam	Dejen	10-07-00-N	38-09-00-E
235	**41323-B**	Amhara	Misrak gojam	Dejen	10-07-00-N	38-09-00-E
236	**41323**	Amhara	Misrak gojam	Dejen	10-07-00-N	38-09-00-E
237	41324-B	Amhara	Misrak gojam	Dejen	10-15-00-N	38-09-00-E
238	41324-C	Amhara	Misrak gojam	Dejen	10-15-00-N	38-09-00-E
239	207138-B	Amhara	Debub Gonder	Kemekem		
240	207143-A	Amhara	Semen Gonder	Chilga		
241	207145-A	Amhara	Debub Gonder	Fogera		
242	**207149-B**	Tigray	Misrakawi	Ganta afeshum		
243	**207149-C**	Tigray	Misrakawi	Ganta afeshum		
244	207155-C	Amhara	Semen Gonder	Gonder zuria		
245	207157-B	Amhara	Debub Gonder	Lay gayint		
246	207161-C	Amhara	Semen Gonder	Dabat		
247	207164-A	Amhara	Semen Gonder	Dabat		
248	207165-B	Amhara	Semen Gonder	Wegera		
249	207166-A	Amhara	Semen Gonder	Belesa		
250	207167-A	Amhara	Debub Gonder	Kemekem		
251	207171-B	Amhara	Semen Gonder	Wegera		
252	207171-C	Amhara	Semen Gonder	Wegera		
253	207173-A	Amhara	Semen Gonder	Wegera		
254	207173-B	Amhara	Semen Gonder	Wegera		
255	207175-A	Amhara	Semen Gonder	Dabat		
256	207175-C	Amhara	Semen Gonder	Dabat		
257	207178-A	Amhara	Semen Gonder	Dabat		
258	207185-B	Amhara	Semen Gonder	Wegera		
259	207186-C	Amhara	Semen Gonder	Dabat		
260	207562-B	Tigray	Misrakawi	Wukro		
261	207607-B	Oromiya	Semen shewa	Gerar jarso	09-37-00-N	38-40-00-E
262	207607-C	Oromiya	Semen shewa	Gerar jarso	09-37-00-N	38-40-00-E
263	207608	Oromiya	Semen shewa	Gerar jarso	09-37-00-N	38-40-00-E
264	207609-A	Amhara	Debub Gonder	Este	11-34-00-N	38-07-00-E
265	207609-B	Amhara	Debub Gonder	Este	11-34-00-N	38-07-00-E
266	**207617-A**	Amhara	Semen Gonder	Chilga	12-33-00-N	37-04-00-E
267	207622	Amhara	Debub Gonder	Lay gayint	11-44-00-N	38-29-00-E
268	207623-A	Amhara	Misrak gojam	Hulet ej enese		
269	207623-B	Amhara	Misrak gojam	Hulet ej enese		
270	**207624-B**	Amhara	Misrak gojam	Hulet ej enese		
271	207635-B	Amhara	Misrak gojam	Hulet ej enese		
272	207635-C	Amhara	Misrak gojam	Hulet ej enese		
273	207636-B	Amhara	Misrak gojam	Hulet ej enese		
274	207637-A	Amhara	Misrak gojam	Hulet ej enese		
275	207637-B	Amhara	Misrak gojam	Hulet ej enese		

Appendix 1: Continued....

	Accession Number	Region	Zone	Woreda/District	Latitude	Longitude
276	207638	Amhara	Misrak gojam	Hulet ej enese		
277	207639-A	Amhara	Misrak gojam	Enarj enawga		
278	207640	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
279	207643-A	Amhara	Semen shewa	Mafudmezezo mojana	09-57-00-N	39-51-00-E
280	207643-B	Amhara	Semen shewa	Mafudmezezo mojana	09-57-00-N	39-51-00-E
281	207648	Amhara	Debub Wollo	Kalu	11-05-00-N	39-45-00-E
282	207649-A	Amhara	Semen shewa	Mafudmezezo mojana	09-58-00-N	39-38-00-E
283	207649-B	Amhara	Semen shewa	Mafudmezezo mojana	09-58-00-N	39-38-00-E
284	207652	Amhara	Semen shewa	Mafudmezezo mojana	09-53-00-N	39-51-00-E
285	207668	Oromiya	Misrak shewa	Akaki	08-53-00-N	38-50-00-E
286	207670	Oromiya	Misrak shewa	Akaki	08-49-00-N	38-49-00-E
287	207679-A	Oromiya	Misrak shewa	Ada'a chukala	08-47-00-N	38-53-00-E
288	207679-B	Oromiya	Misrak shewa	Ada'a chukala	08-47-00-N	38-53-00-E
289	207683-A	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-51-00-E
290	207683-B	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-51-00-E
291	207684	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-51-00-E
292	207688-A	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-51-00-E
293	207691-A	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
294	207692	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
295	207695-C	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
296	207711-A	Oromiya	Mirab shewa	Becho	08-44-00-N	38-20-00-E
297	207711-B	Oromiya	Mirab shewa	Becho	08-44-00-N	38-20-00-E
298	207712	Oromiya	Mirab shewa	Becho	08-44-00-N	38-20-00-E
299	207714	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
300	207715-A	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
301	207715-B	Oromiya	Mirab shewa	Alem gena	08-54-00-N	38-36-00-E
302	207721-B	Amhara	Semen shewa	Moretna jiru	09-53-00-N	39-09-00-E
303	207728-A	Amhara	Misrak gojam	Enemay	10-32-00-N	38-09-00-E
304	207729-A	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
305	207729-B	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
306	207730	Amhara	Misrak gojam	Enarj enawga	10-35-00-N	38-10-00-E
307	207733-A	Amhara	Misrak gojam	Goncha siso enese	10-35-00-N	38-10-00-E
308	207735-A	Amhara	Misrak gojam	Goncha siso enese	10-35-00-N	38-10-00-E
309	207735-B	Amhara	Misrak gojam	Goncha siso enese	10-35-00-N	38-10-00-E
310	207736-B	Amhara	Misrak gojam	Goncha siso enese	10-35-00-N	38-10-00-E
311	207737-A	Amhara	Misrak gojam	Hulet ej enese	11-03-00-N	37-51-00-E
312	207737-B	Amhara	Misrak gojam	Hulet ej enese	11-03-00-N	37-51-00-E
313	207748	Amhara	Semen Gonder	Dabat	12-59-00-N	37-46-00-E
314	207752-A	Amhara	Semen Gonder	Dembia	12-14-00-N	37-16-00-E
315	207753-A	Amhara	Semen Gonder	Gonder zuria	12-36-00-N	37-27-00-E
316	207753-B	Amhara	Semen Gonder	Gonder zuria	12-36-00-N	37-27-00-E
317	207753-C	Amhara	Semen Gonder	Gonder zuria	12-36-00-N	37-27-00-E
318	207766	Oromiya	Mirab shewa	Jeldu		
319	207769-A	Oromiya	Mirab shewa	Jeldu		
320	207769-C	Oromiya	Mirab shewa	Jeldu		
321	207770	Oromiya	Mirab shewa	Jeldu		
322	207893-A	Tigray	Debubawi	Alaje		
323	207893-B	Tigray	Debubawi	Alaje		
324	208454-C	Amhara	Debub Gonder	Este	11-45-00-N	37-35-00-E
325	208988-A	Oromiya	Semen shewa	Berehna aleltu	09-06-00-N	38-50-00-E
326	208988-B	Oromiya	Semen shewa	Berehna aleltu	09-06-00-N	38-50-00-E
327	208994-A	Oromiya	Mirab shewa	Walisona goro		
328	208995-B	Oromiya	Mirab shewa	Walisona goro		
329	208995-D	Oromiya	Mirab shewa	Walisona goro		
330	208997-B	Oromiya	Mirab shewa	Walisona goro		
331	209001-B	SNNP	Semen omo	Offa		
332	209003-B	SNNP	Semen omo	Offa		
333	209008-A	SNNP	Hadiya	Badawacho		
334	209008-B	SNNP	Hadiya	Badawacho		
335	209013-A	SNNP	Hadiya	Badawacho		
336	209014-B	Oromiya	Misrak shewa	Arsi negele		
337	41030	Oromiya	Bale	Robe		
338	41034	Oromiya	Bale	Robe market		
339	209016-C	SNNP	Gurage	Meskanena mareko		
340	209019-A	SNNP	Gurage	Sodo		
341	209022-A	Oromiya	Mirab shewa	Kersana kondaltiiti		
342	209022-B	Oromiya	Mirab shewa	Kersana kondaltiiti		
343	209026-A	Oromiya	Mirab shewa	Kersana kondaltiiti		
344	209026-B	Oromiya	Mirab shewa	Kersana kondaltiiti		
345	209029-C	Oromiya	Mirab shewa	Kersana kondaltiiti		
346	209032-A	Oromiya	Mirab shewa	Kersana kondaltiti		
347	209034-B	Oromiya	Mirab shewa	Kersana kondaltiti		

Appendix 1: Continued....

	Accession Number	Region	Zone	Woreda/District	Latitude	Longitude
348	209034-C	Oromiya	Mirab shewa	Kersana kondaltiti		
349	209038-A	Oromiya	Mirab shewa	Alem gena		
350	209078-A	Oromiya	Mirab harerge	Tulo		
351	209085-A	Oromiya	Mirab harerge	Kuni		
352	209086-A	Oromiya	Mirab harerge	Kuni		
353	209095-A	Oromiya	Arssi	Dodotana sire	08-18-00-N	39-22-00-E
354	209095-B	Oromiya	Arssi	Dodotana sire	08-18-00-N	39-22-00-E
355	**209105-B**	Oromiya	Bale	Goro		
356	209108-A	Oromiya	Misrak shewa	Lome		
357	209112-A	Oromiya	Misrak shewa	Ada'a chukala	08-48-00-N	38-53-00-E
358	209112-B	Oromiya	Misrak shewa	Ada'a chukala	08-48-00-N	38-53-00-E
359	209116-B	Oromiya	Misrak shewa	Ada'a chukala	08-48-00-N	38-53-00-E
360	212477-A	Amhara	Semen shewa	Weremo wajetuna mid	10-14-00-N	39-00-00-E
361	212589-A	Amhara	Debub Wollo	Kalu	11-25-00-N	39-39-00-E
362	212589-B	Amhara	Debub Wollo	Kalu	11-25-00-N	39-39-00-E
363	212685-B	Amhara	Misrak gojam	Shebel berenta	10-31-00-N	38-23-00-E
364	212688-C	Amhara	Debub Gonder	Kemekem	12-05-00-N	37-54-00-E
365	212914-A	SNNP	Bench maji	Dirashe special	05-36-00-N	37-22-00-E
366	212914-B	SNNP	Bench maji	Dirashe special	05-36-00-N	37-22-00-E
367	213050-A	Amhara	Debub Wollo	Were ilu		
368	213050-B	Amhara	Debub Wollo	Were ilu		
369	214621-A	SNNP	Semen omo	Humbo		
370	214623-B	SNNP	Semen omo	Offa		
371	214624-A	SNNP	Semen omo	Humbo		
372	214626-C	SNNP	Semen omo	Humbo		
373	214731-B	Amhara	Debub Wollo	Debresina		
374	214734-A	Amhara	Debub Wollo	Kelala		
375	214734-B	Amhara	Debub Wollo	Kelala		
376	214734-C	Amhara	Debub Wollo	Kelala		
377	215033-B	Oromiya	Borena	Teltele		
378	215067-A	Amhara	Semen shewa	Mama midrina lalo mi		
379	215067-B	Amhara	Semen shewa	Mama midrina lalo mi		
380	215067-C	Amhara	Semen shewa	Mama midrina lalo mi		
381	215190-A	Amhara	Debub Wollo	Kalu	11-04-00-N	39-47-00-E
382	215190-B	Amhara	Debub Wollo	Kalu	11-04-00-N	39-47-00-E
383	215190-C	Amhara	Debub Wollo	Kalu	11-04-00-N	39-47-00-E
384	215289-A	Amhara	Misrak gojam	Hulet ej enese		
385	215289-B	Amhara	Misrak gojam	Hulet ej enese		
386	216854-A	Oromiya	Arssi	Bekoji	07-32-00-N	39-18-00-E
387	225741-C	SNNP	Bench maji	Konso special	06-38-00-N	37-38-00-E
388	225743-A	SNNP	Semen omo	Gofa zuria	06-39-00-N	37-37-00-E
389	225879-A	Amhara	Debub Gonder	Este	11-35-00-N	38-05-00-E
390	225882-A	Amhara	Debub Gonder	Este	11-27-00-N	37-59-00-E
391	225884-A	Amhara	Semen Gonder	Dembia	12-30-00-N	37-24-00-E
392	225887-B	Amhara	Semen Gonder	Dembia	12-32-00-N	37-14-00-E
393	225888-A	Amhara	Mirab gojam	Bahir dar zuria	11-32-00-N	37-25-00-E
394	225889-A	Amhara	Bahir dar special	Bahir dar	11-30-00-N	37-31-00-E
395	225889-C	Amhara	Bahir dar special	Bahir dar	11-30-00-N	37-31-00-E
396	225890-A	Amhara	Bahir dar special	Bahir dar	11-29-00-N	37-33-00-E
397	227151-B	Amhara	Semen Gonder	Debark		
398	227151-C	Amhara	Semen Gonder	Debark		
399	227151-D	Amhara	Semen Gonder	Debark		
400	227152-A	Amhara	Debub Gonder	Kemekem		
401	227152-B	Amhara	Debub Gonder	Kemekem		
402	227156-A	Amhara	Semen Gonder	Gonder zuria		
403	227158-A	Amhara	Semen Gonder	Dembia		
404	227158-C	Amhara	Semen Gonder	Dembia		
405	227160-B	Amhara	Semen Gonder	Alefa		
406	227161-B	Amhara	Semen Gonder	Alefa		
407	228197-C	Oromiya	Mirab shewa	Becho		
408	228197-D	Oromiya	Mirab shewa	Becho		
409	228197-E	Oromiya	Mirab shewa	Becho		
410	228290-A	Amhara	Misrak gojam	Awabel		
411	228293-A	Amhara	Misrak gojam	Shebel berenta		
412	228294-B	Amhara	Misrak gojam	Hulet ej enese		
413	228299-A	Amhara	Misrak gojam	Hulet ej enese		
414	228301-B	Amhara	Misrak gojam	Enbise sar midir		
415	229962-B	Oromiya	Bale	Ginir		
416	230770-A	Oromiya	Borena	Moyale	05-03-00-N	39-28-00-E
417	230770-B	Oromiya	Borena	Moyale	05-03-00-N	39-28-00-E
418	230796-C	Oromiya	Misrak harerge	Girawa	09-08-00-N	41-41-00-E

Appendix 1: Continued....

	Accession Number	**Region**	**Zone**	**Woreda/District**	**Latitude**	**Longitude**
419	231329-B	Oromiya	Arssi	Jeju	08-37-00-N	39-40-00-E
420	231331-A	Oromiya	Arssi	Jeju	08-37-00-N	39-40-00-E
421	231331-C	Oromiya	Arssi	Jeju	08-37-00-N	39-40-00-E
422	234048-A	Tigray	Mirabawi	Medebay zana	14-09-00-N	38-47-00-E
423	234049-A	Tigray	Mehakelegnaw	Laelay maychew	14-09-00-N	38-45-00-E
424	234050-B	Tigray	Mehakelegnaw	Laelay maychew	14-11-00-N	38-47-00-E
425	**235032-A**	Amhara	Semen Wollo	Guba lafto	11-44-00-N	39-31-00-E
426	**235032-B**	Amhara	Semen Wollo	Guba lafto	11-44-00-N	39-31-00-E
427	**235034-C**	Amhara	Semen Wollo	Habru	11-04-00-N	39-45-00-E
428	**235036-A**	Amhara	Semen shewa	Mafudmezezo mojana	11-3 -00-N	39-45-00-E
429	**235036-B**	Amhara	Semen shewa	Mafudmezezo mojana	11-3 -00-N	39-45-00-E
430	**235036-C**	Amhara	Semen shewa	Mafudmezezo mojana	11-3 -00-N	39-45-00-E
431	235391-A	Tigray	Debubawi	Rayaazebo	12-29-00-N	39-38-00-E
432	235392-B	Tigray	Debubawi	Endamehoni		
433	235392-C	Tigray	Debubawi	Endamehoni		
434	235395-A	Tigray	Mehakelegnaw	Kola temben	13-35-00-N	39-10-00-E
435	235825-A	Amhara	Semen Gonder	Alefa	12-23-00-N	37-28-00-E
436	236194-A	Amhara	Debub Wollo	Kutaber		
437	236194-B	Amhara	Debub Wollo	Kutaber		
438	236196-A	Amhara	Debub Wollo	Tehuledere		
439	236196-B	Amhara	Debub Wollo	Tehuledere		
440	236197-A	Amhara	Debub Wollo	Tehuledere		
441	236197-C	Amhara	Debub Wollo	Tehuledere		
442	236198-A	Amhara	Debub Wollo	Kombolcha		
443	236198-B	Amhara	Debub Wollo	Kombolcha		
444	236459-A	Tigray	Misrakawi	Wukro		
445	236459-B	Tigray	Misrakawi	Wukro		
446	236460-A	Tigray	Misrakawi	Wukro		
447	236463-A	Tigray	Mehakelegnaw	Enticho		
448	236463-B	Tigray	Mehakelegnaw	Enticho		
449	236464-A	Tigray	Mehakelegnaw	Laelay maychew		
450	236464-B	Tigray	Mehakelegnaw	Laelay maychew		
451	236465-A	Tigray	Mehakelegnaw	Adwa	14-08-03-N	38-47-12-E
452	236465-C	Tigray	Mehakelegnaw	Adwa	14-08-03-N	38-47-12-E
453	**236467-B**	Tigray	Mirabawi	Medebay zana		
454	**236467-A**	Tigray	Mirabawi	Medebay zana		
455	236469-B	Tigray	Mirabawi	Endaselasie		
456	236470-A	Tigray	Mirabawi	Endaselasie		
457	236470-B	Tigray	Mirabawi	Endaselasie		
458	236471-A	Tigray	Mirabawi	Tahtay koraro		
459	236471-B	Tigray	Mirabawi	Tahtay koraro		
460	236475-A	Amhara	Semen Gonder	Gonder zuria		
461	236475-C	Amhara	Semen Gonder	Gonder zuria		
462	236479-A	Amhara	Mirab gojam	Adet	11-16-00-N	37-29-00-E
463	236479-C	Amhara	Mirab gojam	Adet	11-16-00-N	37-29-00-E
464	236481-B	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
465	236491-A	Oromiya	Semen shewa	Wara jarso		
466	**236493-A**	Amhara	Misrak gojam	Enarj enawga	10-41-05.7-N	038-11-10.6-E
467	236882-B	Oromiya	Semen shewa	Hidabu abote		
468	236882-A	Oromiya	Semen shewa	Hidabu abote		
469	237054-A	Amhara	Semen shewa	Minjarna shenkora	08-50-00-N	39-20-00-E
470	237054-B	Amhara	Semen shewa	Minjarna shenkora	08-50-00-N	39-20-00-E
471	237055-A	Amhara	Semen shewa	Minjarna shenkora	08-50-00-N	39-20-00-E
472	237055-B	Amhara	Semen shewa	Minjarna shenkora	08-50-00-N	39-20-00-E
473	240044-A	Amhara	Misrak gojam	Enemay	10-21-29-N	38-09-36-E
474	240045-A	Amhara	Misrak gojam	Enemay		
475	240050	Amhara	Misrak gojam	Enarj enawga	10-29-22-N	38-11-07-E
476	240054	Amhara	Bahir dar special	Bahir dar		
477	241800-A	Amhara	Semen Gonder	Belesa	12-17-00-N	37-44-00-E
478	241801-A	Amhara	Semen Gonder	Belesa	12-11-00-N	37-50-00-E
479	241801-C	Amhara	Semen Gonder	Belesa	12-11-00-N	37-50-00-E
480	241802-A	Amhara	Debub Gonder	Tach gayint	11-33-00-N	38-31-00-E
481	241804-B	Amhara	Semen Wollo	Bugna	12-02-00-N	39-01-00-E
482	241804-C	Amhara	Semen Wollo	Bugna	12-02-00-N	39-01-00-E
483	241804-D	Amhara	Semen Wollo	Bugna	12-02-00-N	39-01-00-E
484	207687-B	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-51-00-E
485	207133-A	Amhara	Semen Gonder	Wegera		
486	207694	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
487	30341-A	Amhara	South gonder	Fogera	11-57-07.5-N	037-42-51.4-E
488	30339-A	Amhara	South gonder	Fogera	11-56-10.4-N	037-42-30.7-E
489	30338-A	Amhara	Central gonder	Kemkem	12-05-11.1-N	037-45-10.3-E
490	30311-A	Amhara	East gojjam	Mota	11-06-24.5-N	037-47-06.7-E

Appendix 1: Continued....

	Accession Number	Region	Zone	Woreda/District	Latitude	Longitude
491	30333	Amhara	Central gonder	Gonder zuria	12-22-18.2-N	037-33-24.7-E
492	207686	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-50-00-E
493	207664-A	Oromiya	Misrak shewa	Akaki	08-52-00-N	38-47-00-E
494	30317-A	Amhara	Central gonder	Misrak belesa	12-30-52.2-N	038-08-19.6-E
495	30323-A	Amhara	Central gonder	Misrak dendia	12-28-05.1-N	037-21-56.3-E
496	207675-A	Oromiya	Misrak shewa	Ada'a chukala	08-48-00-N	38-37-00-E
497	30321-A	Amhara	Central gonder	Mirab belesa	12-26-23.3-N	037-46-29.1-E
498	30313-C	Amhara	West gojjam	Gonji	11-12-38.4-N	037-06-07.2-E
499	30349-A	Amhara	Semen shewa	Efratana gidim	10-17-39.4-N	039-59-15.7-E
500	30349-B	Amhara	Semen shewa	Efratana gidim	10-17-39.4-N	039-59-15.7-E
501	30349-C	Amhara	Semen shewa	Efratana gidim	10-17-39.4-N	039-59-15.7-E
502	30331-A	Amhara	Central gonder	Chilga	12-32-56.4-N	037-08-14.4-E
503	30304-A	Amhara	East gojjam	Enarji enawiga	10-43-49.1-N	038-09-36.7-E
504	30304-B	Amhara	East gojjam	Enarji enawiga	10-43-49.1-N	038-09-36.7-E
505	30322	Amhara	Central gonder	Mirab belesa	12-26-29.9-N	037-45-34.2-E
506	207717-A	Oromiya	Mirab shewa	Alem gena	08-51-00-N	38-30-00-E
507	207717-B	Oromiya	Mirab shewa	Alem gena	08-51-00-N	38-30-00-E
508	30332-A	Amhara	Central gonder	Chilga	12-32-23.4-N	037-11-02.3-E
509	30287-A	Amhara	East gojjam	Dejen	10-10-06.4-N	038-08-00.6-E
510	30344-A	Amhara	South Wollo	Kobo	12-06-51.5-N	039-37-19.0-E
511	30308-B	Amhara	East gojjam	Mota	11-06-24.5-N	037-47-06.7-E
512	30293-A	Amhara	East gojjam	Dejen	10-14-25.5-N	038-13-18.6-E
513	30312-A	Amhara	East gojjam	Mota	11-07-18.9-N	037-47-55.3-E
514	30312-B	Amhara	East gojjam	Mota	11-07-18.9-N	037-47-55.3-E
515	209032-C	Oromiya	Mirab shewa	Kersana kondaltiti		
516	41156-B	Oromiya	Misrak shewa	Ada'a chukala	08-46-00-N	38-31-00-E
517	207726	Amhara	Semen shewa	Moretna jiru	09-57-00-N	39-13-00-E
518	207726-	Amhara	Semen shewa	Moretna jiru	09-57-00-N	39-13-00-E
519	228196-A	Amhara	Debub Wollo	Debresina		
520	207136-A	Amhara	Semen Gonder	Wegera		
521	30328	Amhara	Central gonder	Takusa	12-15-35.5-N	037-05-18.3-E
522	30348-B	Amhara	Semen shewa	Efratana gidim	10-21-06.9-N	039-56-06.0-E
523	41156-A	Oromiya	Misrak shewa	Ada'a chukala	08-46-00-N	38-31-00-E
524	41127-B	Oromiya	Misrak shewa	Akaki	08-52-00-N	38-47-00-E
525	41026-A	Amhara	Misrak gojam	Hulet ej enese	11-05-00-N	37-50-00-E
526	30345-D	Amhara	North Wollo	Habro	11-28-54.8-N	039-37-15.7-E
527	214732-A	Amhara	Debub Wollo	Sayint		
528	214732-B	Amhara	Debub Wollo	Sayint		
529	30287-C	Amhara	East gojjam	Dejen	10-10-06.4-N	038-08-00.6-E
530	212477-B	Amhara	Semen shewa	Weremo wajetuna mid	10-14-00-N	39-00-00-E
531	41302-A	Amhara	Semen Gonder	Wegera	12-46-00-N	37-37-00-E
532	41059-A	Amhara	Misrak gojam	Hulet ej enese		
533	41059-C	Amhara	Misrak gojam	Hulet ej enese		
534	216855-A	Oromiya	Arssi	Merti	08-34-00-N	39-44-00-E
535	241800-B	Amhara	Semen Gonder	Belesa	12-17-00-N	37-44-00-E
536	207170-A	Amhara	Semen Gonder	Belesa		
537	41263-B	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
538	41003-B	Oromiya	Arssi	Tena	07-44-00-N	39-34-00-E
539	236472-A	Tigray	Mirabawi	Asegede tsimbela		
540	209038-C	Oromiya	Mirab shewa	Alem gena		
541	236472-B	Tigray	Mirabawi	Asegede tsimbela		
542	41301-B	Amhara	Semen Gonder	Wegera	12-46-00-N	37-37-00-E
543	207179-B	Amhara	Semen Gonder	Dabat		
544	30341-B	Amhara	South gonder	Fogera	11-57-07.5-N	037-42-51.4-E
545	207765-B	Oromiya	Mirab shewa	Jeldu		
546	207746	Amhara	Mirab gojam	Adet	11-16-00-N	37-29-00-E
547	207765-A	Oromiya	Mirab shewa	Jeldu		
548	207739-B	Amhara	Misrak gojam	Hulet ej enese	11-04-00-N	37-52-00-E
549	235034-A	Amhara	Semen Wollo	Habru	11-04-00-N	39-45-00-E
550	41281-A	Amhara	Bahir dar special	Bahir dar	11-35-00-N	37-21-00-E
551	30342-B	Amhara	South Wollo	Kobo	12-09-01.6-N	039-37-41.9-E
552	41169-C	Oromiya	Mirab shewa	Ejerie(addis alem)	09-01-00-N	38-15-00-E
553	41026	Amhara	Misrak gojam	Hulet ej enese	11-05-00-N	37-50-00-E
554	228290	Amhara	Misrak gojam	Awabel		
555	227161-C	Amhara	Semen Gonder	Alefa		
556	207691-B	Oromiya	Mirab shewa	Ambo	08-59-00-N	37-46-00-E
557	30334	Amhara	Central gonder	Gonder zuria	12-22-02.3-N	037-33-35.7-E
558	216855	Oromiya	Arssi	Merti	08-34-00-N	39-44-00-E
559	41307-E	Amhara	Semen Gonder	Gonder zuria	12-14-00-N	37-16-00-E
560	207173-C	Amhara	Semen Gonder	Wegera		
561	BBARC-R	Amhara	Semen shewa	Debre birhan zuria	09-43-00-N	39-40-00-E
562	ENEWARI	AMHARA	Semen shewa	Siadiber ean wayau	09-47-03-N	39-04-07-E
563	Tegulet	AMHARA	Semien shewa	Ankober	09-33-06-N	39-46-59-E

Appendix 1: Continued....

	ICARDA Material					
	Crop ID	**Population type**	**Collect country**	**CProvince**		
564	74980	LA	NPL	Mahakali	N28.20	E84.98
565	69690	LA	PRT	Portalegre	N39 16	W07 25
566	7129	LA	TUR	Usak	N38 14	E29 05
567	71875	LA	SYR	Aleppo	N36 08 40	E36 46 50
568	69757	UM	UZB	Tashkent	N 41 26	E 69 25
569	116496	LA	TUR	Canakkale	N39 51 24	E27 19 10
570	73227	LA	SYR	Aleppo	N36 25 40	E37 12 20
571	73249	LA	SYR	Hama	N35 13 44	E36 51 45
572	128699	LA	MDA	Kauschany	N 46 38	E 29 24
573	10165	LA	ESP	Castilla la Mancha	N38 44	W02 00
574	75095	LA	IRN	Kermanshahah	N34 25	E46 40
575	70788	LA	PAK	NWF	N34 30	E71 54
576	9427	LA	AZE	Nagorno-Karabakh (Xankandi)	N39 49	E46 45
577	140941	LA	ARM	Syunik	39 30 33	46 20 24
578	116451	LA	TUR	Canakkale	N40 00 42	E26 51 55
579	141720	LA	ITA	Sardinia	N 40 13	E 09 09
580	73327	LA	JOR	Ma'an	N30 11	E35 44
581	8191	LA	IRN	Fars	N29 45	E51 38
582	8522	LA	AFG	Takhar	N37 24	E69 48
583	9003	LA	TUR	Kutahya	N39 50	E29 34
584	8934	LA	TUR	Urfa	N37 50	E39 46
585	70551	LA	BGR	Grad Sofia	N42 43	E23 20
586	70503	LA	TUR	Mugla	N37 20	E28 35
587	69417	LA	CYP	Nicosia	N35 04	E33 05
588	140383	CV	TJK	Khatlon	38.37761	69.20816
589	6416	LA	LBN	Biqaa Al Gharbi	N33 37 21	E35 48 36
590	72051	LA	TUR	Adiyaman	N37 37	E38 22
591	9632	LA	IND	Himachal Pradesh	N30 55 02	E77 06 55
592	10163	LA	ESP	Castilla la Mancha	N39 15	W01 55
593	5909	LA	IRQ	Dahuk	N36 46	E43 52
594	9402	LA	TUR	Eskisehir	N40 02	E30 37
595	9412	LA	TUR	Afyon	N38 33	E31 13
596	74000	LA	DZA	Tiaret	N35 30	E01 08
597	71861	LA	SYR	Hama	N35 17 50	E36 45 00
598	72130	LA	TUR	Isparta	N37 56	E30 15
599	9049	LA	TUR	Corum	N40 33	E34 57
600	72151	LA	TUR	Denizli	N37 49	E29 17
601	8357	LA	ESP	Castilla la Mancha	N39 58	W04 50
602	139930	LA	AFG	Badakhshan	Market	36 48.58
603	140525	LA	AZE	Ganca	N40 40.617	E46 22.666
604	72034	LA	TUR	Urfa	N37 29	E38 30
605	71897	LA	SYR	Al Hasakah	N37 03	E41 29
606	140393	CV	TJK	Dushanbe	38.56126	69.00889
607	125231	LA	ISR	Northern Israel	N 32 42	E 35 18
608	73241	LA	SYR	Idlib	N35 38	E36 37
609	73396	LA	TUR	Adiyaman	N37 45	E38 24
610	141693	LA	ETH	Shewa	N09 35	E039 44 12
611	128407	LA	AZE	Baku	N 40 30	E 50 06
612	9628	LA	IND	Himachal Pradesh	N31 57 44	E77 06 21
613	72121	LA	TUR	Afyon	N38 18	E30 11
614	6454	LA	TUN	Silyanah	N36 19	E09 10
615	73328	LA	JOR	Amman	N31 47	E35 49
616	71873	LA	SYR	Aleppo	N36 28 30	E37 43 30
617	125187	LA	UZB	Tashkent	N41 23	E69 28
618	75335		SRB	Serbia	N42 48	E22 25
619	140119	CV	TJK	Kulyab	38.09229	69.33821
620	8935	LA	TUR	Elazig	N38 39	E39 41
621	74920	LA	TUR	Afyon	N38 36	E30 .3
622	126302	LA	ARM		N39 46 11	E45 17 18
623	72125	LA	TUR	Afyon	N38 13	E30 10
624	73397	LA	TUR	Urfa	N37 20	E39 22
625	9026	LA	TUR	K. Maras	N38 13	E37 12
626	70753	LA	PAK	NWF	N34 13	E73 08
627	72124	LA	TUR	Afyon	N38 23	E30 13
628	8349	LA	TUN	Zaghwan	N36 22	E09 54
629	73221	LA	SYR	Aleppo	N36 19 00	E37 03 30
630	72038	LA	TUR	Adiyaman	N37 35	E38 35
631	69604	LA	DZA	Tiaret	N 35 29	E 01 37
632	73395	LA	TUR	Gaziantep	N36 54	E37 22
633	140294	CV	TJK	Kulyab	37.90795	69.77456
634	132880	LA	GEO	T'bilisi	N41 50	E44 43

Appendix 1: Continued....

	Crop ID	Population type	Collect country	CProvince	Latitude	Longitude
635	69733	LA	LBY	Yafran	N31 58	E12 34
636	72016	LA	TUR	Urfa	N37 24	E38 37
637	72036	LA	TUR	Urfa	N37 27	E38 28
638	5903	LA	IRQ	As Sulaymaniyah	N35 32 20	E44 50 20
639	69420	LA	CYP	Nicosia	N35 06	E33 06
640	9058	LA	TUR	Adiyaman	N37 48	E38 35
641	132663	LA	AZE	Samaxi	Shamakhi region	N40 41 44
642	71853	LA	SYR	Homs	N34 44	E36 43
643	9415	LA	TUR	Mardin	N37 34	E40 53
644	9427	LA	AZE	Nagorno-Karabakh (Xankandi)	N39 49	E46 45
645	75095	LA	IRN	Kermanshahah	N34 25	E46 40
646	10163	LA	ESP	Castilla la Mancha	N39 15	W01 55

Appendix 1: Continued....

	Improved Varities					
	Variety	Type	Origin	Breeding methods	Year released	
647	Hora	Kabuli	ICARDA	Introdction and hybridization	2016	EAIR
648	Teketay	Desi	ICRISAT	Introdction and hybridization	2013	EAIR
649	Natoli	Desi	ICRISAT	Introdction and hybridization	2007	EAIR
650	Minjar	Desi	ICRISAT	Introdction and hybridization	2010	EAIR
651	Dalota	Desi	ICRISAT	Introdction and hybridization	2013	EAIR
652	Teji	Kabuli	ICARDA	Introdction and hybridization	2005	EAIR
653	Akaki	Desi	ICRISAT	Introduction and selection	1995	EAIR
654	Mastewal	Desi	ICRISAT	Introdction and hybridization	2006	EAIR
655	DZ-10-4	Kabuli	Ethiopia	Local selection	1974	EAIR
656	Shola					EAIR
657	Dhera	Kabuli	ICARDA	Introdction and hybridization	2016	EAIR
658	Ejere	Kabuli	ICARDA	Introdction and hybridization	2005	EAIR
659	Acos Dubie	Kabuli	Mexico	Introduction and aaptation	2009	EAIR
660	Shahso	Kabuli	ICARDA	Introduction and Hybridization	1999	EAIR
661	Habru	Kabuli	ICARDA	Introdction and hybridization	2004	EAIR
662	Arerti	Kabuli	ICARDA	Introduction and Hybridization	1999	EAIR
663	Chefe	Kabuli	ICARDA	Introdction and hybridization	2004	EAIR
664	Dimtu	Desi	ICRISAT	Introdction and hybridization	2016	EAIR
665	DZ-10-11	Desi	Ethiopia	Local selection	1974	EAIR
666	Fetenech	Desi	ICRISAT	Introdction and hybridization	2006	EAIR
667	Kasech	Kabuli	ICRISAT	Introduction and Hybridization	2011	EAIR
668	Akuri	Kabuli	ICRISAT	Introdction and hybridization	2011	EAIR
669	Worku	Desi	ICRISAT	Introduction and selection	1994	EAIR
670	Dubie	Desi	Ethiopia	Local selection	1978	EAIR
671	Kutaye	Desi	ICRISAT	Introdction and hybridization	2005	EAIR
672	Mariye	Desi	ICRISAT	Introduction and Hybridization	1985	EAIR
673	Yelbie	Kabuli	ICRISAT	Introduction and Hybridization	2006	EAIR
674	Kobo	Kabuli	ICRISAT	Introduction and Hybridization	2012	EAIR

Appendix 2. Mean value of quantitative and qualitative traits, and SR and FTR scores of 613 chickpea genotypes (554 Ethiopian genotypes, 32 elite frost resistant genotypes from ICARDA, and 27 improved) grown at Bakelo, Debre Birhan Ethiopia in 2018/2019 (A) and 2019/2020 (B) growing seasons.

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		NPB		NSB		FPPP		IPPP		DTF		DTP		DTM		TSW		GY		SR		FTR		SSS		FC		SLP		SC	
7129	28.5	45.5	4.0	4.8	1.6	3.3	18.8	21.9	7.1	14.7	59.4	76.0	74.9	91.6	133.4	149.5	169.7	170.9	758.1	1747.0	0.6	0.60	6	5	3	3	0	0	0	0	3	3
9026	51.3	52.0	6.2	5.7	5.4	5.9	102.8	102.8	58.3	16.8	49.4	50.0	74.9	73.2	148.0	143.2	124.4	124.9	1072.0	2208.0	0.8	0.70	4	4	4	2	0	0	0	0	3	3
9415	51.7	48.7	4.5	3.8	4.4	3.9	36.8	20.0	31.3	25.5	56.4	59.0	84.9	83.9	154.0	161.2	91.4	92.0	975.9	2059.0	0.8	0.81	3	3	2	2	0	0	0	0	3	3
9427	39.8	48.0	5.6	6.3	1.6	4.1	21.4	24.9	7.9	33.5	51.7	55.0	65.6	73.6	136.7	129.2	173.3	133.0	1178.0	1013.0	0.9	0.75	3	3	1	3	1	1	1	1	2	2
9632	44.3	45.0	3.2	2.7	2.0	2.5	27.8	27.8	22.3	20.8	52.4	53.0	87.9	86.2	149.0	144.2	85.4	85.9	1662.0	2098.0	0.8	0.82	2	2	2	1	0	0	0	0	3	3
9643	39.5	52.6	2.7	7.6	0.3	8.8	23.8	147.5	6.8	60.4	51.4	54.7	57.9	74.6	137.4	128.9	87.3	72.5	1208.0	875.9	0.8	0.78	4	4	1	3	1	1	1	1	2	2
10163	44.0	46.0	2.5	5.8	1.7	5.2	22.8	21.0	6.3	21.5	51.4	77.0	57.9	88.9	131.0	159.2	162.4	163.0	1675.0	2258.0	0.9	0.89	2	2	2	1	0	0	0	0	3	3
10165	43.5	35.8	4.7	5.9	2.0	5.5	36.1	55.5	11.1	42.4	54.4	74.7	76.9	90.6	135.4	137.9	148.5	137.5	752.6	547.0	0.7	0.59	3	5	2	4	0	0	0	0	3	3
30322	38.2	38.7	5.8	4.9	5.2	2.4	65.0	38.3	13.9	11.2	53.7	53.0	61.6	72.9	128.4	130.2	91.0	63.2	1168.0	1058.0	0.6	0.59	4	4	3	3	1	1	1	1	2	2
30328	39.5	47.3	2.3	6.9	0.3	4.5	19.5	36.8	6.1	16.4	55.4	57.7	59.9	73.6	137.4	126.9	86.7	60.3	1132.0	891.9	0.6	0.50	4	4	3	3	1	1	1	1	2	2
30333	43.5	49.2	5.8	4.5	6.2	2.9	51.3	48.0	12.9	35.0	53.7	55.0	61.6	76.6	129.4	129.2	85.8	63.6	1314.0	985.1	0.8	0.81	3	5	2	3	1	1	1	1	2	2
30334	38.5	52.9	4.8	7.1	3.5	3.2	40.6	67.7	12.2	39.0	52.7	62.0	59.6	76.6	130.4	127.2	90.0	60.6	1064.0	867.6	0.7	0.75	4	4	1	3	1	1	1	1	2	2
41026	37.2	38.2	2.8	5.1	1.5	3.2	25.3	52.0	8.6	37.0	51.7	58.0	61.6	82.6	122.4	129.2	78.2	39.9	1274.0	775.1	0.7	0.53	5	7	1	5	1	1	1	1	2	2
41045	36.3	46.6	4.9	5.0	2.3	4.4	42.8	36.3	7.2	29.5	53.7	52.0	61.6	73.6	131.7	127.2	85.3	55.0	1184.0	548.9	0.7	0.73	4	4	2	3	1	1	1	1	2	2
41283	30.5	33.6	3.4	5.8	4.2	2.9	39.0	43.7	11.2	37.0	51.7	71.0	59.6	82.6	129.4	136.2	39.6	38.8	652.7	165.1	0.7	0.67	6	6	5	5	1	1	1	1	2	2
41323	50.0	51.3	7.2	8.3	8.4	9.5	63.5	66.5	58.3	58.4	53.4	55.7	69.9	73.6	130.0	130.9	60.4	59.5	238.4	-8.0	0.6	0.57	5	5	5	4	1	1	1	1	2	2
69420	38.3	35.7	4.8	4.5	4.4	4.5	40.8	21.4	28.3	19.8	62.4	63.4	85.9	84.2	149.0	151.5	132.4	132.6	995.9	1949.0	0.8	0.79	3	3	2	1	0	0	0	0	3	3
69757	32.1	41.2	4.2	4.2	1.7	1.0	35.5	22.9	13.6	26.7	58.4	60.0	64.9	69.6	134.0	135.5	75.8	79.3	733.8	650.3	0.3	0.35	6	5	5	4	0	0	0	0	3	3
70753	44.0	41.0	7.8	7.1	4.7	4.2	25.8	9.0	20.3	24.5	72.4	75.0	69.9	68.9	147.0	154.2	98.4	99.0	993.4	2077.0	0.8	0.76	4	4	2	2	0	0	0	0	3	3
70788	44.0	43.3	6.8	5.0	3.7	3.1	30.8	30.6	8.6	11.5	54.4	76.0	61.9	82.6	138.0	138.2	87.5	87.1	1632.0	1480.0	0.8	0.71	3	3	4	1	1	1	3	3	2	2
71875	43.8	50.3	4.2	5.3	0.6	7.1	21.1	50.9	11.9	13.5	53.7	61.0	63.6	81.6	131.7	127.2	157.7	153.0	1618.0	1388.0	0.8	0.76	3	3	2	2	1	1	3	3	2	2
73227	24.1	22.1	1.1	0.5	0.2	0.8	-3.5	-5.9	-2.9	40.8	53.0	52.4	63.2	60.2	126.7	130.5	67.8	67.2	580.7	562.3	0.3	0.23	7	7	5	5	1	1	1	1	2	2
73249	31.5	33.2	4.4	4.1	2.2	2.2	13.6	14.0	7.2	38.0	54.7	56.0	71.6	73.6	124.4	125.2	64.6	63.2	251.0	269.6	0.3	0.12	8	9	5	4	1	1	1	1	2	2
73327	30.1	48.9	6.8	8.5	7.4	7.9	47.2	53.5	33.3	33.5	74.4	74.0	74.9	72.2	150.0	142.9	92.4	96.4	360.9	367.3	0.4	0.34	6	5	5	4	0	0	0	0	3	3
73328	31.0	39.6	4.8	6.5	3.7	4.2	15.5	21.8	11.3	41.5	64.4	64.0	81.9	79.2	123.0	115.9	31.4	35.4	907.1	913.5	0.5	0.45	6	5	5	4	0	0	0	0	3	3
73395	53.3	50.3	6.5	5.8	8.7	8.2	75.5	58.7	67.3	21.5	55.4	58.0	74.9	73.9	144.0	151.2	81.4	82.0	975.9	1859.0	0.9	0.85	3	3	2	1	0	0	0	0	3	3
73396	45.7	46.4	3.2	2.7	5.4	5.9	30.8	30.8	25.3	13.8	58.4	59.0	76.9	75.2	147.0	142.2	88.4	88.9	752.6	2069.0	0.8	0.82	3	3	2	1	0	0	0	0	3	3
74980	30.0	31.3	6.5	7.6	11.	12	92.5	95.5	24.3	44.9	68.4	70.7	74.9	78.6	127.0	127.9	61.4	60.5	222.1	35.7	0.2	0.10	6	6	5	5	0	0	0	0	3	3
75095	45.8	42.7	4.9	3.5	6.2	5.2	59.9	34.7	21.9	23.8	63.4	63.0	84.6	78.9	140.0	145.2	134.2	103.6	1912.0	1429.0	0.8	0.75	3	3	4	2	1	1	3	3	2	2
116451	48.6	39.0	7.4	6.0	6.3	6.2	24.0	25.8	16.7	14.1	62.4	60.0	68.6	62.2	138.4	133.2	155.1	154.3	1465.0	1321.0	0.8	0.76	4	4	2	1	1	1	2	2	2	2
116496	43.8	43.2	6.9	6.2	9.2	8.0	51.6	30.6	19.6	10.7	62.4	61.0	89.6	89.6	145.0	144.5	110.4	115.3	1560.0	1550.0	0.7	0.72	3	3	2	2	1	1	3	3	2	2
125231	32.0	37.4	4.2	3.9	2.4	2.5	49.2	29.8	34.3	35.8	52.4	53.4	62.9	61.2	129.0	131.5	96.4	96.6	392.1	345.6	0.6	0.53	6	5	5	4	1	1	1	1	2	2
126302	53.7	50.7	5.8	5.1	3.4	2.9	60.5	43.7	37.3	21.5	55.4	58.0	74.9	73.9	147.0	154.2	187.4	188.0	1416.0	1299.0	0.8	0.79	3	4	1	2	1	1	1	1	2	2
128699	43.0	42.0	4.2	5.3	1.0	2.4	27.2	12.3	6.0	8.5	57.4	76.0	68.9	77.6	131.0	129.2	118.6	96.0	1472.0	1234.0	0.8	0.76	4	4	2	2	0	0	0	0	3	3
132663	58.0	35.0	9.2	8.5	9.4	8.9	85.2	68.4	58.3	22.5	55.4	58.0	70.9	69.9	145.0	152.2	198.4	199.0	939.6	1423.0	0.8	0.81	3	3	2	1	0	0	0	0	3	3
140294	55.7	57.0	7.5	8.6	6.4	7.5	80.5	83.5	45.3	15.4	58.4	71.7	76.9	82.6	128.0	128.9	90.4	89.5	877.1	1691.0	0.9	0.85	3	3	3	2	0	0	0	0	3	3
140383	32.2	42.3	4.8	5.9	2.0	3.1	29.5	32.5	23.3	19.4	75.4	77.7	71.9	75.6	130.0	130.9	49.4	48.5	1041.0	1555.0	0.8	0.72	6	5	5	3	0	0	0	0	3	3
140393	55.7	56.4	5.8	5.3	8.0	8.5	44.8	44.8	37.3	15.8	49.4	50.0	85.9	84.2	148.0	143.2	89.4	89.9	837.1	1373.0	0.8	0.81	3	3	2	1	0	0	0	0	3	3
140525	30.2	48.4	5.5	6.9	3.0	3.4	26.5	32.0	22.3	38.2	62.4	65.0	86.9	90.9	148.0	150.2	39.4	39.2	147.1	67.7	0.1	0.08	8	9	5	5	1	1	1	1	2	2
140941	40.0	42.6	5.5	7.5	4.9	4.2	33.0	18.1	2.9	5.5	52.7	74.0	77.6	74.2	136.4	146.9	105.8	103.4	1755.0	1459.0	0.9	0.75	3	3	1	2	1	1	3	3	2	2
141693	46.3	43.7	8.8	8.5	8.7	8.8	93.2	73.8	29.3	20.8	52.4	53.4	69.9	68.2	137.0	139.5	105.4	105.6	869.6	1823.0	0.7	0.68	4	4	2	2	0	0	0	0	3	3
207608	37.6	49.6	4.4	7.2	5.3	3.5	36.4	60.1	8.4	18.5	51.4	51.0	61.6	64.2	141.4	118.9	92.0	87.4	1600.0	1551.0	0.9	0.83	2	3	2	2	1	1	3	2	1	1
207622	38.5	53.0	4.7	5.0	1.6	3.4	27.8	44.3	8.8	9.5	50.4	50.0	56.9	73.6	130.4	164.2	90.1	85.0	1269.0	1160.0	0.9	0.85	2	3	1	1	1	1	2	2	1	1
207638	38.8	40.6	2.9	6.5	0.6	2.5	39.4	75.1	5.9	18.5	48.7	52.0	58.6	65.2	128.7	120.9	98.1	94.4	1003.0	1131.0	0.9	0.85	2	3	1	1	1	1	2	2	1	1
207640	41.1	46.8	3.5	6.8	4.5	3.7	52.9	54.9	18.9	13.7	54.4	60.0	62.6	75.6	133.0	128.5	99.0	103.9	1840.0	1266.0	0.9	0.82	1	3	1	1	1	1	3	2	1	1
207648	35.7	43.4	4.1	5.2	1.9	3.5	10.8	47.8	1.7	14.8	50.0	50.4	56.2	64.2	129.7	128.5	98.4	88.9	1241.0	1223.0	0.9	0.87	1	3	2	2	1	1	3	2	1	1
207652	40.0	50.4	6.2	7.7	6.2	6.9	76.0	126.5	8.2	11.8	49.7	49.0	55.6	64.2	134.4	133.2	109.2	106.9	1829.0	1394.0	1.0	0.89	1	3	1	1	1	1	3	2	1	1
207668	37.1	47.0	5.5	5.3	2.2	4.5	19.2	84.8	1.4	17.8	52.0	53.0	57.2	69.2	122.7	123.2	99.2	98.9	1966.0	1921.0	0.9	0.87	1	2	1	2	1	1	3	3	1	1

Appendix 2: Continued....

Genotypes	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
207670	39.7	38.3	5.2	8.2	3.2	3.9	56.7	115.5	8.9	21.5	50.7	48.0	58.6	63.2	136.4	120.9	104.4	104.4	2095.0	2056.0	0.9	0.85	1	2	1	2	1	1	3	3	1	1
207674	52.0	51.6	4.2	5.9	3.0	3.5	70.2	76.5	27.3	17.5	51.4	51.0	69.9	67.2	126.0	118.9	88.4	92.4	1126.0	1132.0	0.8	0.83	2	3	1	1	1	1	2	2	1	1
207684	35.1	44.6	3.2	8.2	0.3	2.2	39.4	56.1	4.5	17.5	48.7	50.0	59.6	60.2	128.7	120.9	98.3	100.4	1554.0	1532.0	0.9	0.86	1	3	1	1	1	1	3	2	1	1
207686	36.5	40.2	5.1	9.9	2.5	4.1	51.0	72.3	6.9	16.2	50.7	55.0	56.6	73.9	127.4	130.2	93.4	86.1	1294.0	1225.0	0.9	0.87	2	3	1	1	1	1	2	2	1	1
207692	40.5	42.9	4.5	6.5	1.4	2.5	33.5	43.7	5.1	15.0	51.7	55.0	61.2	76.6	136.7	127.2	86.3	85.6	2487.0	1940.0	1.0	0.81	1	2	1	1	1	1	3	3	1	1
207694	41.2	35.3	5.5	4.3	2.7	1.8	43.8	52.9	8.7	25.8	52.7	53.7	61.2	75.2	134.7	125.2	87.5	41.3	1240.0	960.3	0.7	0.19	4	8	2	5	1	1	1	1	2	2
207712	36.0	47.6	4.5	6.3	5.9	4.1	48.0	70.9	16.9	3.5	48.7	51.0	56.6	71.6	131.4	129.2	109.2	108.0	2531.0	2604.0	0.9	0.85	2	2	1	1	1	1	3	3	1	1
207714	47.3	53.5	5.1	4.5	9.8	1.5	146.0	32.7	20.5	17.0	53.7	59.0	61.9	74.6	139.4	129.2	91.6	65.6	1493.0	1240.0	0.8	0.83	1	3	2	2	1	1	3	2	1	1
207726	20.3	32.8	0.2	3.5	-0.2	2.5	5.0	31.1	-3.1	30.8	50.7	50.4	57.6	69.2	136.4	127.5	48.2	47.6	828.8	780.6	0.8	0.71	8	9	4	3	1	1	1	1	2	2
207730	35.2	37.8	3.2	5.7	-0.4	3.7	20.8	49.6	2.2	15.5	53.7	52.0	60.6	73.6	131.7	129.2	108.3	106.0	1486.0	1555.0	0.9	0.82	2	3	2	2	1	1	2	2	1	1
207746	42.5	43.0	4.2	7.0	2.7	4.4	41.2	80.6	9.1	15.5	52.7	53.0	61.2	71.6	138.7	126.2	80.3	81.0	1762.0	1965.0	0.9	0.81	2	2	1	1	1	1	3	3	1	1
207748	41.6	43.9	5.1	4.5	6.3	2.9	56.0	28.0	18.1	17.0	53.4	59.0	62.6	74.6	141.4	127.2	73.9	70.6	1314.0	1166.0	0.9	0.83	2	3	2	2	1	1	3	2	1	1
207766	38.8	51.6	3.6	6.6	2.3	4.5	42.1	56.2	8.9	18.4	49.7	58.7	59.6	79.6	131.7	128.9	93.3	72.5	1230.0	1165.0	0.8	0.80	2	3	1	2	1	1	2	2	1	1
207770	44.2	44.2	4.2	4.0	2.1	1.8	32.2	47.9	5.4	-3.2	51.7	50.7	61.2	62.2	149.7	146.2	80.7	82.5	2008.0	1991.0	0.9	0.84	1	2	2	2	1	1	3	3	1	1
216855	31.7	38.2	4.2	4.3	2.1	3.1	38.8	30.9	8.7	38.8	51.7	53.7	62.2	75.2	131.7	125.2	39.1	40.6	1015.0	424.0	0.5	0.50	6	6	4	5	1	1	1	1	2	2
228290	43.2	50.8	5.8	5.8	3.9	5.0	40.6	51.2	13.6	30.7	54.7	59.0	61.6	77.6	129.4	129.5	81.4	68.9	1341.0	867.4	0.7	0.50	3	7	2	3	1	1	1	1	2	2
240050	38.1	45.2	3.9	4.8	6.5	2.3	37.9	57.2	14.6	32.7	54.4	54.0	65.6	76.6	136.0	129.5	75.2	33.9	737.1	337.4	0.6	0.47	5	6	4	4	1	1	1	1	2	2
240054	31.5	31.0	4.4	2.8	4.2	-0.1	23.6	3.7	9.2	37.5	51.7	53.0	59.6	70.9	124.4	155.2	40.6	40.0	952.7	272.7	0.1	0.12	8	9	4	4	1	1	1	1	2	2
16341-A	38.1	42.7	5.5	5.8	6.5	2.2	72.6	33.7	15.3	18.5	53.4	52.0	62.6	64.9	138.0	136.2	93.0	95.0	1925.0	1935.0	0.9	0.86	1	2	1	2	1	1	3	3	1	1
16341-B	31.1	39.7	3.6	6.3	0.3	3.2	17.8	55.8	4.2	33.8	53.7	51.0	59.6	64.2	124.7	121.2	47.9	39.9	423.5	480.7	0.6	0.57	7	7	4	4	1	1	1	1	2	2
16585-B	38.5	38.3	5.0	8.3	2.0	6.5	30.8	75.5	12.1	42.4	51.4	54.7	56.9	74.6	126.4	130.9	80.5	43.5	1038.0	873.2	0.7	0.43	4	5	3	4	1	1	1	1	2	2
16586-A	35.1	36.3	3.2	8.3	4.0	3.7	30.4	89.3	5.9	44.5	51.7	50.0	59.6	73.6	138.7	138.2	102.1	48.0	1234.0	198.9	0.7	0.49	4	5	2	4	1	1	1	1	2	2
16588-A	42.0	53.3	4.2	7.0	1.2	4.7	36.3	101.6	4.5	17.5	50.7	54.0	59.6	75.6	131.4	138.2	76.6	64.5	1389.0	1085.0	0.7	0.65	4	4	3	3	1	1	1	1	2	2
16589-A	36.5	38.3	5.0	7.6	4.6	6.8	47.1	76.2	9.1	43.4	53.4	54.7	59.9	87.6	137.4	128.9	71.0	45.5	267.6	-8.0	0.6	0.21	4	6	5	5	1	1	1	1	2	2
16591-A	35.0	38.6	7.8	5.5	4.2	4.2	33.7	132.5	4.5	37.5	53.7	51.0	60.6	63.2	134.4	121.9	88.6	70.4	1155.0	967.3	0.8	0.70	4	6	2	3	1	1	1	1	2	2
16591-B	38.3	41.6	3.5	8.3	0.9	4.4	31.7	101.3	3.9	43.5	50.7	54.0	58.6	76.6	128.4	129.2	79.4	36.0	927.1	358.9	0.6	0.56	4	4	3	4	1	1	1	1	2	2
207133-A	30.5	32.4	3.5	4.2	2.4	2.1	14.5	30.0	7.4	36.2	52.7	53.0	62.2	61.9	131.7	133.2	46.3	47.2	549.9	526.0	0.4	0.31	8	8	5	5	1	1	1	1	2	2
207136-A	40.3	37.1	6.5	9.2	6.6	4.9	56.0	88.5	12.9	44.5	51.7	51.0	56.6	63.2	134.4	121.9	53.2	50.4	489.6	207.3	0.3	0.13	6	8	5	5	1	1	1	1	2	2
207138-B	31.5	41.1	4.1	4.2	3.2	3.1	36.6	54.3	9.9	32.2	49.7	55.0	58.6	75.9	153.4	130.2	45.9	46.2	711.0	120.2	0.3	0.32	6	5	4	5	1	1	1	1	2	2
207143-A	36.1	41.5	3.6	3.7	2.0	2.8	34.1	37.2	7.9	27.8	50.7	51.7	58.6	63.2	136.7	133.2	95.7	64.5	881.0	929.0	0.5	0.26	5	5	4	3	1	1	1	1	2	2
207145-A	43.3	47.1	3.7	5.6	1.8	0.8	17.0	35.7	12.5	30.2	52.7	54.0	59.9	73.9	133.4	128.2	79.2	53.2	1206.0	610.2	0.9	0.50	3	4	1	3	1	1	1	1	2	2
207149-B	42.6	46.7	7.4	5.5	7.0	4.5	51.7	64.4	24.4	54.8	53.4	53.4	63.6	68.2	138.4	131.5	99.5	47.6	1084.0	600.6	0.7	0.64	4	5	3	3	1	1	1	1	2	2
207149-C	32.6	34.6	6.1	4.0	4.8	2.1	32.0	24.2	17.1	32.8	52.7	53.7	60.9	80.2	126.4	126.2	47.2	46.1	941.5	570.3	0.4	0.33	7	7	4	5	1	1	1	1	2	2
207155-C	38.5	30.7	5.5	2.8	3.7	0.5	27.5	12.7	5.1	37.5	50.7	54.0	61.2	64.9	139.7	154.2	83.6	46.0	1038.0	119.0	0.5	0.51	4	6	1	5	1	1	1	1	2	2
207157-B	43.6	40.7	4.7	5.2	3.0	3.1	33.0	54.1	11.1	33.8	52.4	50.4	61.6	65.2	138.4	128.5	84.3	46.6	1054.0	796.9	0.7	0.63	5	5	3	3	1	1	1	1	2	2
207161-C	38.8	44.0	6.0	7.7	3.0	3.4	50.8	54.6	13.8	27.5	49.4	50.0	57.9	73.6	133.4	127.2	98.1	45.0	1016.0	750.1	0.8	0.71	3	4	3	3	1	1	1	1	2	2
207164-A	36.8	48.6	4.6	8.0	1.3	5.4	35.4	49.9	7.2	29.5	49.7	52.0	57.6	73.6	134.7	138.2	86.9	35.0	722.7	153.9	0.6	0.53	4	7	4	4	1	1	1	1	2	2
207165-B	38.1	38.2	4.9	4.0	1.3	2.2	40.4	58.2	6.2	35.8	49.7	51.0	58.6	64.2	131.7	121.2	86.1	47.9	398.5	455.7	0.6	0.17	4	8	5	5	1	1	1	1	2	2
207166-A	41.9	46.8	6.1	5.0	6.8	5.1	35.4	69.9	15.8	26.8	51.7	54.7	59.9	77.2	136.4	123.2	71.2	64.1	866.5	306.5	0.4	0.34	7	8	4	4	1	1	1	1	2	2
207167-A	37.5	48.3	3.3	5.3	1.6	2.7	35.5	46.6	6.5	32.5	53.4	50.0	57.9	73.6	133.4	127.2	91.3	56.0	1089.0	565.1	0.7	0.68	4	4	3	3	1	1	1	1	2	2
207170-A	31.6	36.3	4.1	3.5	2.5	1.2	32.6	23.7	8.6	39.5	51.7	51.0	58.6	69.9	130.4	155.2	44.0	45.0	1103.0	106.5	0.5	0.51	6	7	4	5	1	1	1	1	2	2
207171-B	30.2	30.1	2.5	5.1	0.7	0.5	0.8	23.4	3.7	40.5	51.7	51.0	61.2	62.9	135.7	135.2	41.3	43.0	306.6	145.2	0.4	0.33	7	8	5	4	1	1	1	1	2	2
207171-C	44.8	39.8	6.1	7.2	5.5	3.4	52.6	51.7	14.6	37.2	49.7	59.0	57.6	73.9	121.4	128.2	76.6	35.2	607.7	140.2	0.6	0.37	4	8	4	5	1	1	1	1	2	2
207173-A	37.7	44.3	4.5	4.8	1.2	2.5	16.7	49.4	2.2	43.5	48.7	55.0	56.6	72.9	131.4	144.2	130.2	130.0	825.4	466.5	0.7	0.64	4	4	4	2	1	1	1	1	2	2
207173-B	36.8	42.2	4.5	7.1	5.9	3.2	56.9	67.7	13.3	38.0	54.4	56.0	61.6	74.6	160.0	125.2	81.6	13.6	627.1	290.1	0.6	0.42	4	4	4	5	1	1	1	1	2	2
207173-C	46.3	46.6	5.5	7.0	5.4	6.1	41.2	40.3	25.3	11.5	71.4	73.0	74.9	80.6	126.0	127.2	80.4	80.0	1185.0	1133.0	0.8	0.81	2	3	2	2	1	1	2	2	1	1
207175-A	38.4	46.4	6.9	4.5	11	2.5	70.9	39.1	18.9	9.8	54.4	53.4	61.6	68.2	138.0	128.5	101.0	102.6	1583.0	1569.0	0.8	0.75	3	3	1	1	1	1	3	3	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		NPB		NSB		FPPP		IPPP		DTF		DTP		DTM		TSW		GY		SR		FTR		SSS		FC		SLP		SC	
207178-A	48.5	51.3	3.9	5.5	0.3	3.2	24.8	86.8	8.9	41.5	51.7	52.0	59.6	69.2	131.7	130.9	95.3	36.4	796.0	347.3	0.6	0.50	4	6	4	4	1	1	1	1	2	2
207179-B	36.6	33.6	8.1	2.5	7.6	3.5	72.4	36.0	20.4	27.0	53.4	60.0	60.6	74.6	132.4	129.2	75.9	42.6	1005.0	327.5	0.6	0.50	4	6	4	5	1	1	1	1	2	2
207185-B	38.1	49.6	4.1	5.5	3.5	5.5	45.8	83.5	4.4	12.5	51.0	51.0	54.2	63.2	129.7	118.9	99.2	102.4	1836.0	1361.0	0.8	0.73	3	4	1	2	1	1	3	2	2	2
207186-C	37.5	33.0	4.0	4.2	2.3	1.9	24.8	61.5	7.1	35.5	52.4	50.0	55.9	63.2	130.4	120.9	94.5	49.4	1169.0	971.0	0.7	0.56	4	7	3	4	1	1	1	1	2	2
207562-B	27.7	41.8	2.8	3.2	1.0	1.7	27.8	28.2	9.0	22.7	50.4	52.0	55.9	72.6	124.0	162.5	71.8	67.9	613.8	271.1	0.2	0.16	7	9	4	4	1	1	1	1	2	2
207607-B	42.8	40.8	7.1	4.8	2.9	5.2	36.6	55.7	12.9	35.0	50.7	55.0	59.6	74.6	126.4	125.2	77.8	39.6	999.4	188.8	0.7	0.38	5	5	1	4	1	1	1	1	2	2
207607-C	40.8	37.2	3.4	4.5	5.2	3.2	38.3	46.7	11.9	36.0	50.7	59.0	56.6	76.6	126.4	127.2	94.2	48.6	807.7	458.8	0.6	0.31	5	5	4	4	1	1	1	1	2	2
207609-A	42.5	37.5	7.9	6.7	6.7	4.1	65.8	87.5	9.1	48.8	51.7	51.7	60.2	77.2	131.7	141.2	93.1	24.1	1327.0	721.5	0.7	0.38	4	5	2	5	1	1	1	1	2	2
207609-B	41.5	35.2	7.9	4.0	5.1	0.8	47.5	51.2	6.4	26.8	51.7	51.7	59.2	75.2	139.7	123.2	99.1	45.1	1385.0	390.3	0.7	0.23	4	6	2	5	1	1	1	1	2	2
207617-A	40.4	30.4	4.5	2.8	2.2	2.5	53.2	32.7	14.3	37.5	56.4	55.0	62.6	70.9	140.0	136.2	79.6	44.0	1254.0	591.5	0.7	0.67	4	6	2	5	1	1	1	1	2	2
207623-A	34.8	34.3	5.2	5.0	2.3	2.4	34.1	51.3	1.9	24.5	52.7	52.0	59.6	75.6	136.7	127.2	94.1	40.0	1281.0	678.9	0.6	0.56	4	5	2	4	1	1	1	1	2	2
207623-B	38.1	33.5	3.2	5.2	4.0	4.0	50.1	46.9	12.5	31.7	50.7	54.0	58.6	73.6	126.7	129.5	92.5	49.9	1159.0	392.4	0.7	0.51	4	7	2	5	1	1	1	1	2	2
207624-B	35.9	42.4	5.1	3.9	3.8	3.5	19.7	27.1	13.5	37.8	53.7	50.4	60.9	69.2	146.4	131.5	63.2	61.6	761.5	443.1	0.2	0.19	8	9	4	4	1	1	1	1	2	2
207635-B	39.1	52.6	7.5	4.6	6.5	3.1	70.9	56.2	14.9	47.4	53.4	52.7	61.6	73.6	160.0	128.9	72.8	44.5	908.8	497.0	0.6	0.52	5	5	3	3	1	1	1	1	2	2
207635-C	40.3	34.1	6.1	3.5	3.6	4.1	36.0	14.1	11.7	20.8	52.4	62.4	61.6	84.2	127.4	152.5	89.1	82.7	1400.0	1373.0	0.8	0.75	4	4	1	1	1	1	2	2	2	2
207636-B	39.8	40.6	5.0	5.6	4.3	8.1	47.1	49.5	11.5	34.4	51.4	52.7	57.9	73.6	130.4	126.9	86.9	44.5	1264.0	282.0	0.6	0.43	4	5	2	5	1	1	1	1	2	2
207637-A	39.5	37.0	3.5	4.9	1.1	4.5	27.2	93.8	5.7	31.5	52.7	52.0	61.2	67.2	136.7	129.9	90.7	56.4	1270.0	823.5	0.7	0.67	4	5	2	3	1	1	1	1	2	2
207637-B	36.5	36.7	2.5	5.3	1.1	4.9	2.8	51.8	3.4	39.8	52.7	51.0	63.2	64.2	136.7	123.2	70.7	44.9	804.9	475.7	0.5	0.49	6	6	4	4	1	1	1	1	2	2
207639-A	36.6	39.2	3.7	4.5	3.5	2.7	24.4	62.6	8.8	34.7	55.7	52.0	64.9	76.6	141.4	130.5	87.2	48.9	1045.0	782.4	0.7	0.67	5	7	3	3	1	1	1	1	2	2
207643-A	36.5	35.7	3.9	4.7	0.3	4.2	54.4	66.5	9.2	41.8	48.7	51.0	58.6	64.2	136.7	121.2	87.5	45.9	937.7	180.7	0.6	0.14	4	9	3	5	1	1	1	1	2	2
207643-B	35.2	51.6	4.7	3.6	1.6	2.8	37.5	32.2	10.5	29.4	50.4	52.7	57.9	70.6	133.4	129.9	80.5	71.5	919.3	745.7	0.5	0.40	6	6	4	3	1	1	1	1	2	2
207649-A	39.5	49.3	4.5	7.0	2.4	5.1	29.5	71.9	3.4	12.5	50.7	51.0	62.2	74.6	134.7	138.2	100.3	101.0	1147.0	1054.0	0.7	0.65	4	4	3	2	1	1	2	2	2	2
207649-B	37.0	39.0	5.2	7.0	3.2	5.7	30.0	83.3	4.5	42.5	48.7	51.0	57.6	99.6	132.4	126.2	88.6	46.0	622.1	331.4	0.5	0.43	4	5	3	4	1	1	1	1	2	2
207664-A	48.8	45.6	6.5	7.0	3.4	5.4	58.8	63.9	6.4	17.5	51.7	52.0	61.2	68.6	131.7	127.2	95.1	91.0	1787.0	1890.0	0.9	0.86	1	2	1	1	1	1	3	3	1	1
207675-A	39.8	34.6	5.9	3.7	3.7	3.8	41.2	49.5	5.7	26.8	54.7	51.7	61.2	75.2	138.7	122.2	83.1	47.1	1395.0	575.3	0.7	0.19	5	6	1	5	1	1	1	1	2	2
207679-A	38.4	42.8	3.9	2.5	5.2	0.3	43.9	39.6	12.9	29.7	51.4	57.0	61.6	76.6	136.0	127.5	72.4	53.9	1105.0	750.0	0.8	0.75	4	4	1	3	1	1	1	1	2	2
207679-B	38.0	48.5	4.5	4.2	1.0	2.7	40.2	54.2	10.3	31.7	50.4	57.0	58.9	73.6	131.0	129.5	80.6	53.9	1289.0	589.9	0.7	0.67	4	4	2	3	1	1	1	1	2	2
207683-A	38.9	47.5	6.1	4.1	7.2	1.2	38.0	43.4	14.1	15.0	53.7	59.0	60.9	82.6	139.4	129.2	88.0	84.6	1193.0	1238.0	0.7	0.71	4	4	3	2	1	1	2	2	2	2
207683-B	38.6	39.2	6.4	4.5	6.2	2.2	27.0	46.0	10.8	27.0	52.7	59.0	59.9	74.6	139.4	129.2	81.6	37.6	758.2	213.8	0.6	0.46	4	6	4	5	1	1	1	1	2	2
207687-B	40.8	51.1	4.9	4.9	4.7	2.4	37.5	37.3	13.1	33.2	52.7	62.0	62.2	75.9	141.7	128.2	70.3	32.2	941.6	120.2	0.3	0.21	5	5	4	4	1	1	1	1	2	2
207688-A	40.3	41.1	8.1	2.9	10	2.5	74.0	81.1	21.1	19.8	53.4	53.4	62.6	68.2	141.4	132.5	114.7	113.6	1382.0	1356.0	0.8	0.81	2	3	1	1	1	1	2	2	1	1
207691-A	37.7	47.8	5.2	5.5	2.9	4.7	45.0	84.2	7.2	38.7	50.7	52.0	57.6	74.6	134.4	129.5	103.2	105.9	1412.0	839.9	0.9	0.67	3	4	1	2	1	1	1	1	2	2
207691-B	41.8	35.7	3.4	4.8	2.5	3.9	33.0	58.4	11.9	36.0	49.7	55.0	58.6	76.6	129.4	127.2	75.9	41.6	519.4	130.1	0.6	0.50	3	7	4	5	1	1	1	1	2	2
207695-C	37.1	46.8	4.2	5.8	0.5	5.3	40.2	53.9	9.6	32.7	51.4	54.0	61.6	76.6	138.0	129.5	85.6	37.9	720.5	357.4	0.6	0.49	3	6	4	4	1	1	1	1	2	2
207711-A	39.0	43.5	4.2	4.8	4.7	1.7	40.8	34.9	18.0	27.7	48.4	53.0	56.9	73.6	133.0	143.5	75.0	54.1	1077.0	158.6	0.7	0.41	5	5	3	4	1	1	1	1	2	2
207711-B	37.6	47.4	6.1	5.6	3.8	2.1	27.0	35.7	14.5	35.2	52.7	53.0	58.9	72.9	137.4	128.2	76.8	31.2	1201.0	255.2	0.6	0.59	4	4	3	4	1	1	1	1	2	2
207715-A	29.5	35.0	4.1	4.0	0.9	0.8	30.3	32.2	13.6	37.8	51.7	51.7	58.6	61.2	130.4	129.2	48.0	49.1	917.7	939.0	0.2	0.15	8	9	4	4	1	1	1	1	2	2
207715-B	47.5	33.7	4.5	3.8	2.4	-0.1	25.2	6.7	3.4	42.5	51.7	57.0	60.2	68.9	136.7	134.2	63.0	47.0	464.9	145.2	0.5	0.13	4	8	4	4	1	1	1	1	2	2
207717-A	39.5	42.0	4.5	3.5	2.1	0.2	13.5	23.0	3.7	32.5	49.7	53.0	58.2	69.9	130.7	136.2	95.1	67.0	586.6	284.0	0.5	0.17	4	8	4	4	1	1	1	1	2	2
207717-B	39.5	36.6	3.0	5.5	2.6	2.2	41.5	40.5	9.8	27.5	54.4	50.0	57.9	63.2	130.4	120.9	98.5	43.5	1028.0	306.0	0.8	0.58	4	5	1	4	1	1	1	1	2	2
207721-B	41.8	46.2	3.5	8.8	2.1	10	18.2	122.6	7.1	63.7	53.7	54.0	64.2	72.6	136.7	127.5	77.3	44.9	1037.0	678.6	0.7	0.69	5	6	3	3	1	1	1	1	2	2
207728-A	43.6	51.7	7.4	7.9	9.5	4.1	52.0	72.0	10.8	17.2	51.7	55.0	58.9	74.9	140.4	128.2	82.2	80.2	1500.0	1349.0	0.8	0.81	1	3	1	2	1	1	3	2	1	1
207729-A	44.1	36.2	10.	3.8	9.5	3.9	83.9	55.0	22.3	35.0	53.4	58.0	62.6	78.6	133.0	139.2	83.2	45.6	1322.0	202.6	0.7	0.43	4	5	2	4	1	1	1	1	2	2
207729-B	36.1	36.5	5.5	5.1	3.9	1.5	53.9	45.7	11.9	36.0	53.4	55.0	62.6	71.6	138.0	127.2	89.2	58.6	1197.0	371.3	0.7	0.09	4	5	2	4	1	1	1	1	2	2
207733-A	36.2	50.3	4.0	5.5	2.0	2.2	33.5	57.8	8.1	33.5	54.4	50.0	60.9	65.2	130.4	120.9	84.1	60.4	1326.0	689.8	0.6	0.62	4	4	2	3	1	1	1	1	2	2
207735-A	38.0	52.6	3.8	5.6	1.9	4.5	27.0	53.5	-0.5	35.9	50.7	54.7	58.6	75.6	133.4	128.9	87.0	30.5	1050.0	389.5	0.7	0.59	5	5	3	4	1	1	1	1	2	2
207735-B	41.8	40.2	5.4	4.5	6.5	5.2	51.3	78.7	10.9	57.0	50.7	60.0	58.6	80.6	122.4	125.2	85.4	40.6	947.7	183.8	0.6	0.50	4	6	3	4	1	1	1	1	2	2
207736-B	36.0	38.5	5.5	4.8	3.4	1.3	46.2	49.2	15.3	41.7	52.4	54.0	58.9	73.6	126.0	129.5	77.8	21.4	1467.0	677.4	0.7	0.33	4	6	3	5	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		**SC**	
207737-A	36.8	33.6	4.1	3.8	3.2	0.7	26.3	8.6	9.9	26.7	51.7	62.0	59.6	82.6	126.4	129.5	87.4	50.9	912.7	521.1	0.5	0.43	6	7	4	5	1	1	1	1	2	2
207737-B	33.3	36.9	2.5	6.9	1.4	0.8	32.8	26.0	10.0	32.2	49.4	53.0	56.9	75.9	124.0	130.2	48.4	41.2	413.8	120.7	0.2	0.13	7	9	5	5	1	1	1	1	2	2
207739-B	37.5	41.5	4.1	4.1	3.5	2.9	21.3	62.7	8.9	15.0	51.7	58.0	59.6	94.6	123.4	136.2	226.0	224.6	1354.0	1373.0	0.8	0.78	3	4	1	1	1	1	2	2	2	2
207752-A	36.6	36.7	3.7	3.5	1.5	1.5	17.0	15.7	8.8	45.5	52.7	54.0	58.9	71.9	129.4	153.2	85.6	49.3	1023.0	444.0	0.6	0.44	5	6	1	4	1	1	1	1	2	2
207753-A	43.6	45.1	10.	5.2	8.5	5.4	54.0	44.0	23.1	29.2	52.7	55.0	58.9	73.9	129.4	130.2	82.8	57.2	1076.0	551.5	0.6	0.50	4	4	3	3	1	1	1	1	2	2
207753-B	35.8	38.8	2.9	5.3	1.3	3.8	35.4	52.2	12.5	46.9	48.7	53.7	59.6	73.6	124.7	128.9	87.3	54.7	1189.0	375.7	0.7	0.34	4	6	2	4	1	1	1	1	2	2
207753-C	32.6	35.0	1.9	0.5	0.4	-0.8	1.5	-5.3	2.4	39.5	50.7	51.0	60.2	54.9	132.7	139.2	46.5	46.8	674.9	613.5	0.4	0.38	8	8	4	4	1	1	1	1	2	2
207765-A	38.5	41.0	2.5	3.5	1.1	1.2	15.5	25.4	4.4	39.5	50.7	52.0	60.2	63.9	128.7	136.2	97.1	56.0	1442.0	582.7	0.6	0.54	4	4	2	3	1	1	1	1	2	2
207765-B	41.5	31.0	3.5	2.8	3.4	4.2	34.5	55.7	3.7	58.5	50.7	52.0	61.2	70.9	133.7	154.2	105.9	48.5	1365.0	436.5	0.8	0.54	3	5	2	4	1	1	1	1	2	2
207769-A	36.0	45.3	5.8	6.5	6.2	3.9	57.3	69.8	7.9	39.5	52.7	51.0	58.6	63.2	134.4	99.9	80.2	51.5	1257.0	391.0	0.7	0.53	4	5	2	4	1	1	1	1	2	2
207769-C	40.4	39.8	4.2	5.5	5.5	1.3	58.6	27.6	18.6	38.7	51.4	54.0	60.6	71.6	132.0	129.5	62.3	64.9	1369.0	107.4	0.7	0.16	4	9	3	4	1	1	1	1	2	2
207893-A	37.8	38.7	4.2	5.9	2.0	3.2	33.4	85.8	5.5	6.5	48.7	50.0	59.6	63.2	136.7	118.9	99.3	92.4	1703.0	1431.0	0.8	0.71	3	3	2	2	1	1	3	3	2	2
208454-C	36.2	37.2	5.1	6.5	2.5	2.9	34.0	44.4	8.9	27.0	49.7	56.0	57.6	75.6	126.4	127.2	83.8	45.4	1128.0	205.1	0.8	0.52	4	5	1	4	1	1	1	1	2	2
208988-A	39.5	43.8	3.2	3.7	2.1	1.4	12.8	47.9	6.1	36.8	52.7	51.7	61.2	84.2	141.7	123.2	76.3	56.3	1380.0	442.8	0.9	0.78	3	3	1	3	1	1	1	1	2	2
208988-B	43.5	42.3	3.9	6.2	5.1	3.5	12.5	84.5	5.4	48.5	51.7	50.0	60.2	63.2	130.7	121.9	63.9	56.4	1360.0	686.0	0.7	0.62	3	6	2	3	1	1	1	1	2	2
208994-A	42.1	38.8	5.5	5.3	5.5	5.4	55.6	52.9	14.6	0.8	54.4	52.7	61.6	62.2	136.0	131.2	88.8	90.9	1299.0	1359.0	0.9	0.78	3	4	1	1	1	1	2	2	2	2
208995-B	43.5	46.6	4.2	6.9	1.6	2.5	42.1	79.8	5.9	16.5	51.7	52.0	59.6	67.2	126.7	120.9	94.5	72.4	1028.0	1156.0	0.7	0.65	4	4	3	3	1	1	2	2	2	2
208995-D	41.3	35.7	4.8	6.7	3.2	4.1	41.3	68.3	9.9	27.5	50.7	51.0	57.6	71.6	136.4	129.2	78.6	48.1	1344.0	738.9	0.7	0.56	4	5	2	4	1	1	1	1	2	2
208997-B	37.0	40.3	5.8	6.3	3.9	4.1	40.3	50.5	3.5	38.8	52.7	53.7	57.6	63.2	128.4	125.2	83.8	43.7	627.1	679.0	0.8	0.85	6	6	4	3	1	1	1	1	2	2
209001-B	37.1	38.5	3.2	6.5	2.5	1.3	40.9	46.9	11.6	30.7	53.4	53.0	63.6	72.6	138.0	129.5	91.6	35.9	870.5	228.6	0.5	0.43	5	5	4	4	1	1	1	1	2	2
209003-B	35.8	45.9	6.5	4.1	3.9	3.5	43.9	48.7	13.6	32.0	52.4	53.0	62.6	74.6	134.0	142.2	88.8	34.6	1355.0	115.1	0.8	0.58	4	4	1	4	1	1	1	1	2	2
209008-A	32.0	36.1	3.8	5.3	5.9	3.1	49.3	47.6	2.5	23.5	51.7	51.0	57.6	73.6	131.4	129.2	43.2	38.0	1289.0	683.9	0.6	0.42	6	6	4	5	1	1	1	1	2	2
209008-B	38.8	39.9	6.9	7.0	3.0	3.8	52.4	55.5	5.2	-4.2	51.7	52.7	58.6	63.2	131.7	128.2	89.3	68.9	1003.0	1051.0	0.8	0.76	4	4	3	3	1	1	2	2	2	2
209013-A	37.8	38.8	5.0	5.9	1.3	4.1	52.5	49.8	22.1	37.4	51.4	52.7	57.9	73.6	130.4	126.9	94.5	45.5	1304.0	220.7	0.8	0.63	3	6	1	5	1	1	1	1	2	2
209014-B	29.8	29.6	2.9	4.2	2.0	2.9	29.4	33.1	6.5	38.5	52.7	52.0	59.6	56.2	124.7	116.9	68.5	70.6	269.3	396.8	0.4	0.33	8	8	5	4	1	1	1	1	2	2
209016-A	36.1	35.2	6.9	4.1	6.5	1.5	52.9	41.4	16.3	27.0	52.4	55.0	62.6	76.6	133.0	142.2	88.0	42.6	1089.0	113.8	0.6	0.34	4	6	3	5	1	1	1	1	2	2
209016-B	39.1	49.2	4.2	4.8	1.2	2.2	45.9	61.0	13.3	17.0	52.4	55.0	60.6	74.6	133.0	127.2	87.6	82.6	1137.0	1188.0	0.9	0.85	2	3	1	2	1	1	2	2	1	1
209016-C	35.4	51.8	5.9	6.8	2.5	3.0	47.9	72.2	26.3	40.7	53.4	52.0	62.6	74.6	136.0	129.5	86.0	66.5	1057.0	849.9	0.7	0.65	4	6	3	3	1	1	1	1	2	2
209019-A	39.5	36.5	3.7	6.5	3.0	2.7	43.8	52.2	10.5	32.7	52.4	52.0	57.9	74.6	133.4	129.5	88.9	48.1	1203.0	352.4	0.5	0.45	4	6	3	5	1	1	1	1	2	2
209022-A	39.9	52.5	6.4	4.8	6.5	2.9	58.0	50.0	15.8	18.0	52.7	55.0	60.9	77.6	135.4	126.2	80.0	81.6	2146.0	2191.0	0.9	0.87	2	2	1	2	1	1	3	3	1	1
209026-A	44.1	35.8	4.6	6.7	2.3	6.7	56.1	97.3	4.9	13.5	49.7	50.0	57.6	73.6	134.7	130.2	108.1	100.0	1583.0	1938.0	0.8	0.81	1	2	1	1	1	1	3	3	1	1
209026-B	31.8	34.3	5.2	3.5	3.2	0.9	43.9	15.0	13.6	39.5	53.4	55.0	63.6	68.9	136.0	147.2	61.0	63.0	562.1	332.7	0.5	0.48	6	6	4	4	1	1	1	1	2	2
209029-C	39.1	52.4	5.8	5.0	3.2	8.2	20.5	126.2	1.1	10.8	51.0	49.0	58.2	74.2	126.7	132.2	101.2	100.9	1218.0	1182.0	0.8	0.75	4	4	4	2	1	1	2	2	2	2
209032-A	37.0	37.3	6.5	9.2	5.2	4.2	62.0	82.1	8.5	40.5	50.7	51.0	56.6	63.2	134.4	120.9	76.6	50.2	847.7	427.3	0.5	0.46	5	5	4	5	1	1	1	1	2	2
209032-C	33.8	39.7	1.9	6.8	1.6	3.2	18.1	81.7	6.5	43.0	51.7	59.0	59.6	84.6	131.7	138.2	71.7	42.6	779.3	102.6	0.5	0.57	6	6	4	5	1	1	1	1	2	2
209034-B	39.2	41.3	3.9	3.1	2.4	2.5	28.2	34.0	4.4	35.5	50.7	52.0	59.2	64.9	130.7	144.2	104.3	106.0	1373.0	814.0	0.6	0.59	4	4	2	2	1	1	1	1	2	2
209034-C	44.5	35.3	3.5	4.3	1.4	2.1	12.8	33.8	6.1	37.9	52.7	53.7	63.2	72.6	137.7	126.9	77.1	45.5	1440.0	364.5	0.8	0.56	3	5	1	4	1	1	1	1	2	2
209038-A	36.9	30.9	6.1	4.5	5.3	3.8	51.0	38.4	14.1	31.8	53.4	50.4	62.6	64.2	128.4	131.5	76.1	48.6	1440.0	848.1	0.7	0.59	4	5	3	4	1	1	1	1	2	2
209038-C	40.6	44.1	2.7	4.5	0.6	3.5	18.7	32.8	8.1	37.8	53.4	50.4	63.6	64.2	130.4	131.5	94.1	38.6	870.4	296.9	0.6	0.56	4	5	4	4	1	1	1	1	2	2
209078-A	20.9	22.5	1.4	0.8	0.5	0.2	3.7	15.7	7.5	38.0	56.7	56.0	64.9	65.6	123.4	123.2	46.2	42.6	201.5	246.3	0.2	0.10	8	9	5	4	1	1	1	1	2	2
209085-A	39.8	39.4	5.2	7.2	3.7	4.1	51.5	53.7	6.7	34.2	54.7	55.0	63.2	75.9	135.7	128.2	71.1	45.7	1278.0	139.0	0.8	0.45	3	6	1	4	1	1	1	1	2	2
209086-A	29.4	45.8	6.2	4.8	4.9	1.0	54.9	53.2	18.9	31.7	55.4	54.0	63.6	76.6	138.0	131.5	75.6	66.9	618.8	307.4	0.4	0.31	6	8	4	4	1	1	1	1	2	2
209095-A	39.8	36.4	4.5	3.9	1.1	2.5	15.5	31.8	5.4	36.9	51.7	52.7	58.2	73.6	130.7	126.9	54.3	44.5	956.6	20.7	0.6	0.53	5	7	4	5	1	1	1	1	2	2
209095-B	43.5	38.5	3.5	8.3	2.7	6.8	39.8	70.2	9.7	43.8	48.7	53.7	61.2	75.2	136.7	123.2	105.1	39.1	1217.0	170.3	0.5	0.46	4	5	3	5	1	1	1	1	2	2
209105-B	39.6	32.8	4.1	4.2	5.0	3.5	43.4	27.8	11.4	38.8	51.4	50.4	60.6	65.2	131.4	127.5	84.9	43.6	1344.0	419.4	0.9	0.15	3	9	1	4	1	1	1	1	2	2
209108-A	30.8	48.3	6.2	6.9	3.0	2.9	57.4	69.8	15.9	47.5	52.7	52.0	58.6	65.2	128.7	155.9	66.3	68.4	946.0	548.5	0.5	0.56	7	7	4	4	1	1	1	1	2	2
209112-A	42.8	48.0	3.9	6.6	1.4	5.1	13.5	75.2	7.7	48.4	52.7	51.7	61.2	71.6	130.7	128.9	73.3	35.5	1023.0	352.0	0.7	0.57	4	4	3	4	1	1	1	1	2	2
209112-B	40.2	39.5	4.5	5.0	2.4	4.8	52.5	55.5	7.4	21.8	52.7	53.7	61.2	75.2	134.7	126.2	80.7	34.1	1037.0	187.8	0.7	0.32	4	5	1	5	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		NPB		NSB		FPPP		IPPP		DTF		DTP		DTM		TSW		GY		SR		FTR		SSS		FC		SLP		SC	
209116-B	40.7	49.0	5.2	6.0	7.6	6.2	54.7	64.2	4.9	36.8	51.7	49.0	56.6	74.2	134.4	132.2	61.0	33.9	715.4	375.7	0.5	0.47	6	6	4	4	1	1	1	1	2	2
212477-A	37.5	51.6	3.2	7.2	1.6	4.9	26.8	92.8	6.2	16.5	48.7	50.0	57.6	65.2	128.7	120.9	98.1	72.4	968.8	1096.0	0.7	0.73	4	4	3	3	1	1	2	2	2	2
212477-B	35.8	36.8	3.7	6.9	0.3	4.5	25.1	54.5	8.8	29.5	49.4	50.0	56.9	65.2	130.4	120.9	104.5	52.4	1053.0	867.3	0.7	0.51	4	5	3	4	1	1	1	1	2	2
212589-A	36.9	43.7	5.7	5.2	6.6	2.8	53.0	53.4	15.7	29.8	52.4	50.4	61.6	65.2	134.4	128.5	144.3	67.6	998.8	945.6	0.6	0.64	5	5	4	3	1	1	1	1	2	2
212589-B	35.7	44.5	4.4	4.0	4.6	4.4	35.4	42.9	12.1	-0.5	51.4	49.7	59.6	60.2	132.4	129.2	87.9	87.3	1269.0	1216.0	0.8	0.83	2	3	2	2	1	1	2	2	1	1
212685-B	39.4	46.0	5.5	4.3	6.2	5.2	67.5	81.2	12.1	35.8	52.0	56.0	60.2	72.2	131.7	124.2	76.2	62.9	924.0	272.0	0.5	0.38	7	8	4	4	1	1	1	1	2	2
212688-C	42.1	46.0	5.8	4.7	-0.6	4.2	14.8	57.8	3.4	15.8	51.0	53.0	57.2	64.2	124.7	123.2	108.2	107.9	1020.0	1984.0	0.7	0.73	3	4	3	2	0	0	0	0	3	3
212914-A	35.9	38.7	3.4	3.7	0.8	2.5	7.4	80.2	6.8	11.8	51.7	49.0	57.9	64.2	123.4	121.2	81.2	45.9	1286.0	1343.0	0.7	0.67	5	5	4	4	1	1	1	1	2	2
212914-B	41.3	44.7	8.4	4.2	8.2	2.8	70.0	32.0	21.8	18.2	52.7	55.0	58.9	79.9	137.4	130.2	87.7	85.7	1930.0	1335.0	0.9	0.86	1	3	1	2	1	1	3	2	1	1
213050-A	27.6	28.5	3.7	3.3	1.5	1.1	20.4	33.9	12.5	37.8	51.7	49.7	67.9	69.2	123.4	121.2	50.4	49.3	304.8	352.3	0.3	0.13	6	9	5	4	1	1	1	1	2	2
213050-B	38.9	37.8	6.7	5.6	6.8	5.1	59.7	62.0	25.1	44.2	53.7	53.0	61.9	73.9	130.4	128.2	85.6	40.2	1326.0	61.5	0.9	0.43	3	7	1	5	1	1	1	1	2	2
214621-A	38.4	41.7	5.8	4.3	4.2	2.9	28.2	61.2	6.4	34.8	51.0	50.0	57.2	67.2	128.7	123.2	79.8	47.9	892.3	614.5	0.6	0.60	5	5	4	3	1	1	1	1	2	2
214623-B	40.4	36.1	4.5	6.0	3.5	3.2	57.2	69.2	5.1	30.8	50.0	50.0	57.2	64.2	129.7	121.2	84.4	47.9	1329.0	884.5	0.9	0.14	3	9	1	4	1	1	1	1	2	2
214624-A	35.3	34.4	3.8	5.7	6.6	3.4	24.7	50.3	2.2	30.5	49.7	51.0	56.6	75.6	128.4	129.2	75.8	47.0	812.1	211.4	0.7	0.65	6	6	4	5	1	1	1	1	2	2
214626-C	34.4	41.4	3.8	4.2	-0.2	3.8	7.5	55.4	0.4	36.8	50.0	50.4	55.2	63.2	122.7	128.5	62.6	42.6	792.3	604.4	0.7	0.64	4	4	4	3	1	1	1	1	2	2
214731-B	36.8	50.0	2.9	4.3	0.0	2.9	32.1	64.2	6.9	16.8	48.7	53.0	57.6	64.2	127.7	121.2	85.7	89.9	1321.0	1778.0	0.9	0.82	2	2	1	2	1	1	3	3	1	1
214732-A	36.8	39.3	3.6	7.3	0.3	4.4	35.4	66.3	5.5	25.5	49.7	51.0	59.6	75.6	136.7	129.2	93.3	46.0	1108.0	876.4	0.7	0.65	4	6	3	5	1	1	1	1	2	2
214732-B	37.0	50.0	2.8	7.0	2.2	5.1	12.7	68.3	1.9	26.5	48.7	51.0	57.6	71.6	131.4	127.2	104.2	103.0	783.4	856.4	0.6	0.63	4	4	4	2	1	1	1	1	2	2
214734-A	36.9	49.7	5.1	7.9	4.2	3.1	25.7	61.7	17.1	12.2	52.7	55.0	59.9	75.9	138.4	128.2	70.8	64.2	1095.0	1136.0	0.8	0.83	2	3	1	2	1	1	3	2	1	1
214734-B	38.9	34.9	8.1	4.3	4.5	2.8	62.7	46.2	18.1	16.8	55.7	52.7	60.9	77.2	135.4	123.2	76.8	46.9	1110.0	122.8	0.7	0.50	4	5	3	4	1	1	1	1	2	2
214734-C	40.9	34.9	9.4	6.7	11	6.8	90.7	65.2	32.5	35.8	56.7	52.7	63.9	84.2	135.4	123.2	74.8	45.1	1243.0	147.8	0.7	0.14	4	6	3	5	1	1	1	1	2	2
215033-B	30.6	38.2	6.1	7.1	4.0	2.5	37.7	46.0	16.1	33.0	53.4	55.0	62.6	76.6	131.4	128.2	62.8	8.6	720.4	322.8	0.6	0.35	4	7	4	5	1	1	1	1	2	2
215067-A	34.8	50.0	5.2	6.3	-0.7	4.4	23.1	90.3	4.2	11.5	48.7	52.0	57.6	73.6	131.7	127.2	100.5	73.0	1270.0	1339.0	0.9	0.85	2	3	1	2	1	1	2	2	1	1
215067-B	35.7	39.0	3.8	6.0	0.9	4.1	30.0	49.3	2.9	30.5	51.7	56.0	57.6	71.6	128.4	129.2	86.2	17.4	1347.0	530.1	0.7	0.47	4	5	2	5	1	1	1	1	2	2
215067-C	32.3	43.7	3.2	5.5	3.2	4.5	23.3	71.1	-0.1	47.8	50.7	50.4	57.6	69.2	124.4	128.5	65.2	64.6	913.4	991.9	0.7	0.64	8	9	4	3	1	1	1	1	2	2
215190-A	38.4	37.4	8.5	4.5	2.2	5.1	15.2	46.1	3.4	16.8	52.0	51.4	58.2	66.2	126.7	128.5	91.2	90.6	1704.0	1986.0	0.8	0.81	1	2	1	1	1	1	3	3	1	1
215190-B	29.1	44.1	5.5	7.9	2.5	6.1	14.8	54.4	5.7	28.8	56.0	53.4	62.2	69.2	128.7	139.5	65.2	64.6	976.5	958.1	0.6	0.64	6	6	4	3	1	1	1	1	2	2
215190-C	38.0	38.4	1.8	2.3	1.0	1.4	25.8	31.5	10.3	28.8	49.4	50.7	56.9	62.2	131.0	128.2	66.4	67.1	840.4	367.3	0.6	0.44	4	5	4	4	1	1	1	1	2	2
215289-A	38.9	39.9	4.7	6.9	3.5	5.1	27.4	54.0	9.5	33.2	53.7	55.0	58.9	73.9	135.4	143.2	88.0	43.2	1320.0	141.6	0.7	0.49	3	7	2	5	1	1	1	1	2	2
215289-B	37.6	39.3	3.7	3.5	2.8	0.9	22.4	22.0	13.8	24.5	52.7	54.0	58.9	71.9	129.4	153.2	118.8	112.0	1718.0	1789.0	0.9	0.85	1	2	1	1	1	1	3	3	1	1
216854-A	39.5	37.3	4.7	9.9	2.6	7.1	38.1	66.2	5.8	39.4	57.4	54.7	57.9	76.6	130.4	128.9	79.7	44.5	892.6	143.2	0.6	0.16	5	9	4	5	1	1	1	1	2	2
216855-A	36.4	38.3	4.5	5.7	4.9	3.7	16.2	60.9	0.4	13.5	52.0	53.0	58.2	73.6	130.7	126.2	81.0	60.8	1396.0	1230.0	0.7	0.50	4	6	2	3	1	1	1	1	2	2
225741-C	32.7	31.8	4.1	6.9	2.9	4.8	14.2	47.1	-0.3	31.8	52.0	53.4	59.2	69.2	131.7	128.5	48.2	47.6	1196.0	731.9	0.5	0.51	6	7	4	5	1	1	1	1	2	2
225743-A	43.4	35.4	6.8	4.7	18	2.2	100.2	31.2	12.7	41.8	53.0	56.0	59.2	71.2	131.7	123.2	86.2	18.9	1072.0	298.2	0.7	0.67	5	6	3	5	1	1	1	1	2	2
225879-A	23.9	18.8	1.9	1.7	1.5	1.4	19.6	16.9	5.6	31.8	53.4	51.7	69.6	70.2	126.0	121.2	45.6	37.1	220.5	280.7	0.1	0.10	7	9	5	4	1	1	1	1	2	2
225884-A	42.1	44.7	6.9	2.1	7.2	0.9	74.9	26.7	16.9	41.5	56.4	55.0	66.6	71.9	138.0	156.2	80.4	30.1	1384.0	234.0	0.7	0.25	4	5	2	4	1	1	1	1	2	2
225887-B	41.5	39.0	3.5	7.7	1.7	7.1	16.2	72.5	5.1	42.8	52.7	53.7	61.2	77.2	138.7	134.2	87.5	47.1	1362.0	876.5	0.6	0.36	4	6	2	4	1	1	1	1	2	2
225888-A	39.5	40.7	4.0	4.0	2.6	2.4	45.1	49.5	10.1	-0.9	50.4	50.7	57.9	64.2	123.4	121.2	83.3	81.7	1139.0	1139.0	0.8	0.72	4	4	3	2	1	1	2	2	2	2
225889-A	30.9	39.7	3.8	4.3	0.9	4.5	28.6	79.8	10.2	34.8	49.7	53.0	57.6	64.2	130.4	124.2	46.0	46.9	975.2	1006.0	0.2	0.13	7	9	4	4	1	1	1	1	2	2
225889-C	35.3	50.4	2.5	6.5	2.2	3.8	33.7	51.8	3.5	47.8	49.7	50.4	56.6	69.2	131.4	128.5	121.2	120.6	1327.0	973.1	0.8	0.72	5	6	2	2	1	1	1	1	2	2
225890-A	30.6	35.9	3.1	5.7	1.5	5.8	12.7	67.9	9.5	26.8	52.7	53.7	59.9	74.2	122.4	140.2	77.6	29.2	764.8	196.5	0.5	0.48	6	7	4	5	1	1	1	1	2	2
227151-B	37.7	44.1	5.2	4.5	4.0	2.1	46.2	43.8	9.3	48.8	49.4	53.4	57.9	69.2	131.0	142.5	77.8	47.6	1099.0	710.6	0.5	0.61	6	7	3	3	1	1	1	1	2	2
227151-C	37.4	40.7	8.1	4.2	8.2	4.1	52.5	36.8	3.4	44.8	50.0	54.4	54.2	64.2	132.7	131.5	72.2	43.1	929.0	509.4	0.5	0.56	6	6	4	4	1	1	1	1	2	2
227151-D	35.1	40.3	4.1	7.2	0.5	1.9	-1.5	67.5	-4.3	38.5	50.0	50.0	57.2	63.2	129.7	129.9	93.8	45.7	1447.0	477.3	0.7	0.41	3	5	2	4	1	1	1	1	2	2
227152-A	35.4	32.7	6.1	5.2	4.9	4.5	237.5	37.4	9.1	35.8	50.0	49.4	54.2	61.2	129.7	131.5	48.7	44.9	839.0	313.1	0.4	0.14	7	9	4	4	1	1	1	1	2	2
227152-B	38.0	50.3	3.5	8.2	1.6	3.9	22.0	71.5	4.5	11.5	48.7	50.0	56.6	62.2	124.4	120.9	94.2	90.4	1137.0	1207.0	0.8	0.74	4	3	1	2	1	1	2	2	2	2
227156-A	39.0	49.0	3.2	6.3	1.6	4.4	29.0	69.9	0.9	12.5	49.7	51.0	57.6	71.6	131.4	127.2	78.2	68.8	1977.0	1079.0	0.9	0.71	3	4	1	3	1	1	3	2	2	2
227158-A	38.7	42.4	6.5	4.0	7.2	4.5	47.5	63.2	7.7	35.8	51.0	50.0	57.2	67.2	129.7	123.2	67.8	38.9	1037.0	330.7	0.6	0.37	7	8	3	4	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
227160-B	45.7	48.7	8.8	6.7	6.9	5.5	70.5	99.2	7.7	14.8	53.0	50.0	57.2	64.2	132.7	123.2	84.2	68.8	1384.0	1038.0	0.7	0.64	4	4	2	3	1	1	2	2	2	2
227161-B	36.4	34.5	4.5	3.8	4.2	1.2	55.6	31.4	13.6	39.0	51.4	59.0	61.6	74.6	133.0	127.2	89.2	46.6	1067.0	105.1	0.7	0.31	4	6	3	4	1	1	1	1	2	2
227161-C	37.2	38.0	7.4	5.8	5.2	6.0	61.6	132.6	17.6	26.7	66.7	54.0	77.6	73.6	131.4	129.5	89.0	71.5	882.7	893.6	0.6	0.67	4	4	4	3	1	1	1	1	2	2
228196-A	36.0	42.9	2.9	5.5	-0.4	2.2	25.4	54.8	1.9	36.5	49.7	50.0	58.6	62.2	136.7	120.9	105.3	58.4	1279.0	628.5	0.9	0.69	3	6	1	3	1	1	1	1	2	2
228197-C	35.1	48.7	6.2	3.8	2.5	2.2	49.9	46.4	12.3	55.5	53.4	55.0	62.6	70.9	160.0	144.2	64.8	34.1	1135.0	390.2	0.7	0.57	4	5	3	4	1	1	1	1	2	2
228197-D	29.7	32.8	3.1	3.2	2.2	6.5	0.8	29.4	2.7	36.8	52.0	50.4	58.2	67.2	122.7	130.5	45.2	44.6	720.7	145.6	0.4	0.35	7	8	4	5	1	1	1	1	2	2
228197-E	34.8	32.8	4.8	7.5	1.5	3.5	3.5	38.1	0.4	38.8	51.0	50.4	56.2	64.2	122.7	130.5	55.4	45.6	794.0	446.9	0.6	0.56	5	5	4	4	1	1	1	1	2	2
228290-A	39.7	37.7	5.8	6.9	6.9	7.8	63.8	79.8	6.4	47.4	54.0	73.7	60.2	78.6	131.7	128.9	82.6	47.5	1101.0	378.2	0.6	0.55	5	5	3	4	1	1	1	1	2	2
228293-A	35.1	35.0	3.6	6.0	-0.7	3.4	29.4	52.6	2.5	32.5	52.7	52.0	59.6	75.6	133.7	127.2	102.5	39.2	1396.0	333.9	0.6	0.37	4	6	2	5	1	1	1	1	2	2
228294-B	37.7	48.4	3.8	5.3	4.5	5.2	45.5	92.2	3.4	15.8	52.0	50.0	58.2	64.2	129.7	123.2	104.2	103.9	1075.0	1039.0	0.8	0.75	4	4	3	2	1	1	2	2	2	2
228299-A	35.0	43.6	3.5	4.5	1.9	3.9	24.3	53.8	1.5	30.5	48.7	50.0	57.6	63.2	134.4	118.9	93.8	67.9	1190.0	954.8	0.6	0.61	4	6	2	3	1	1	1	1	2	2
229962-B	37.5	37.5	6.1	7.3	5.2	10	41.0	88.2	11.2	55.8	50.7	53.7	58.6	74.2	123.4	126.2	69.4	48.1	792.7	151.5	0.5	0.47	5	7	4	5	1	1	1	1	2	2
230770-A	39.1	45.2	5.2	5.5	2.5	1.7	40.6	41.6	15.3	30.7	53.4	54.0	60.6	74.6	133.0	129.5	85.6	35.9	802.1	97.4	0.5	0.41	5	5	4	4	1	1	1	1	2	2
230770-B	39.1	43.2	7.2	5.8	7.5	0.3	79.6	41.6	23.3	29.7	55.4	53.0	66.6	76.6	142.0	129.5	78.8	41.9	915.5	167.4	0.7	0.47	5	6	3	4	1	1	1	1	2	2
230796-C	35.3	37.3	4.8	6.3	3.6	5.7	25.3	99.3	4.5	65.5	49.7	54.0	56.6	78.6	128.4	127.2	68.2	44.1	948.8	436.4	0.7	0.42	5	6	3	5	1	1	1	1	2	2
231329-B	36.8	37.1	3.6	7.9	0.3	8.9	32.8	96.5	5.9	46.5	49.7	52.0	57.6	65.2	134.7	118.9	106.1	50.4	837.7	349.8	0.5	0.53	4	7	4	5	1	1	1	1	2	2
231331-A	31.9	45.4	2.4	3.0	3.3	1.9	17.0	43.5	8.4	39.8	53.4	50.0	63.6	64.2	141.4	121.2	78.3	60.9	1389.0	450.7	0.6	0.38	4	8	3	4	1	1	1	1	2	2
231331-C	37.6	40.7	6.1	4.9	7.0	2.8	60.7	27.1	16.4	36.8	52.4	51.4	63.6	63.2	133.4	128.5	63.1	37.6	725.4	369.4	0.6	0.53	4	4	4	4	1	1	1	1	2	2
234048-A	39.5	48.6	3.0	5.7	2.3	5.1	30.5	108.9	8.1	28.5	50.4	50.0	56.9	73.6	126.4	127.2	86.9	62.5	820.6	841.4	0.7	0.71	4	4	4	3	1	1	1	1	2	2
234049-A	37.5	37.1	3.7	8.5	1.3	9.3	50.1	123.6	7.8	57.7	52.4	54.0	57.9	78.6	137.4	138.5	102.5	49.9	1431.0	303.6	0.8	0.53	3	7	1	5	1	1	1	1	2	2
234050-B	30.4	41.4	5.1	4.9	2.2	5.5	20.2	51.8	5.7	37.8	51.0	50.4	56.2	66.2	129.7	131.5	65.4	45.6	630.7	571.9	0.7	0.61	6	6	4	3	1	1	1	1	2	2
235032-A	35.6	32.8	6.7	6.5	12	5.1	72.0	30.4	19.4	35.8	53.4	51.4	63.6	64.2	134.4	128.5	87.9	46.6	558.8	445.6	0.6	0.51	4	5	4	4	1	1	1	1	2	2
235032-B	35.7	35.7	4.5	6.7	3.2	2.4	41.7	50.9	1.5	28.5	49.7	52.0	57.6	73.6	131.4	127.2	77.8	7.0	1145.0	751.4	0.7	0.46	4	5	3	5	1	1	1	1	2	2
235034-A	39.0	30.4	3.8	7.5	3.2	4.9	29.7	52.4	3.9	39.5	48.7	55.0	57.6	70.9	131.4	136.2	82.4	48.0	1050.0	267.7	0.6	0.17	4	9	1	5	1	1	1	1	2	2
235034-C	30.8	45.9	6.2	7.5	3.3	4.5	54.4	84.5	2.5	31.5	48.7	51.0	59.6	63.2	128.7	121.9	78.9	57.4	1249.0	794.8	0.7	0.64	4	5	3	3	1	1	1	1	2	2
235036-A	40.3	41.4	5.1	2.9	8.6	3.5	59.0	31.8	16.7	19.8	52.4	52.4	61.6	64.2	126.4	131.5	84.7	104.6	1267.0	1087.0	0.8	0.76	4	4	2	2	1	1	1	2	2	2
235036-B	34.6	34.4	5.1	4.9	5.6	4.5	54.4	61.8	16.4	19.8	53.4	50.4	63.6	64.2	131.4	128.5	71.5	45.6	1060.0	1033.0	0.6	0.65	5	7	4	4	1	1	1	1	2	2
235036-C	40.6	32.4	6.4	5.9	8.6	1.5	65.7	10.8	13.7	37.8	54.4	52.4	62.6	64.2	128.4	131.5	82.7	16.6	837.1	573.1	0.5	0.51	6	6	4	5	1	1	1	1	2	2
235391-A	43.1	37.2	3.8	4.7	4.5	5.2	24.5	55.5	4.4	36.8	53.0	53.0	59.2	64.2	122.7	123.2	75.0	39.9	1472.0	118.2	0.9	0.64	3	6	1	5	1	1	1	1	2	2
235392-B	26.1	17.2	4.1	4.3	1.5	2.4	6.5	29.2	-1.9	27.8	50.0	49.7	59.2	63.2	129.7	128.2	48.2	48.1	580.7	535.7	0.3	0.33	7	7	4	5	1	1	1	1	2	2
235392-C	42.4	34.7	8.5	8.3	7.2	7.1	96.9	94.2	33.6	9.8	54.4	52.7	63.6	64.2	133.0	128.2	64.4	47.1	1160.0	1221.0	0.9	0.61	3	5	1	4	1	1	1	1	2	2
235395-A	39.2	38.9	6.9	9.2	5.7	4.8	66.5	86.3	6.7	40.2	52.7	55.0	65.2	73.9	138.7	130.2	93.9	46.2	879.9	149.0	0.6	0.32	4	5	4	5	1	1	1	1	2	2
235825-A	36.1	49.0	4.8	10	4.9	8.7	47.2	117.6	8.4	68.5	50.0	53.0	54.2	73.6	129.7	129.2	67.0	56.0	1307.0	722.6	0.7	0.69	4	6	3	3	1	1	1	1	2	2
236194-A	37.1	30.7	4.5	3.1	8.2	2.9	50.2	33.0	11.9	22.5	53.4	53.0	61.6	61.9	136.0	171.2	78.0	42.0	1329.0	976.5	0.6	0.63	3	6	2	4	1	1	1	1	2	2
236194-B	39.1	46.3	5.2	6.8	6.2	5.5	43.9	76.0	13.9	49.5	53.4	55.0	62.6	71.9	160.0	147.2	75.2	38.9	323.8	191.5	0.5	0.47	5	6	5	4	1	1	1	1	2	2
236196-A	36.5	41.6	3.7	9.2	1.0	3.9	25.5	98.5	12.5	30.5	52.4	52.0	56.9	71.2	139.4	99.9	77.3	55.4	821.0	813.5	0.6	0.64	4	4	4	3	1	1	1	1	2	2
236196-B	42.4	42.3	7.2	3.5	12	1.5	100.9	45.0	18.9	25.5	53.4	55.0	62.6	64.9	134.0	147.2	141.0	143.0	1641.0	1758.0	0.9	0.81	1	2	1	2	1	1	3	3	1	1
236197-A	26.4	23.3	3.2	1.8	0.2	-0.8	19.6	-5.6	8.3	43.5	55.4	55.0	63.6	57.9	126.0	131.2	47.1	49.1	235.5	251.9	0.3	0.25	6	6	5	4	1	1	1	1	2	2
236197-C	38.1	32.8	4.2	5.5	1.2	5.1	33.2	44.8	11.6	32.8	51.4	53.4	60.6	70.2	138.0	142.5	85.3	44.6	1247.0	778.1	0.9	0.14	3	9	1	5	1	1	1	1	2	2
236198-A	36.1	31.5	5.5	8.5	0.9	5.5	44.9	61.4	12.9	71.5	52.4	55.0	61.6	70.9	126.0	136.2	84.4	46.0	757.1	125.5	0.4	0.28	5	5	4	4	1	1	1	1	2	2
236198-B	44.9	41.4	5.4	5.6	4.8	5.4	47.4	51.7	16.1	32.2	51.7	53.0	59.9	73.9	126.4	128.2	77.6	61.2	1103.0	301.5	0.7	0.45	4	7	3	4	1	1	1	1	2	2
236459-A	42.2	40.3	3.7	7.9	2.3	7.5	56.8	83.5	13.5	46.4	49.4	52.7	57.9	72.6	133.4	128.9	97.7	17.9	1196.0	328.2	0.7	0.43	4	6	1	5	1	1	1	1	2	2
236459-B	38.5	49.6	3.7	4.3	1.3	3.1	44.8	40.5	7.5	19.4	51.4	52.7	55.9	73.6	123.4	126.9	90.9	83.7	1317.0	1203.0	0.9	0.83	2	3	1	2	1	1	2	2	1	1
236460-A	38.4	48.6	1.5	7.9	1.5	5.8	2.8	52.5	-3.6	38.4	52.0	54.7	57.2	73.6	134.7	128.9	113.0	62.5	872.3	244.5	0.6	0.46	4	6	4	4	1	1	1	1	2	2
236463-A	37.5	37.3	2.3	7.9	1.6	11	27.8	119.8	9.1	73.4	53.4	52.7	57.9	73.6	130.4	130.9	98.9	45.5	1056.0	515.7	0.7	0.58	4	5	3	4	1	1	1	1	2	2
236463-B	39.1	48.5	5.9	5.5	5.0	2.2	35.4	64.4	14.2	35.0	50.7	53.0	57.6	76.6	136.7	127.2	100.9	47.7	977.7	347.6	0.8	0.56	4	4	1	4	1	1	1	1	2	2
236464-A	37.4	37.2	4.5	7.3	3.9	6.5	19.8	82.8	6.4	11.8	50.0	49.0	58.2	74.2	132.7	124.2	108.2	107.9	1226.0	1191.0	0.7	0.69	4	4	4	2	1	1	2	2	2	2
236464-B	28.5	40.7	3.4	3.9	4.0	4.1	12.4	27.1	6.1	39.8	53.4	50.4	64.6	65.2	141.4	127.5	78.7	61.6	1432.0	365.6	0.7	0.39	4	8	3	4	1	1	1	1	2	2
236465-A	37.2	39.3	5.0	6.3	1.6	5.1	57.1	53.8	16.1	37.4	49.4	52.7	57.9	72.6	137.4	128.9	114.9	41.5	1226.0	195.7	0.9	0.35	3	6	1	5	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
236465-C	38.5	48.9	5.3	6.2	3.6	3.2	34.8	75.8	5.5	47.5	51.4	50.0	56.9	65.2	137.4	120.9	105.3	54.4	792.6	741.0	0.6	0.61	4	4	4	3	1	1	1	1	2	2
236467-A	42.3	39.1	7.4	3.9	12	3.5	82.0	27.1	16.1	34.8	54.4	51.4	62.6	63.2	141.4	128.5	79.9	30.6	870.4	388.1	0.6	0.59	4	4	4	4	1	1	1	1	2	2
236467-B	41.6	46.1	5.4	3.9	6.0	4.5	49.7	38.4	11.4	38.8	52.4	51.4	59.6	64.2	133.4	131.5	91.5	37.6	1484.0	495.6	0.9	0.51	3	4	1	4	1	1	1	1	2	2
236469-B	35.6	35.8	5.1	6.2	4.2	2.8	25.0	55.0	6.5	33.2	54.7	55.0	62.9	80.9	139.4	130.2	77.8	10.2	1138.0	184.9	0.8	0.13	4	9	1	5	1	1	1	1	2	2
236470-A	29.2	40.3	4.3	4.6	0.3	5.5	25.1	51.8	5.1	34.4	51.4	52.7	57.9	73.6	125.4	126.9	67.1	45.5	984.3	74.5	0.6	0.47	4	7	3	5	1	1	1	1	2	2
236470-B	34.7	45.6	0.8	1.0	0.2	1.1	-10.5	12.2	-1.3	-3.9	60.0	59.7	76.2	80.2	137.7	136.2	127.4	127.3	1321.0	1276.0	0.7	0.76	4	4	2	2	1	1	1	1	2	2
236471-A	32.8	45.2	3.5	3.5	2.2	1.0	34.6	35.9	9.9	29.7	53.4	54.0	60.6	73.6	136.0	127.5	73.2	33.9	1052.0	172.4	0.7	0.42	4	5	3	4	1	1	1	1	2	2
236471-B	40.7	42.0	5.2	5.1	4.2	6.5	47.7	47.0	6.9	45.5	49.7	54.0	54.6	69.9	131.4	136.2	85.8	49.0	1345.0	610.2	0.9	0.71	3	4	1	3	1	1	1	1	2	2
236472-A	32.6	35.4	9.7	5.1	9.0	4.9	66.0	71.7	22.4	59.0	52.4	55.0	62.6	78.6	141.4	129.2	40.8	37.7	777.1	453.6	0.4	0.37	7	8	5	5	1	1	1	1	2	2
236472-B	36.3	36.6	3.4	4.1	4.3	1.5	26.0	33.7	11.7	40.0	53.4	59.0	63.6	74.6	136.4	127.2	77.5	12.6	1104.0	122.6	0.7	0.33	4	6	1	5	1	1	1	1	2	2
236475-A	35.7	34.6	3.8	7.5	3.2	7.5	21.0	89.8	1.9	47.5	48.7	50.0	56.6	63.2	128.4	120.9	77.8	51.4	1329.0	257.3	0.9	0.42	3	5	1	4	1	1	1	1	2	2
236475-C	40.5	38.0	6.3	5.6	3.6	5.1	54.1	69.5	13.5	40.4	52.4	54.7	57.9	73.6	139.4	130.9	96.9	18.0	1406.0	123.2	0.7	0.56	4	7	2	5	1	1	1	1	2	2
236479-A	35.7	38.7	3.0	7.3	1.3	6.1	25.8	115.8	7.1	43.4	54.4	52.7	56.9	73.6	133.4	130.9	97.3	50.5	916.0	473.2	0.5	0.57	5	6	4	3	1	1	1	1	2	2
236479-C	37.8	47.5	3.2	3.8	1.6	2.0	47.1	36.9	6.2	10.7	51.7	54.0	58.6	73.6	131.7	129.5	92.1	84.9	1442.0	1480.0	0.8	0.81	2	3	1	1	1	1	3	2	1	1
236481-B	35.1	37.8	4.1	7.0	2.2	7.9	15.2	102.2	2.4	63.8	55.0	50.0	59.2	64.2	132.7	123.2	93.8	45.9	1401.0	465.7	0.9	0.16	3	9	1	4	1	1	1	1	2	2
236491-A	38.3	38.3	3.8	5.0	0.2	2.7	30.7	41.9	3.2	32.5	51.7	51.0	56.6	80.6	131.4	129.2	84.6	47.0	1427.0	305.1	0.8	0.57	3	5	1	4	1	1	1	1	2	2
236493-A	36.8	34.7	2.9	3.3	1.0	2.5	37.8	55.8	7.2	35.8	52.7	53.0	58.6	66.2	127.7	121.2	102.1	46.9	1151.0	503.2	0.7	0.54	4	5	3	4	1	1	1	1	2	2
236882-A	42.3	34.6	5.7	6.3	6.8	5.8	41.0	47.2	17.5	25.8	51.7	54.7	58.9	77.2	133.4	126.2	81.6	42.4	1268.0	129.0	0.7	0.55	4	6	2	5	1	1	1	1	2	2
236882-B	43.8	40.4	4.8	9.2	2.5	4.1	38.3	52.7	10.2	33.2	49.7	62.0	57.6	78.9	139.4	128.2	86.2	39.2	837.7	166.5	0.4	0.33	5	7	4	5	1	1	1	1	2	2
237054-A	31.2	41.4	3.2	4.9	2.7	2.1	39.5	43.7	12.0	30.2	49.4	53.0	57.9	73.9	133.0	130.2	39.4	26.0	623.4	544.0	0.4	0.39	7	8	5	5	1	1	1	1	2	2
237054-B	37.6	50.3	6.7	5.9	8.6	3.2	43.4	63.8	15.4	18.5	52.4	50.0	60.6	60.2	134.4	121.9	109.5	85.4	1982.0	2009.0	0.8	0.82	1	2	2	2	1	1	3	3	1	1
237055-A	42.8	34.9	4.9	7.0	2.4	6.8	46.8	54.9	6.1	26.8	50.7	51.7	61.2	74.2	137.7	126.2	86.7	35.7	217.9	200.3	0.4	0.33	3	5	5	5	1	1	1	1	2	2
237055-B	36.8	37.7	2.6	5.0	0.0	2.5	38.4	62.2	6.9	35.8	48.7	51.0	58.6	69.2	137.7	121.2	99.3	47.9	967.7	382.0	0.7	0.58	5	7	3	5	1	1	1	1	2	2
240044-A	35.4	35.4	4.8	4.2	2.5	6.1	17.5	48.4	-2.6	32.8	52.0	53.4	59.2	69.2	131.7	131.5	79.4	17.6	1129.0	906.9	0.6	0.58	5	7	3	5	1	1	1	1	2	2
240045-A	38.5	39.0	3.7	6.9	1.3	5.5	33.8	126.2	6.5	20.4	50.4	52.7	55.9	72.6	133.4	130.9	104.7	101.5	1347.0	1233.0	0.7	0.76	5	5	1	2	1	1	1	1	2	2
24159-B	39.0	49.3	4.2	7.0	3.9	4.7	44.0	56.9	9.2	15.5	48.7	51.0	56.6	71.6	131.4	127.2	106.2	105.0	1089.0	1079.0	0.8	0.74	4	4	1	2	1	1	1	1	2	2
24159-C	48.2	38.3	3.2	4.0	2.4	2.1	52.8	29.9	10.4	28.5	49.7	53.0	61.2	74.6	129.7	129.2	101.5	47.0	1463.0	846.4	0.9	0.42	3	6	1	5	1	1	1	1	2	2
241800-A	44.6	37.2	3.1	3.3	2.2	4.2	26.4	84.8	10.1	32.8	53.7	50.0	61.9	64.2	140.4	122.2	87.5	8.9	1361.0	130.7	0.9	0.13	3	9	1	5	1	1	1	1	2	2
241800-B	37.1	39.2	7.5	6.1	3.9	3.2	79.6	86.0	20.3	40.0	53.4	57.0	62.6	73.6	133.0	127.2	79.6	54.6	995.5	210.1	0.8	0.42	4	5	1	4	1	1	1	1	2	2
241801-A	44.4	43.7	3.1	6.5	2.2	5.5	22.2	55.1	3.4	28.8	52.0	50.4	57.2	64.2	126.7	131.5	101.2	100.6	1109.0	1009.0	0.8	0.76	5	5	3	2	1	1	1	1	2	2
241801-C	37.5	54.3	3.3	8.6	0.3	5.1	29.8	89.2	8.5	17.4	52.4	54.7	57.9	73.6	130.4	130.9	89.3	62.0	1092.0	978.2	0.7	0.66	4	4	4	3	1	1	2	2	2	2
241802-A	29.8	31.3	3.2	3.9	-0.7	0.8	27.8	28.2	8.2	41.3	51.7	53.7	57.6	60.6	131.7	131.9	66.5	63.7	679.3	614.0	0.3	0.28	7	7	4	4	1	1	1	1	2	2
241804-B	41.1	35.9	5.2	3.9	7.2	1.8	87.9	35.8	17.9	27.4	52.4	53.7	61.6	73.6	135.0	128.9	79.2	49.5	1355.0	139.5	0.6	0.31	4	6	2	4	1	1	1	1	2	2
241804-C	37.8	49.8	6.9	4.2	7.2	2.7	69.6	65.9	13.3	32.7	53.4	54.0	60.6	74.6	133.0	129.5	97.6	33.9	1375.0	344.9	0.9	0.47	3	4	1	4	1	1	1	1	2	2
241804-D	40.8	36.1	8.9	5.2	7.2	3.1	90.2	49.8	16.6	30.8	52.4	51.4	61.6	66.2	133.0	128.5	80.0	40.6	1342.0	830.6	0.7	0.55	4	7	2	4	1	1	1	1	2	2
26918-A	41.8	34.7	4.9	7.0	3.0	6.4	41.4	68.3	7.9	33.5	50.7	52.0	58.6	73.6	138.7	127.2	85.6	46.0	1323.0	413.9	0.8	0.67	3	6	1	5	1	1	1	1	2	2
30287-A	36.2	49.0	3.7	6.6	2.0	5.5	34.8	60.2	7.8	34.4	53.4	62.7	57.9	81.6	133.4	130.9	94.5	44.1	1168.0	133.2	0.7	0.56	4	4	3	4	1	1	1	1	2	2
30287-C	36.8	45.1	4.6	5.6	4.0	4.4	56.1	38.3	14.9	10.2	52.7	55.0	60.6	76.9	134.7	130.2	92.1	66.2	1649.0	1308.0	0.8	0.85	1	5	2	3	1	1	3	1	2	2
30288-A	37.8	34.9	5.2	4.0	1.6	2.2	35.4	38.5	6.5	27.8	50.7	53.0	59.6	64.2	131.7	121.2	103.7	45.9	1189.0	819.5	0.7	0.42	4	5	2	5	1	1	1	1	2	2
30288-B	37.8	38.3	4.2	7.5	1.3	8.5	32.8	92.5	9.9	29.5	51.7	57.0	61.6	73.2	136.7	130.9	85.7	54.4	742.7	786.0	0.7	0.64	4	4	4	3	1	1	1	1	2	2
30289-A	37.6	35.8	5.1	3.9	2.5	3.1	18.7	39.0	7.1	14.2	53.7	63.0	62.9	83.9	137.4	130.2	88.4	50.2	1353.0	1022.0	0.7	0.63	4	6	2	3	1	1	1	1	2	2
30289-B	38.3	40.2	4.7	4.8	3.3	3.5	41.0	53.0	18.4	16.0	53.4	61.0	62.6	76.6	136.4	129.2	88.3	50.6	1337.0	1003.0	0.8	0.78	4	6	2	3	1	1	1	1	2	2
30290-A	37.9	45.7	4.4	6.2	2.2	4.8	26.0	47.3	7.5	31.2	55.7	59.0	61.9	81.9	133.4	130.2	93.6	71.3	1270.0	952.0	0.9	0.81	3	6	1	3	1	1	1	1	2	2
30290-C	30.5	53.3	2.4	6.6	0.2	4.5	16.0	73.8	4.6	45.4	50.7	63.7	59.6	88.6	126.4	130.9	72.2	35.5	977.7	22.0	0.6	0.56	4	4	3	4	1	1	1	1	2	2
30293-A	36.5	46.1	5.1	6.9	3.2	2.8	48.0	64.0	7.2	14.2	51.7	65.0	60.6	75.9	136.4	128.2	102.0	102.2	1389.0	1343.0	0.8	0.79	3	4	1	2	1	1	1	1	2	2
30296-A	37.9	31.8	6.4	4.5	3.3	4.1	54.7	54.8	16.7	8.8	53.4	50.4	63.6	68.2	124.4	131.5	66.1	43.6	1345.0	1418.0	0.7	0.61	4	5	3	4	1	1	1	1	2	2
30300-A	39.3	45.6	4.5	5.3	3.6	4.4	41.0	78.9	3.9	34.5	50.7	51.0	57.6	73.6	128.4	129.2	79.0	34.0	1040.0	236.4	0.7	0.33	5	6	3	4	1	1	1	1	2	2
30300-B	31.7	35.0	3.8	3.7	2.2	2.1	27.3	29.2	10.2	33.8	51.7	51.7	57.6	60.2	129.4	128.2	47.9	49.2	779.4	800.7	0.5	0.48	6	6	4	4	1	1	1	1	2	2
30301-A	30.5	35.0	3.9	4.6	2.3	3.8	26.1	26.5	8.5	40.9	50.7	52.7	61.6	64.6	131.7	131.9	49.4	43.5	824.3	759.0	0.3	0.36	7	7	4	4	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		NPB		NSB		FPPP		IPPP		DTF		DTP		DTM		TSW		GY		SR		FTR		SSS		FC		SLP		SC	
30301-C	39.5	40.0	3.3	3.9	2.0	2.5	20.8	22.5	7.8	8.4	52.4	53.7	58.9	63.6	133.4	134.9	92.7	89.5	1729.0	1116.0	0.5	0.52	4	4	4	2	1	1	2	2	2	2
30302-A	37.1	37.3	2.9	7.9	-0.7	3.5	28.1	95.5	4.9	27.5	48.7	50.0	57.6	65.2	131.7	120.9	104.9	68.4	1094.0	1027.0	0.6	0.62	4	4	3	3	1	1	1	1	2	2
30302-C	41.1	51.6	4.9	7.9	2.6	4.2	46.8	61.8	22.2	34.5	50.7	52.0	57.6	65.2	131.7	129.9	89.7	56.4	1376.0	629.8	0.8	0.64	3	6	1	3	1	1	1	1	2	2
30304-A	42.2	36.8	4.1	6.6	4.5	7.4	48.6	70.7	4.9	41.2	51.7	55.0	61.6	75.9	125.4	145.2	67.6	36.2	1256.0	120.2	0.7	0.45	4	7	2	5	1	1	1	1	2	2
30304-B	39.2	30.3	3.9	1.8	5.7	-0.8	23.8	9.0	4.1	44.5	54.7	53.0	65.2	69.9	135.7	144.2	82.5	18.0	1028.0	419.0	0.6	0.19	4	9	1	5	1	1	1	1	2	2
30307-A	40.3	34.6	9.1	4.1	7.8	2.9	57.4	42.4	13.5	26.0	53.7	60.0	60.9	76.6	135.4	127.2	69.2	19.6	979.8	127.6	0.7	0.48	4	7	1	5	1	1	1	1	2	2
30307-D	42.3	34.9	5.1	5.2	5.5	3.1	51.0	32.7	16.1	39.2	51.7	67.0	62.9	83.9	139.4	130.2	80.4	25.7	1333.0	112.7	0.7	0.41	4	5	3	5	1	1	1	1	2	2
30308-B	34.8	45.5	3.8	3.8	3.5	2.0	26.3	28.9	9.9	28.7	51.7	54.0	58.6	73.6	125.4	129.5	82.6	45.9	1373.0	573.6	0.7	0.67	4	5	2	3	1	1	1	1	2	2
30309-A	37.0	35.7	2.5	6.7	4.2	3.1	30.3	49.3	5.9	26.5	52.7	53.0	58.6	74.6	131.4	127.2	69.8	48.0	1162.0	725.1	0.9	0.59	3	5	1	4	1	1	1	1	2	2
30309-C	36.5	34.9	4.6	5.9	3.6	5.9	44.8	100.8	6.9	30.5	48.7	50.0	58.6	63.2	136.7	118.9	83.8	22.9	1094.0	901.0	0.6	0.43	4	5	1	5	1	1	1	1	2	2
30310-A	31.0	35.2	8.1	3.8	6.2	2.9	80.7	36.4	17.5	47.0	55.7	61.0	63.9	83.6	139.4	129.2	44.0	26.6	379.0	423.8	0.2	0.11	8	9	4	5	1	1	1	1	2	2
30311-A	38.8	40.0	4.2	3.5	2.4	0.5	16.8	15.4	3.4	31.5	50.7	51.0	60.2	75.9	134.7	144.2	97.9	63.0	1375.0	915.2	0.8	0.63	3	4	1	3	1	1	1	1	2	2
30312-A	31.9	36.9	6.8	5.2	3.9	3.8	80.0	40.7	17.6	29.2	53.7	55.0	60.6	73.9	129.4	142.2	47.7	24.7	1026.0	541.5	0.6	0.32	6	6	4	5	1	1	1	1	2	2
30312-B	40.2	40.4	3.4	6.9	2.2	3.8	56.3	49.0	8.9	30.2	51.7	53.0	59.6	72.9	130.4	145.2	83.5	24.6	1211.0	131.5	0.8	0.51	3	7	1	5	1	1	1	1	2	2
30313-C	45.2	37.0	5.1	6.9	4.9	2.4	90.3	44.3	6.2	31.2	51.7	53.0	59.6	79.9	139.4	127.2	62.5	41.2	1339.0	546.5	0.9	0.51	3	7	1	5	1	1	1	1	2	2
30314-A	30.1	35.3	4.9	6.7	0.3	3.7	36.4	62.6	8.9	52.5	51.7	55.0	59.6	73.6	131.7	164.2	46.1	15.0	984.3	307.6	0.6	0.15	7	9	4	5	1	1	1	1	2	2
30314-B	36.3	47.2	4.2	4.8	4.2	3.9	31.7	53.4	3.2	33.0	57.7	61.0	72.6	75.6	131.4	136.2	156.6	31.6	992.1	138.8	0.7	0.59	4	4	3	4	1	1	1	1	2	2
30315-C	37.5	47.8	4.9	7.8	1.1	5.0	26.2	81.6	8.7	40.7	52.7	76.0	63.2	76.6	136.7	129.5	90.7	35.9	1083.0	174.9	0.7	0.41	4	5	3	4	1	1	1	1	2	2
30316-A	36.1	35.1	3.6	5.9	3.0	4.2	56.8	70.1	7.2	52.5	51.7	52.0	59.6	74.2	131.7	129.9	69.8	52.4	1404.0	564.8	0.7	0.34	4	8	3	4	1	1	1	1	2	2
30316-B	35.0	35.6	3.2	5.0	2.9	5.4	20.3	61.3	3.9	34.5	50.7	51.0	58.6	68.6	131.4	126.2	81.4	46.0	1097.0	301.4	0.7	0.56	4	6	3	4	1	1	1	1	2	2
30317-A	46.5	40.2	3.4	4.1	3.5	2.5	68.3	53.0	19.9	40.0	53.7	59.0	61.6	76.6	129.4	127.2	83.1	18.6	1333.0	383.8	0.9	0.48	3	7	1	5	1	1	1	1	2	2
30318-B	40.3	39.3	6.8	5.3	3.9	4.4	46.3	69.3	5.2	50.5	52.7	52.0	60.6	73.6	131.4	129.2	84.5	35.0	1370.0	961.4	0.9	0.43	3	6	1	5	1	1	1	1	2	2
30319-A	42.6	49.1	5.1	6.2	3.0	6.1	37.0	97.8	7.7	33.8	54.4	51.4	63.6	66.2	128.4	131.5	87.5	67.6	985.4	876.9	0.7	0.71	4	4	4	3	1	1	1	1	2	2
30319-B	38.2	35.1	4.9	5.3	3.7	3.4	30.2	32.5	8.7	32.8	54.7	54.7	65.2	75.2	141.7	123.2	83.9	25.2	1392.0	669.3	0.8	0.14	3	9	1	5	1	1	1	1	2	2
30320-C	46.3	35.0	11	6.7	11	7.4	98.0	62.9	21.5	37.8	55.7	62.7	64.9	85.2	140.4	125.2	73.4	25.1	1305.0	506.1	0.8	0.34	3	6	1	5	1	1	1	1	2	2
30321-A	43.5	38.5	6.1	7.7	3.9	3.8	63.3	46.9	8.6	23.8	53.7	69.7	59.6	78.2	130.4	147.2	90.2	41.1	1018.0	79.0	0.6	0.53	4	6	1	5	1	1	1	1	2	2
30323-A	42.2	34.9	6.1	6.2	5.2	2.0	55.0	44.9	10.9	29.7	51.7	61.0	61.6	74.6	132.4	129.5	83.2	19.9	1224.0	251.1	0.9	0.43	3	5	1	5	1	1	1	1	2	2
30324-A	36.5	38.7	3.8	5.8	1.5	3.9	27.6	42.0	10.6	28.0	52.7	53.0	61.6	76.6	129.4	127.2	84.2	30.6	852.7	300.1	0.5	0.51	4	4	4	4	1	1	1	1	2	2
30325-A	35.1	37.7	6.9	9.7	0.3	6.5	49.1	55.2	5.9	32.8	52.7	53.0	59.6	64.2	138.7	121.2	89.5	32.9	1329.0	274.5	0.8	0.41	3	5	1	5	1	1	1	1	2	2
30325-C	36.5	36.9	4.3	9.9	3.3	6.2	39.5	84.8	13.1	45.5	54.4	50.0	59.9	72.2	130.4	120.9	83.3	51.4	921.0	239.8	0.6	0.55	4	5	4	4	1	1	1	1	2	2
30326-A	43.7	34.1	5.8	5.9	4.6	3.2	48.0	67.1	4.9	33.5	51.7	50.0	57.6	65.2	131.4	120.9	92.2	51.4	1175.0	664.8	0.6	0.41	4	6	2	4	1	1	1	1	2	2
30327-A	37.5	39.0	2.3	8.6	1.3	8.8	18.1	126.2	6.8	112.4	58.4	52.7	58.9	72.6	137.4	137.9	102.5	18.0	1246.0	513.2	0.8	0.54	3	7	1	5	1	1	1	1	2	2
30329-A	38.8	34.8	6.4	6.6	5.9	4.4	81.6	44.3	17.2	30.2	52.7	62.0	61.6	76.9	132.4	131.2	68.2	28.2	1091.0	564.9	0.7	0.45	4	5	3	5	1	1	1	1	2	2
30329-B	43.7	38.5	4.2	7.8	-0.3	5.2	2.5	64.4	2.3	37.0	57.4	62.0	73.9	92.6	128.0	136.2	64.7	43.6	407.1	331.3	0.3	0.15	7	9	5	4	1	1	1	1	2	2
30331-A	44.5	37.8	5.4	6.9	4.9	1.4	59.0	58.3	5.9	34.2	51.7	53.0	60.6	75.9	125.4	130.2	80.6	23.5	1048.0	610.0	0.7	0.67	5	6	3	5	1	1	1	1	2	2
30332-A	29.8	34.7	6.9	6.3	3.4	1.4	44.2	30.2	8.7	27.8	52.7	54.7	65.2	78.2	138.7	123.2	46.3	25.4	604.9	231.3	0.2	0.12	8	9	4	5	1	1	1	1	2	2
30334-B	35.7	39.6	5.5	7.3	3.9	5.2	45.7	114.2	1.2	56.8	52.7	53.0	60.6	64.2	131.4	123.2	91.0	31.9	1374.0	398.2	0.8	0.54	3	4	1	4	1	1	1	1	2	2
30334-C	35.5	35.1	4.6	6.5	3.3	2.9	47.4	78.1	6.9	48.5	51.7	50.0	58.6	63.2	136.7	120.9	95.3	59.4	1051.0	738.5	0.8	0.75	4	3	2	3	1	1	1	1	2	2
30335-A	41.3	46.1	6.1	4.9	8.3	5.5	92.7	60.4	15.7	33.8	53.4	52.4	63.6	64.2	141.4	131.5	102.7	101.6	1305.0	916.9	0.6	0.61	4	4	2	2	1	1	1	1	2	2
30335-B	37.9	39.8	7.4	8.2	5.5	5.4	25.0	48.0	8.1	14.2	54.7	59.0	60.9	74.9	133.4	130.2	98.2	65.2	1660.0	1283.0	0.7	0.82	3	5	2	3	1	1	3	1	2	2
30336-A	40.5	35.2	5.5	5.0	2.4	2.4	25.2	35.9	9.1	2.8	54.7	53.7	63.2	75.2	132.7	123.2	75.9	57.1	1309.0	1291.0	0.8	0.85	2	3	2	2	1	1	1	1	1	1
30336-B	35.7	43.9	5.2	5.9	5.2	2.5	44.0	65.1	6.5	19.5	52.7	50.0	57.6	65.2	131.4	120.9	88.6	97.4	1410.0	1900.0	0.8	0.81	2	2	2	2	1	1	3	3	1	1
30337-A	45.3	36.0	5.2	6.2	3.6	1.5	73.7	61.5	8.5	36.5	51.7	48.0	59.6	62.2	133.4	118.9	90.2	51.4	1297.0	559.8	0.7	0.57	3	7	2	5	1	1	1	1	2	2
30337-C	36.2	38.8	4.2	8.9	2.7	13.	22.2	149.8	6.1	59.4	53.7	74.7	62.2	78.6	138.7	137.9	82.7	30.5	906.6	85.7	0.5	0.41	5	6	4	4	1	1	1	1	2	2
30338-A	40.2	38.2	4.1	6.5	2.2	4.9	26.3	60.7	9.2	36.0	50.7	61.0	59.6	76.6	123.4	129.2	75.0	18.7	806.0	232.6	0.7	0.69	5	6	4	5	1	1	1	1	2	2
30339-A	30.7	38.2	5.8	5.1	2.2	5.5	45.3	59.0	14.6	46.0	53.7	55.0	61.6	76.6	128.4	129.2	41.8	38.6	792.7	40.1	0.4	0.38	8	8	4	5	1	1	1	1	2	2
30340-A	37.6	39.7	8.7	3.2	8.0	5.5	91.7	37.4	16.1	38.8	56.4	53.4	63.6	68.2	136.4	131.5	61.7	60.6	957.1	259.4	0.4	0.11	7	9	4	4	1	1	1	1	2	2
30340-C	32.1	39.4	5.4	7.6	4.2	3.4	24.4	56.0	5.1	36.2	55.7	60.0	62.9	74.9	140.4	130.2	46.0	27.8	1216.0	953.9	0.5	0.33	6	5	4	5	1	1	1	1	2	2
30341-A	38.2	39.7	6.4	8.5	2.9	3.5	40.0	57.4	11.9	36.0	49.7	60.0	60.6	74.6	123.4	127.2	83.4	23.1	1161.0	192.6	0.6	0.49	4	7	3	5	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
30341-B	38.8	43.3	5.2	3.1	4.7	0.5	45.5	25.4	10.1	41.5	49.7	51.0	61.2	68.9	134.7	137.2	77.5	60.0	938.3	572.7	0.6	0.61	6	7	4	3	1	1	1	1	2	2
30342-B	36.8	37.8	4.2	9.2	5.7	2.4	50.5	49.0	8.4	36.2	52.7	55.0	64.2	75.9	138.7	145.2	61.7	34.4	1342.0	232.8	0.6	0.36	4	5	3	5	1	1	1	1	2	2
30343-A	35.6	43.1	6.1	5.9	8.3	4.8	47.7	88.1	15.1	13.8	53.4	50.4	63.6	64.2	138.4	131.5	97.7	96.6	1319.0	1224.0	0.8	0.74	3	4	1	2	1	1	2	2	2	2
30344-A	32.0	38.6	4.9	4.3	4.1	6.5	49.5	69.8	17.4	65.8	52.7	51.0	63.2	64.2	138.7	120.2	45.2	46.9	1068.0	647.7	0.5	0.14	7	9	4	5	1	1	1	1	2	2
30345-B	35.5	38.7	2.2	2.9	0.3	1.8	14.4	14.8	5.5	7.7	50.7	52.7	57.6	60.6	131.7	131.9	85.3	82.5	1654.0	1289.0	0.7	0.71	3	3	4	1	1	1	3	3	2	2
30345-C	38.0	33.0	2.8	6.2	1.2	3.5	34.3	57.8	-1.5	32.5	48.7	48.0	56.6	63.2	131.4	118.9	103.4	21.8	1239.0	822.3	0.7	0.59	4	7	2	5	1	1	1	1	2	2
30345-D	33.2	42.8	4.0	2.5	0.3	1.0	28.1	29.2	5.5	20.7	49.4	54.0	56.9	75.6	130.4	129.5	64.7	65.9	961.0	367.4	0.5	0.45	7	7	4	4	1	1	1	1	2	2
30346-A	39.6	48.4	4.4	6.0	4.3	4.5	46.7	96.5	17.1	60.8	53.4	50.0	60.6	64.2	140.4	124.2	109.7	108.9	1149.0	989.7	0.8	0.76	4	5	3	2	1	1	1	1	2	2
30346-C	38.6	47.7	5.7	5.9	5.2	6.1	23.4	66.1	15.5	48.8	51.7	50.4	59.9	65.2	136.4	128.5	97.6	38.6	854.8	523.1	0.6	0.54	5	7	4	4	1	1	1	1	2	2
30347-A	43.6	43.7	5.4	5.0	8.3	4.5	27.7	90.2	12.7	31.8	53.4	50.0	62.6	68.2	138.4	121.2	84.3	45.9	654.6	610.7	0.6	0.60	5	8	5	3	1	1	1	1	2	2
30347-B	36.3	39.5	5.4	6.7	3.2	1.8	32.7	54.5	10.8	24.8	52.7	53.7	60.9	75.2	137.4	123.2	76.8	35.2	1343.0	399.0	0.6	0.40	4	6	3	5	1	1	1	1	2	2
30348-B	37.8	43.8	3.2	4.8	1.6	4.0	32.4	30.6	8.9	11.7	51.7	54.0	59.6	73.6	136.7	129.5	89.3	69.9	1943.0	1379.0	0.8	0.81	1	3	1	2	1	1	3	2	1	1
30348-C	41.3	37.6	5.8	8.2	4.2	5.9	43.3	92.5	4.2	48.5	51.7	50.0	59.6	63.2	131.4	120.9	80.6	49.4	1062.0	363.5	0.6	0.44	4	7	3	5	1	1	1	1	2	2
30349-A	38.5	34.8	5.4	4.9	3.2	3.8	35.3	47.7	10.2	24.2	49.7	53.0	58.6	76.9	126.4	127.2	78.2	43.2	919.4	187.7	0.6	0.31	5	5	4	5	1	1	1	1	2	2
30349-B	39.2	47.1	5.2	5.9	2.4	4.8	33.5	69.3	7.1	16.2	52.7	55.0	62.2	73.9	139.7	131.2	90.7	85.2	1980.0	1989.0	0.9	0.83	1	2	1	2	1	1	3	3	1	1
30349-C	31.9	33.8	5.9	5.3	7.1	1.8	51.5	38.5	7.4	33.8	52.7	54.7	68.2	75.2	139.7	126.2	80.7	31.3	1405.0	238.7	0.5	0.13	7	9	3	5	1	1	1	1	2	2
30350-A	32.0	38.7	4.7	9.3	3.6	5.1	39.5	64.5	8.8	48.4	51.4	54.7	56.9	73.6	137.4	126.9	50.8	42.5	927.6	329.6	0.6	0.54	7	7	4	4	1	1	1	1	2	2
30350-B	37.0	48.5	3.8	4.2	0.9	1.0	21.3	17.9	2.5	27.7	49.7	54.0	57.6	82.6	128.4	129.5	83.8	49.9	1200.0	554.9	0.8	0.78	4	3	2	3	1	1	1	1	2	2
30350-C	33.3	50.0	5.2	5.0	1.9	3.7	23.3	51.9	2.2	33.5	49.7	53.0	58.6	74.6	124.4	131.2	75.0	36.0	867.1	493.9	0.6	0.58	4	4	4	4	1	1	1	1	2	2
41001-A	36.4	35.4	5.8	5.7	2.9	2.5	23.5	31.2	-0.3	37.8	50.0	53.0	58.2	64.2	118.7	123.2	85.4	46.9	1067.0	237.0	0.7	0.64	5	6	3	5	1	1	1	1	2	2
41003-B	34.6	33.5	7.1	4.7	5.5	2.4	50.7	46.5	13.1	25.8	52.7	53.7	57.9	76.2	135.4	134.2	75.6	32.6	724.8	380.3	0.6	0.58	6	7	4	5	1	1	1	1	2	2
41003-C	36.9	37.2	6.4	3.7	3.2	1.8	50.7	34.5	20.1	8.8	51.7	54.7	58.9	79.2	129.4	126.2	85.6	84.1	1375.0	1105.0	0.8	0.72	3	4	1	1	1	1	2	2	2	2
41004-C	38.5	52.3	4.7	3.6	0.6	2.1	24.8	35.5	7.5	17.4	49.4	53.7	55.9	73.6	130.4	130.9	93.7	61.3	1899.0	955.7	0.8	0.82	1	3	2	2	1	1	3	2	1	1
41018-B	40.8	51.5	4.0	6.2	2.6	3.0	40.8	70.2	12.1	41.7	54.4	52.0	59.9	74.6	133.4	129.5	86.1	49.9	991.0	633.6	0.6	0.41	5	5	3	3	1	1	1	1	2	2
41020-A	38.0	36.2	5.8	3.3	4.6	2.9	38.7	55.8	3.2	34.8	50.7	53.0	57.6	70.2	131.4	123.2	93.4	18.4	960.4	190.7	0.6	0.53	5	6	3	5	1	1	1	1	2	2
41020-B	36.0	48.9	3.7	6.9	0.3	3.2	20.5	69.8	6.5	32.5	51.4	48.0	56.9	61.2	133.4	118.9	104.5	65.4	804.3	883.5	0.8	0.74	4	4	4	3	1	1	1	1	2	2
41021-A	35.6	47.1	5.7	6.5	9.3	7.5	35.0	97.1	11.4	33.8	54.4	50.4	62.6	67.2	138.4	131.5	91.9	68.6	889.6	863.1	0.7	0.69	4	5	4	3	1	1	1	1	2	2
41021-B	38.6	37.2	4.4	5.8	2.5	3.5	14.7	52.0	10.5	39.0	52.7	71.0	60.9	83.6	129.4	127.2	71.2	16.6	874.8	298.8	0.6	0.64	5	6	4	5	1	1	1	1	2	2
41026-A	38.5	37.0	2.3	5.8	0.6	4.0	25.1	63.2	9.1	35.7	53.4	54.0	57.9	74.6	137.4	129.5	90.5	59.9	1119.0	154.9	0.8	0.41	4	5	1	4	1	1	1	1	2	2
41026-C	31.2	36.9	3.5	4.9	0.7	1.1	14.8	20.3	12.3	28.2	59.4	62.0	72.9	76.9	128.0	130.2	40.4	34.2	189.1	105.7	0.4	0.18	8	9	5	5	1	1	1	1	2	2
41029-A	37.3	51.2	7.1	5.3	3.8	5.1	49.7	75.5	18.8	49.8	53.7	53.7	59.9	77.2	122.4	136.2	74.4	35.1	789.8	292.8	0.6	0.54	4	4	4	4	1	1	1	1	2	2
41029-B	45.3	36.9	4.7	4.6	5.8	3.8	48.4	48.3	11.8	30.2	52.7	55.0	58.9	72.9	135.4	128.2	86.8	63.2	559.8	116.5	0.3	0.29	4	5	4	4	1	1	1	1	2	2
41030-A	35.8	36.3	3.6	5.9	6.6	1.2	61.8	45.1	12.5	28.5	53.7	50.0	61.6	62.2	134.7	118.9	87.3	51.4	566.0	391.0	0.3	0.27	4	7	4	5	1	1	1	1	2	2
41036-A	36.8	41.0	7.2	7.1	5.2	3.2	50.2	33.4	15.9	18.5	53.4	52.0	62.6	71.9	132.0	136.2	73.6	61.0	1132.0	970.2	0.8	0.81	4	6	3	3	1	1	1	1	2	2
41036-B	39.9	47.4	6.7	5.2	4.5	0.8	38.0	30.3	14.8	16.2	53.7	55.0	59.9	73.9	132.4	128.2	90.3	88.3	1219.0	1260.0	0.8	0.81	2	3	1	2	1	1	2	2	1	1
41036-C	37.1	49.0	5.1	6.3	5.9	4.9	31.5	60.8	4.4	30.8	51.0	50.0	57.2	66.2	132.7	123.2	100.2	99.9	1387.0	844.5	0.7	0.75	4	6	3	2	1	1	1	1	2	2
41043-B	40.1	37.9	4.2	9.9	3.0	9.5	73.8	137.5	7.2	7.5	51.7	51.0	57.6	71.2	134.7	130.9	105.3	107.4	1495.0	1422.0	0.7	0.72	3	3	2	2	1	1	3	3	2	2
41044-A	29.6	25.7	3.1	2.5	3.9	4.5	1.8	-0.6	1.4	41.8	54.0	53.4	59.2	56.2	128.7	132.5	45.2	44.6	864.0	845.6	0.2	0.19	7	8	4	4	1	1	1	1	2	2
41046-B	31.7	38.5	4.2	4.7	3.1	6.4	33.2	67.9	11.1	25.8	53.7	53.7	63.2	75.2	136.7	126.2	47.4	25.6	941.6	146.5	0.5	0.41	6	6	4	5	1	1	1	1	2	2
41051-A	36.6	42.6	4.3	4.9	2.0	2.8	32.5	32.5	9.8	19.4	52.4	54.7	58.9	74.6	130.4	128.9	84.5	65.5	1147.0	1033.0	0.9	0.83	2	3	2	2	1	1	3	2	1	1
41051-C	31.8	34.7	4.7	5.3	2.3	2.8	27.5	41.5	7.5	28.4	51.4	52.7	60.9	71.6	132.4	129.9	42.9	18.0	851.0	234.5	0.6	0.52	6	6	4	5	1	1	1	1	2	2
41057-B	46.1	35.3	5.8	6.7	8.5	7.4	71.2	101.6	15.4	57.5	52.0	53.0	57.2	73.6	130.7	129.2	74.2	45.0	1204.0	292.6	0.7	0.41	4	5	3	4	1	1	1	1	2	2
41058-A	35.3	37.8	7.5	6.9	2.6	5.1	24.7	73.5	-0.5	30.4	51.7	58.7	58.6	78.6	138.4	137.9	72.6	68.4	1252.0	699.5	0.8	0.81	4	6	2	3	1	1	1	1	2	2
41058-B	39.0	34.3	3.8	6.3	4.6	3.7	49.0	55.6	10.5	32.5	56.7	52.0	63.6	82.6	131.4	127.2	104.6	46.0	710.4	440.1	0.6	0.51	4	5	4	4	1	1	1	1	2	2
41059-A	33.1	34.9	7.1	4.2	7.6	3.3	46.0	26.6	12.1	20.7	52.4	62.0	61.6	76.6	130.4	127.5	49.8	20.9	898.8	108.6	0.4	0.13	8	9	5	5	1	1	1	1	2	2
41059-C	38.7	39.2	7.5	6.8	4.5	5.5	39.5	77.0	6.7	58.0	53.0	63.0	57.2	77.6	120.7	138.2	84.6	43.6	1376.0	565.1	0.7	0.43	4	6	2	4	1	1	1	1	2	2
41073-A	38.5	36.7	4.0	3.9	3.3	5.5	47.5	58.2	14.1	37.4	53.4	54.7	57.9	74.6	130.4	130.9	97.9	47.5	899.3	222.0	0.5	0.37	4	6	4	4	1	1	1	1	2	2
41073-B	40.5	48.4	3.8	5.0	1.2	5.2	37.3	93.2	10.9	28.8	49.7	53.0	58.6	64.2	122.4	124.2	90.2	66.9	850.2	880.7	0.6	0.65	5	6	4	3	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
41074-A	36.6	35.4	6.4	6.2	7.0	6.5	58.7	74.8	17.4	30.8	54.4	50.4	63.6	67.2	142.4	131.5	86.3	48.6	1124.0	840.6	0.7	0.57	4	5	3	4	1	1	1	1	2	2
41074-B	29.9	37.4	3.8	6.6	2.9	5.1	35.3	42.3	14.2	23.2	51.7	64.0	59.6	75.9	129.4	128.2	46.4	26.7	1373.0	121.5	0.7	0.62	6	6	4	5	1	1	1	1	2	2
41075-A	40.5	37.1	5.0	4.9	3.0	4.2	28.1	60.8	19.5	34.5	54.4	52.0	59.9	67.2	137.4	120.9	88.5	33.4	849.3	542.3	0.6	0.57	4	4	4	4	1	1	1	1	2	2
41075-C	38.5	51.6	3.3	5.2	1.0	3.9	26.5	116.8	8.5	59.5	52.4	55.0	58.9	69.2	133.4	129.9	111.7	113.4	1264.0	972.8	0.8	0.78	4	4	2	2	1	1	1	1	2	2
41076-A	43.3	37.9	7.4	3.8	2.3	2.2	55.4	41.0	22.7	45.0	53.4	60.0	63.6	82.6	131.4	127.2	84.3	12.6	1009.0	135.1	0.7	0.60	5	6	3	5	1	1	1	1	2	2
41076-C	37.2	36.9	4.1	5.2	2.2	2.4	26.6	45.0	8.2	28.2	49.7	63.0	58.6	75.9	122.4	128.2	71.4	53.2	839.4	661.5	0.6	0.45	4	5	4	4	1	1	1	1	2	2
41077-B	38.8	48.9	3.2	7.5	1.0	3.2	27.4	76.8	5.2	11.5	50.7	52.0	58.6	67.2	127.7	120.9	86.3	88.4	1433.0	1561.0	0.8	0.75	4	4	3	2	1	1	3	3	2	2
41078-A	37.1	39.3	7.5	4.8	3.5	1.5	45.2	30.4	12.6	22.5	53.4	55.0	61.6	69.9	140.0	153.2	93.2	62.0	1159.0	1169.0	0.8	0.79	4	5	2	3	1	1	1	1	2	2
41078-B	40.0	48.3	5.2	5.9	2.2	3.5	40.7	44.1	7.9	32.5	51.7	55.0	56.6	66.2	134.4	120.9	94.6	55.4	1257.0	878.5	0.8	0.84	4	5	2	3	1	1	1	1	2	2
41079-A	37.6	46.7	3.7	5.5	4.0	1.9	38.7	9.7	8.4	45.5	56.4	53.0	62.6	72.9	139.4	136.2	91.1	32.2	933.8	385.7	0.6	0.55	5	5	4	4	1	1	1	1	2	2
41080-A	34.5	49.7	5.1	5.3	2.9	4.9	31.0	81.2	7.9	51.8	52.7	51.0	61.6	70.2	129.4	123.2	83.8	36.9	642.7	489.5	0.5	0.51	5	6	4	4	1	1	1	1	2	2
41080-B	38.9	47.4	3.7	5.9	4.2	7.1	27.7	64.0	15.8	34.2	51.7	64.0	60.9	73.9	126.4	130.2	74.4	34.2	1288.0	319.0	0.7	0.46	4	5	3	4	1	1	1	1	2	2
41081-A	36.7	47.1	3.8	6.2	2.9	6.1	19.2	81.1	0.7	21.8	54.0	50.4	59.2	64.2	129.7	131.5	96.2	95.6	1751.0	1933.0	0.8	0.80	1	2	2	2	1	1	3	3	1	1
41086-A	36.6	37.8	6.1	6.9	7.8	4.4	72.4	69.7	20.8	40.2	53.7	54.0	58.9	73.9	130.4	128.2	87.2	38.2	1430.0	474.2	0.7	0.59	4	4	2	4	1	1	1	1	2	2
41086-B	38.4	45.1	4.1	6.5	4.2	6.8	40.2	48.1	3.1	33.8	50.0	50.4	56.2	64.2	122.7	128.5	75.0	66.6	1249.0	896.9	0.9	0.85	5	6	2	3	1	1	1	1	2	2
41089-A	38.5	43.2	1.7	1.5	1.0	0.9	26.8	29.7	6.5	35.7	52.4	54.0	55.9	61.6	125.4	125.2	92.5	61.6	937.6	934.6	0.6	0.61	5	6	4	3	1	1	1	1	2	2
41089-B	37.5	50.2	3.3	5.8	0.3	5.9	23.8	75.4	5.8	56.0	53.4	55.0	57.9	74.6	130.4	127.2	86.5	50.6	1041.0	693.8	0.8	0.50	4	4	1	3	1	1	1	1	2	2
41090-A	35.1	47.3	3.5	6.5	3.2	3.2	19.2	47.7	-1.3	34.5	54.0	55.0	60.2	72.9	129.7	136.2	98.6	45.0	1417.0	681.5	0.7	0.71	3	4	2	3	1	1	1	1	2	2
41090-B	36.6	40.7	5.1	4.8	3.0	2.9	37.4	30.4	11.4	40.5	56.4	53.0	64.6	72.9	138.4	136.2	94.7	39.2	1499.0	374.0	0.7	0.55	4	4	2	4	1	1	1	1	2	2
41091-A	37.1	46.4	6.9	4.9	5.5	3.1	59.6	19.4	10.9	12.8	55.4	53.4	65.6	68.2	136.0	128.5	90.0	66.6	1098.0	1184.0	0.6	0.61	5	6	3	3	1	1	1	1	2	2
41092-B	31.9	34.4	3.8	4.8	1.5	4.5	6.5	50.0	-2.6	38.0	52.0	62.0	59.2	89.6	118.7	136.2	76.6	48.6	1361.0	775.1	0.6	0.51	6	5	3	4	1	1	1	1	2	2
41093-B	41.1	51.4	6.2	6.3	4.5	6.5	55.9	115.2	12.6	10.8	53.4	52.0	63.6	69.2	133.0	132.2	91.0	92.9	1288.0	1357.0	0.9	0.76	3	4	1	1	1	1	3	3	2	2
41093-C	38.1	36.3	6.2	7.9	8.5	6.8	70.2	54.5	11.9	38.4	52.4	63.7	61.6	75.6	140.0	137.9	86.0	27.5	848.8	-81.8	0.5	0.51	5	7	4	5	1	1	1	1	2	2
41094-B	39.3	40.2	3.7	7.2	4.6	2.0	27.4	56.6	14.1	42.7	52.4	61.0	60.6	74.6	130.4	127.5	91.9	56.9	1294.0	906.1	0.8	0.81	2	3	2	3	1	1	2	2	1	1
41094-C	36.4	48.0	6.1	3.7	3.2	7.2	30.2	33.8	-0.3	13.8	51.0	74.0	58.2	72.2	129.7	143.2	161.8	131.9	2000.0	1364.0	0.7	0.73	4	4	1	1	1	1	3	3	2	2
41097-B	33.1	35.4	3.1	6.3	1.9	5.5	22.5	43.8	0.1	35.4	54.0	52.7	63.2	72.6	129.7	126.9	67.4	48.5	652.3	440.7	0.2	0.15	6	9	4	4	1	1	1	1	2	2
41101-A	38.0	42.2	2.8	4.8	0.4	3.0	28.2	60.2	8.6	33.7	48.4	63.0	56.9	74.6	131.0	130.5	90.6	34.9	1015.0	224.9	0.5	0.50	5	6	4	4	1	1	1	1	2	2
41104-B	37.3	41.3	6.4	3.5	2.5	0.2	25.4	21.4	10.1	45.5	52.7	55.0	59.9	73.9	129.4	136.2	85.6	31.2	969.8	344.0	0.7	0.57	5	5	3	4	1	1	1	1	2	2
41107-A	38.0	44.3	3.8	8.0	2.2	7.1	27.3	95.3	1.5	44.5	49.7	53.0	57.6	75.6	134.4	129.2	85.4	37.0	1359.0	277.6	0.7	0.54	4	5	2	4	1	1	1	1	2	2
41107-B	35.8	52.7	4.2	4.0	0.6	2.2	30.8	54.5	0.9	14.8	51.7	53.0	59.6	64.2	134.7	121.2	100.9	85.5	1654.0	1244.0	0.9	0.87	1	3	1	2	1	1	3	2	1	1
41116-A	38.5	50.3	3.0	5.3	2.0	5.1	35.8	50.8	10.1	36.4	53.4	54.7	55.9	74.6	133.4	126.9	92.9	65.5	929.3	267.0	0.5	0.51	4	7	4	4	1	1	1	1	2	2
41121-A	35.8	36.3	3.0	3.9	1.0	3.1	23.5	64.8	9.5	37.4	51.4	52.7	57.9	78.6	130.4	128.9	83.7	10.7	857.6	37.0	0.6	0.62	5	6	4	5	1	1	1	1	2	2
41121-B	41.2	39.3	4.4	8.3	2.9	5.7	39.0	65.3	14.9	37.5	49.7	54.0	58.6	78.6	139.4	129.2	86.6	54.0	1369.0	807.4	0.8	0.78	4	4	2	3	1	1	1	1	2	2
41124-C	38.5	50.6	4.7	5.6	2.0	4.5	32.8	62.8	13.5	36.4	54.4	53.7	58.9	75.6	133.4	130.9	104.9	30.5	1459.0	336.3	0.9	0.56	3	6	1	4	1	1	1	1	2	2
41127-A	36.8	36.1	6.9	7.3	3.0	6.5	54.1	53.5	6.5	45.8	50.7	53.0	58.6	68.2	131.7	121.2	83.7	30.9	401.0	349.5	0.5	0.15	4	9	4	5	1	1	1	1	2	2
41127-B	38.5	45.2	3.7	3.5	0.6	1.7	24.5	44.9	7.1	14.7	51.4	54.0	57.9	74.6	130.4	129.5	115.7	116.9	1713.0	1902.0	0.9	0.83	2	2	1	1	1	1	3	3	1	1
41133-A	41.1	38.2	5.1	5.7	4.5	5.9	28.2	86.2	1.7	10.8	50.0	50.0	57.2	64.2	129.7	123.2	122.2	121.9	1667.0	1411.0	0.8	0.81	1	3	1	1	1	1	3	2	1	1
41133-B	37.4	53.6	2.1	7.3	-0.2	5.1	-1.5	58.2	0.1	44.4	50.0	60.7	58.2	75.6	129.7	126.9	87.8	67.0	1216.0	725.2	0.8	0.78	4	4	3	3	1	1	1	1	2	2
41136-A	37.6	46.5	8.1	4.8	5.3	2.5	54.7	56.0	22.1	33.0	53.4	55.0	61.6	76.6	134.4	127.2	78.3	50.6	1242.0	506.3	0.7	0.62	5	6	3	3	1	1	1	1	2	2
41136-B	37.6	43.5	6.7	5.8	10	6.5	56.4	94.7	14.7	47.0	53.4	55.0	60.6	76.6	138.4	127.2	73.1	35.6	817.1	171.3	0.6	0.55	4	4	4	4	1	1	1	1	2	2
41138-A	39.0	38.5	4.2	5.7	2.9	4.1	15.3	63.6	1.9	30.5	49.7	54.0	58.6	72.6	128.4	129.2	69.0	57.0	907.1	781.4	0.7	0.65	4	4	3	3	1	1	1	1	2	2
41143-B	38.7	47.0	6.5	5.9	2.5	5.1	28.2	50.2	4.7	36.4	50.0	53.7	58.2	72.6	129.7	126.9	81.0	51.5	1002.0	422.0	0.7	0.61	5	6	3	3	1	1	1	1	2	2
41145-A	37.6	35.9	7.1	7.0	3.5	3.1	40.7	41.9	9.1	22.8	53.7	53.7	60.9	73.2	140.4	123.2	80.8	39.1	488.2	45.3	0.5	0.40	4	5	4	4	1	1	1	1	2	2
41153-A	40.6	39.7	5.4	3.2	6.0	5.8	72.7	37.8	15.1	30.8	53.4	53.4	63.6	63.2	127.4	131.5	85.5	45.6	1410.0	754.4	0.7	0.67	4	4	2	3	1	1	1	1	2	2
41153-B	36.6	42.7	8.1	6.0	5.0	5.5	49.0	37.5	16.4	32.8	52.4	51.0	61.6	70.2	131.4	123.2	91.9	60.0	1335.0	653.2	0.8	0.73	3	4	1	3	1	1	1	1	2	2
41156-A	32.2	35.1	4.0	7.2	1.0	5.0	28.1	68.6	8.1	51.7	51.4	54.0	55.9	76.6	137.4	129.5	60.9	61.9	791.4	780.7	0.6	0.58	6	5	4	4	1	1	1	1	2	2
41156-B	43.0	46.3	3.2	5.3	0.6	4.7	34.0	69.6	4.2	43.5	50.7	51.0	58.6	69.6	131.4	126.2	82.6	44.0	1399.0	682.6	0.9	0.76	3	5	1	3	1	1	1	1	2	2
41158-A	39.8	46.7	4.4	5.9	3.5	2.4	56.3	37.0	16.9	41.2	49.7	55.0	58.6	73.9	122.4	128.2	86.2	64.2	831.0	252.3	0.6	0.55	5	6	4	4	1	1	1	1	2	2
41163-A	38.7	37.1	4.2	6.5	5.9	4.5	35.3	70.8	4.2	56.5	51.7	50.0	58.6	64.2	134.4	118.9	90.2	69.2	1267.0	1069.0	0.8	0.76	3	4	2	3	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
41165-D	32.8	46.7	8.5	5.3	5.5	5.5	58.2	54.2	12.6	11.8	54.4	52.0	63.6	69.2	133.0	123.2	89.0	90.9	1395.0	1258.0	0.7	0.71	3	3	2	2	1	1	3	3	2	2
41166-A	40.8	48.8	7.5	4.8	5.5	6.7	74.2	56.9	15.6	33.7	55.4	54.0	62.6	74.6	136.0	127.5	75.6	47.9	867.1	580.3	0.8	0.71	5	5	4	3	1	1	1	1	2	2
41167-B	38.4	34.5	6.5	7.8	5.5	3.2	84.2	44.0	15.6	36.5	53.4	58.0	61.6	66.9	138.0	136.2	75.2	37.2	1327.0	242.7	0.6	0.51	4	4	3	4	1	1	1	1	2	2
41167-C	37.8	40.3	6.2	3.5	6.2	1.9	56.2	29.4	11.3	17.5	56.4	55.0	63.6	73.9	140.0	159.2	107.0	109.0	1865.0	1873.0	1.0	0.87	1	2	1	1	1	1	3	3	1	1
41169-A	42.6	41.0	6.1	3.5	3.0	3.9	32.0	43.7	13.7	38.5	55.4	53.0	62.6	69.9	136.4	136.2	86.7	51.0	860.4	551.5	0.7	0.68	4	4	4	3	1	1	1	1	2	2
41169-C	38.5	44.8	5.0	3.8	3.0	1.0	42.5	32.2	8.8	32.7	49.4	54.0	57.9	73.6	127.4	127.5	95.3	34.9	1356.0	332.4	0.7	0.52	4	4	2	4	1	1	1	1	2	2
41173-A	37.4	45.7	4.8	4.0	0.5	4.2	7.2	46.5	-0.3	36.8	51.0	49.0	57.2	65.2	128.7	121.2	83.4	45.0	1437.0	370.7	0.6	0.41	5	6	2	4	1	1	1	1	2	2
41173-D	34.7	40.8	3.5	6.0	1.5	3.1	9.8	56.5	-0.9	27.8	52.0	57.7	58.2	75.2	122.7	139.2	88.2	59.4	1396.0	651.5	0.7	0.65	4	5	2	3	1	1	1	1	2	2
41180-A	38.6	46.4	8.7	3.7	6.0	4.2	33.0	71.8	10.4	46.8	55.4	49.0	62.6	65.2	141.4	124.2	91.1	35.0	1225.0	473.2	0.6	0.56	4	5	3	4	1	1	1	1	2	2
41180-B	37.3	46.7	4.1	4.2	3.6	3.1	21.0	45.8	9.4	28.8	52.4	50.4	60.6	65.2	138.4	128.5	86.2	68.6	1010.0	983.1	0.7	0.64	4	4	4	3	1	1	1	1	2	2
41198-A	41.6	41.5	7.1	6.7	11	10	61.7	69.2	18.1	37.8	51.4	49.7	63.6	64.2	131.4	128.2	84.3	33.1	917.1	464.0	0.5	0.53	4	4	4	4	1	1	1	1	2	2
41198-B	40.4	34.1	3.5	4.2	2.9	4.1	13.5	27.8	1.4	35.8	51.0	55.4	58.2	63.2	128.7	131.5	69.0	53.6	1344.0	683.6	0.7	0.68	4	4	2	3	1	1	1	1	2	2
41200-B	38.3	42.2	7.1	6.2	2.8	0.4	55.0	25.3	14.1	14.2	53.7	59.0	59.9	81.9	135.4	144.2	82.8	77.3	1770.0	1411.0	0.8	0.73	3	3	2	2	1	1	3	3	2	2
41200-C	33.8	38.7	1.9	8.3	-0.7	7.1	23.4	70.6	3.5	44.5	49.7	52.0	57.6	75.6	130.7	129.2	78.1	42.6	371.0	440.1	0.6	0.56	4	4	5	4	1	1	1	1	2	2
41203-B	38.8	37.1	4.2	4.7	1.3	2.5	28.1	25.8	4.5	42.8	48.7	50.0	57.6	69.2	134.7	121.2	87.4	38.0	422.7	65.7	0.5	0.54	4	7	4	4	1	1	1	1	2	2
41203-C	39.4	40.6	9.2	9.6	8.5	9.1	71.6	66.2	21.9	19.7	52.4	51.7	62.6	61.6	130.0	128.9	82.4	82.9	1850.0	1297.0	0.8	0.72	3	3	2	2	1	1	3	3	2	2
41206-A	38.8	42.0	7.5	5.1	7.9	3.9	76.6	47.4	17.6	55.5	55.4	52.0	61.6	62.9	138.0	136.2	85.2	57.0	1182.0	609.0	0.7	0.67	4	6	2	3	1	1	1	1	2	2
41206-B	37.1	42.4	4.5	4.2	4.9	2.5	50.9	29.8	13.9	19.8	54.4	51.4	62.6	66.2	132.0	128.5	117.0	118.6	1699.0	1998.0	0.9	0.84	1	2	1	1	1	1	3	3	1	1
41206-C	37.8	45.4	7.5	5.9	5.5	3.5	72.6	43.4	15.3	31.8	53.4	51.4	62.6	66.2	132.0	131.5	89.2	56.6	860.5	733.1	0.7	0.71	4	4	4	3	1	1	1	1	2	2
41209-A	36.2	45.5	4.5	5.0	2.4	2.8	47.2	49.9	4.7	24.8	53.7	51.7	63.2	74.2	140.7	126.2	75.5	38.1	1267.0	157.8	0.9	0.45	3	4	1	4	1	1	1	1	2	2
41209-C	37.7	40.3	5.2	5.3	3.9	5.2	37.0	39.2	4.2	31.8	52.7	53.0	60.6	64.2	131.4	123.2	72.6	53.9	1150.0	802.0	0.7	0.56	4	4	3	3	1	1	1	1	2	2
41212-B	42.8	48.4	5.4	3.3	3.5	5.2	50.0	85.8	12.9	9.8	50.7	49.0	58.6	68.2	119.4	124.2	109.0	109.9	1330.0	1361.0	0.8	0.76	4	4	4	2	1	1	2	2	2	2
41221-A	36.0	38.8	5.5	5.5	7.4	2.3	87.8	37.6	11.3	32.7	49.4	53.0	55.9	76.6	132.0	145.5	84.6	63.9	1524.0	851.1	0.9	0.67	3	4	1	3	1	1	1	1	2	2
41221-B	31.2	36.9	5.2	6.6	3.4	3.8	43.2	48.7	36.3	32.2	51.4	54.0	69.9	73.9	128.0	130.2	53.4	53.2	185.9	106.5	0.5	0.45	6	6	5	4	1	1	1	1	2	2
41222-A	38.2	50.8	5.4	4.2	6.2	4.3	43.0	59.2	9.6	43.7	51.7	62.0	61.6	76.6	126.4	129.5	86.6	43.9	1071.0	779.5	0.8	0.74	4	4	3	3	1	1	1	1	2	2
41222-B	36.2	44.8	4.4	3.8	4.5	3.7	33.0	42.2	9.9	42.7	52.7	54.0	61.6	74.6	129.4	127.5	90.6	72.9	1349.0	949.9	0.8	0.75	4	4	2	3	1	1	1	1	2	2
41223-A	39.1	40.7	4.9	2.9	2.9	2.1	63.6	5.1	19.6	32.8	59.4	50.4	66.6	68.2	133.0	128.5	83.6	46.6	1017.0	810.6	0.7	0.71	4	4	3	3	1	1	1	1	2	2
41223-B	41.4	50.2	7.2	4.2	10	2.3	110.9	28.9	19.3	29.7	54.4	54.0	62.6	76.6	138.0	129.5	88.4	67.7	1315.0	889.9	0.9	0.82	4	5	2	3	1	1	1	1	2	2
41225-B	38.6	42.2	4.4	3.5	3.6	2.9	34.4	42.7	12.1	26.0	52.4	62.0	60.6	76.6	136.4	136.2	77.5	53.6	1075.0	581.3	0.6	0.63	4	5	3	3	1	1	1	1	2	2
41226-A	31.5	35.0	9.2	9.0	7.9	7.8	57.9	55.2	18.9	33.8	55.4	53.7	63.6	64.2	130.0	125.2	47.2	39.1	628.8	689.0	0.5	0.45	6	5	4	4	1	1	1	1	2	2
41226-B	37.5	45.5	8.1	4.5	7.5	3.5	72.0	51.7	17.6	32.0	51.7	58.0	58.6	77.6	129.4	125.2	89.4	27.6	1306.0	488.8	0.6	0.59	4	4	2	4	1	1	1	1	2	2
41228-A	41.5	49.7	4.1	7.9	2.5	3.1	39.0	50.0	12.2	34.2	52.7	64.0	58.6	80.9	128.4	130.2	89.0	54.2	1434.0	594.0	0.8	0.82	4	5	2	3	1	1	1	1	2	2
41228-B	40.5	50.7	5.8	4.9	1.9	3.4	43.0	37.0	8.9	36.2	53.7	53.0	61.6	76.9	127.4	131.2	80.2	61.2	1028.0	285.3	0.7	0.57	4	4	3	4	1	1	1	1	2	2
41228-C	37.5	39.3	3.9	6.9	5.7	5.5	18.5	56.5	0.4	44.4	52.7	62.7	63.2	75.6	136.7	128.9	39.1	15.0	919.9	485.7	0.6	0.56	7	7	4	5	1	1	1	1	2	2
41229-A	35.3	40.7	6.1	5.9	2.0	2.8	106.4	39.1	18.1	41.8	54.4	51.4	63.6	63.2	136.4	128.5	71.9	33.6	940.4	459.4	0.6	0.55	4	4	4	4	1	1	1	1	2	2
41230-A	32.8	50.9	6.1	2.8	5.0	3.5	39.7	71.4	13.1	38.0	55.4	64.0	63.6	85.6	138.4	147.2	55.5	36.6	732.1	322.6	0.4	0.41	6	5	4	4	1	1	1	1	2	2
41231-A	43.6	53.5	3.1	5.1	0.3	3.9	24.4	35.0	8.4	34.0	54.4	61.0	62.6	76.6	124.4	127.2	87.5	36.6	987.1	376.3	0.6	0.54	4	4	4	4	1	1	1	1	2	2
41231-B	41.6	42.1	5.4	3.5	2.2	1.1	31.7	36.4	14.1	32.8	53.7	52.4	60.9	64.2	140.4	129.5	88.8	44.6	1440.0	756.9	0.7	0.67	4	4	2	3	1	1	1	1	2	2
41233-B	39.4	41.3	7.9	4.6	6.2	3.5	75.6	35.8	17.3	28.4	53.4	63.7	62.6	73.6	133.0	137.9	82.0	36.5	943.8	3.2	0.6	0.58	5	7	3	4	1	1	1	1	2	2
41234-B	39.5	52.5	1.3	5.2	0.3	5.3	17.1	45.9	3.8	29.7	54.4	54.0	59.9	77.6	135.4	129.5	94.9	36.9	1089.0	224.9	0.6	0.56	4	6	3	4	1	1	1	1	2	2
41234-C	37.0	49.5	3.3	4.2	1.6	2.3	33.1	22.6	7.1	21.7	52.4	54.0	57.9	77.6	137.4	129.5	103.3	36.9	1374.0	264.9	0.8	0.49	3	4	1	4	1	1	1	1	2	2
41236-A	30.7	31.4	5.1	3.5	5.5	2.9	10.2	22.4	-1.6	37.5	53.0	55.0	59.2	70.9	126.7	136.2	79.0	44.0	1077.0	537.7	0.6	0.58	4	5	3	4	1	1	1	1	2	2
41241-A	39.9	40.8	7.4	4.0	5.2	1.8	44.4	23.9	15.8	31.8	55.7	53.7	62.9	77.2	137.4	123.2	72.0	62.4	1016.0	451.5	0.7	0.14	5	9	3	4	1	1	1	1	2	2
41241-B	36.9	34.9	5.4	5.0	3.8	3.1	31.0	49.9	10.5	29.8	54.7	60.7	62.9	79.2	135.4	123.2	66.8	36.1	1040.0	856.1	0.7	0.43	4	5	3	4	1	1	1	1	2	2
41242-A	31.0	47.6	4.5	5.9	6.5	4.1	36.8	51.5	11.7	38.4	54.0	63.7	60.2	78.6	132.7	128.9	74.2	72.5	884.6	726.3	0.7	0.64	6	5	4	3	1	1	1	1	2	2
41245-A	43.2	40.3	3.5	2.1	1.1	0.5	25.5	22.7	2.4	34.5	52.7	51.0	63.2	74.9	132.7	136.2	93.5	64.0	1400.0	835.2	0.7	0.62	4	4	2	3	1	1	1	1	2	2
41245-B	48.8	42.7	3.5	3.8	6.1	1.9	48.2	22.4	9.7	37.5	52.7	52.0	63.2	72.9	136.7	136.2	101.1	51.0	1240.0	749.7	0.8	0.81	4	5	2	3	1	1	1	1	2	2
41247-A	48.8	41.7	6.5	4.8	7.7	2.5	80.2	22.0	6.1	32.5	53.7	52.0	63.2	72.9	140.7	144.2	88.3	69.0	1400.0	802.7	0.8	0.69	3	4	1	3	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
41247-B	43.5	49.5	6.1	4.8	4.2	2.5	53.0	63.4	16.2	35.0	52.7	60.0	60.6	73.6	129.4	136.2	91.8	28.6	1414.0	261.3	0.9	0.43	3	5	1	4	1	1	1	1	2	2
41250-A	55.0	35.8	8.2	9.7	5.4	6.1	109.5	108.6	77.3	73.5	52.4	54.0	69.9	75.6	128.0	129.2	97.4	44.0	678.4	626.4	0.7	0.73	4	4	4	3	1	1	1	1	2	2
41251-B	46.7	50.3	7.5	6.9	10	4.2	96.5	60.1	30.4	33.5	52.0	49.0	58.2	71.2	132.7	121.9	81.0	55.4	1501.0	612.3	0.7	0.55	4	4	2	3	1	1	1	1	2	2
41255-B	37.4	37.4	4.2	5.2	8.5	4.5	61.9	38.4	18.9	17.8	56.4	53.4	73.6	67.2	133.0	131.5	106.0	105.6	1109.0	1087.0	0.8	0.78	4	4	1	1	1	1	1	1	2	2
41256-A	41.8	42.7	8.2	3.1	9.9	4.2	83.6	65.7	18.6	16.5	57.4	53.0	63.6	73.9	130.0	144.2	94.0	96.0	1224.0	1240.0	0.8	0.78	4	4	3	2	1	1	2	2	2	2
41257-A	35.9	50.4	3.7	6.2	4.5	3.4	33.0	50.0	13.8	32.2	54.7	55.0	59.9	73.9	132.4	128.2	85.2	64.2	1425.0	910.9	0.9	0.79	3	4	1	3	1	1	1	1	2	2
41258-A	35.6	40.0	4.4	5.0	5.5	7.1	23.0	52.5	14.1	25.8	53.7	53.7	60.9	74.2	126.4	140.2	82.4	63.1	1230.0	219.0	0.6	0.57	4	4	2	4	1	1	1	1	2	2
41258-B	38.6	50.8	4.4	5.7	3.2	2.1	25.4	55.2	15.5	24.8	53.7	53.7	62.9	74.2	122.4	141.2	69.2	65.1	829.8	182.8	0.5	0.53	4	4	4	4	1	1	1	1	2	2
41261-B	38.7	52.5	5.5	4.2	3.7	1.0	62.8	24.6	13.3	36.7	49.4	52.0	57.9	74.6	126.0	129.5	80.2	62.9	1380.0	947.8	0.8	0.83	4	5	2	3	1	1	1	1	2	2
41263-A	38.2	46.7	6.8	6.6	2.5	3.4	48.0	58.3	13.2	44.2	49.7	63.0	56.6	81.9	122.4	128.2	86.6	55.2	1003.0	650.8	0.7	0.71	4	4	3	3	1	1	1	1	2	2
41263-B	37.6	54.7	8.4	9.6	6.8	6.8	41.4	77.0	11.5	52.2	53.7	53.0	60.9	73.9	130.4	128.2	89.6	44.2	1080.0	766.2	0.8	0.76	4	4	1	3	1	1	1	1	2	2
41264-B	35.6	36.2	3.4	7.0	2.3	5.5	36.7	89.5	11.1	12.8	52.4	49.0	60.6	68.2	136.4	124.2	83.1	63.9	1129.0	1029.0	0.8	0.82	4	4	3	3	1	1	2	2	2	2
41265-B	44.7	45.3	5.2	6.3	4.2	3.7	51.7	46.3	5.9	26.5	49.7	52.0	57.6	73.6	131.4	127.2	79.8	73.0	985.4	967.2	0.7	0.65	4	4	3	3	1	1	1	1	2	2
41265-C	43.0	39.5	2.2	4.7	0.9	2.1	29.7	34.6	4.9	42.5	49.7	51.0	58.6	97.6	128.4	126.2	79.0	66.8	1279.0	840.3	0.7	0.64	5	6	2	3	1	1	1	1	2	2
41266-A	40.2	41.7	2.9	3.8	2.4	3.9	19.5	34.0	3.4	39.5	51.7	53.0	60.2	64.9	136.7	133.2	95.1	58.0	1233.0	557.7	0.7	0.71	4	4	2	3	1	1	1	1	2	2
41266-C	36.5	35.2	3.2	4.7	0.7	2.1	13.8	40.5	5.4	16.8	52.7	53.7	63.2	75.2	133.7	123.2	86.3	45.1	956.6	107.8	0.6	0.45	5	5	4	5	1	1	1	1	2	2
41267-A	34.4	53.6	4.8	4.3	0.9	3.1	10.5	57.5	2.1	38.4	51.0	53.7	58.2	72.6	122.7	128.9	85.4	52.5	1006.0	562.0	0.9	0.83	5	5	3	3	1	1	1	1	2	2
41268-A	37.6	36.9	7.7	6.6	3.8	2.1	42.4	47.7	17.1	42.2	51.7	53.0	57.9	75.9	123.4	146.2	88.0	58.2	1188.0	820.5	0.7	0.58	4	5	2	4	1	1	1	1	2	2
41269-B	35.8	42.3	3.6	7.3	1.6	8.4	26.8	80.3	8.2	24.5	49.7	52.0	57.6	75.6	136.7	138.2	91.3	65.8	916.0	985.1	0.7	0.67	4	4	5	3	1	1	1	1	2	2
41270-A	37.1	34.9	7.1	2.3	3.5	1.9	29.8	37.2	2.1	44.8	51.0	53.0	57.2	69.2	122.7	123.2	79.8	46.9	1157.0	255.7	0.7	0.44	4	6	3	4	1	1	1	1	2	2
41270-B	39.1	49.4	5.5	2.0	5.5	2.5	69.5	38.2	4.1	44.8	52.0	51.0	58.2	64.2	134.7	121.2	81.4	37.9	1497.0	303.2	0.7	0.58	4	4	2	4	1	1	1	1	2	2
41278-A	43.5	41.6	5.3	5.6	3.0	2.5	56.5	58.8	16.5	39.4	51.4	53.7	57.9	81.6	131.4	130.9	95.3	43.5	1149.0	547.0	0.7	0.69	4	5	3	3	1	1	1	1	2	2
41279-A	29.8	47.7	3.8	3.8	1.2	3.2	9.2	48.0	1.7	40.5	52.0	55.0	58.2	70.9	126.7	133.2	59.0	57.0	712.3	506.5	0.8	0.75	7	7	4	3	1	1	1	1	2	2
41281-A	41.5	42.7	4.8	6.2	2.9	3.1	53.6	32.7	16.9	42.2	47.7	55.0	60.6	72.9	126.4	130.2	80.2	56.2	1086.0	696.1	0.7	0.65	5	5	3	3	1	1	1	1	2	2
41281-C	39.5	47.7	4.9	5.9	3.1	2.4	39.5	36.0	8.7	24.2	50.7	55.0	61.2	81.9	136.7	146.2	78.7	37.2	1073.0	454.0	0.7	0.43	5	6	3	4	1	1	1	1	2	2
41282-A	41.6	47.0	3.1	4.0	2.3	4.5	19.4	58.8	12.1	36.8	52.4	49.0	60.6	64.2	130.4	121.2	84.3	46.9	927.1	708.2	0.9	0.80	5	5	4	3	1	1	1	1	2	2
41282-B	35.6	50.4	5.1	4.3	3.3	2.9	37.7	71.2	14.1	39.8	53.4	49.0	60.6	62.2	138.4	124.2	77.1	61.9	905.4	363.2	0.4	0.15	6	9	4	4	1	1	1	1	2	2
41292-A	38.6	35.8	4.4	5.2	3.2	3.8	33.7	48.0	11.1	34.2	53.7	63.0	60.9	74.9	135.4	128.2	70.0	44.2	1186.0	637.7	0.7	0.47	4	7	3	5	1	1	1	1	2	2
41292-B	36.6	34.6	5.7	4.7	6.8	1.1	56.0	24.2	21.5	33.8	53.7	57.7	59.9	82.2	136.4	125.2	85.6	38.2	783.2	525.3	0.5	0.15	4	9	4	5	1	1	1	1	2	2
41294-B	32.7	35.2	5.7	5.1	9.0	8.9	38.4	44.4	15.1	38.0	52.4	52.0	61.6	61.6	136.4	135.2	50.7	47.6	682.1	626.3	0.2	0.15	8	8	4	4	1	1	1	1	2	2
41295-A	42.2	49.7	4.4	7.6	2.2	5.4	38.6	42.3	13.9	28.2	51.7	59.0	60.6	78.9	124.4	130.2	81.4	51.2	857.7	550.2	0.7	0.67	5	6	4	3	1	1	1	1	2	2
41295-B	35.0	38.9	3.5	4.0	4.2	4.4	30.7	40.9	3.2	-1.2	50.7	51.7	58.6	64.2	136.4	133.2	64.6	64.5	1200.0	1252.0	0.8	0.81	5	5	1	3	1	1	1	1	2	2
41301-A	40.3	42.2	5.7	5.8	8.0	0.3	137.0	51.6	30.4	36.7	52.4	53.0	63.6	75.6	136.4	127.5	88.3	40.9	1505.0	375.7	0.9	0.56	3	4	1	4	1	1	1	1	2	2
41301-B	36.9	39.1	7.7	6.8	11.	6.9	62.4	162.7	16.4	116.0	52.4	55.0	61.6	74.6	134.4	127.2	101.1	62.6	1152.0	933.8	0.8	0.79	4	3	1	3	1	1	1	1	2	2
41302-A	21.7	21.9	0.8	2.2	0.5	1.5	-5.5	17.8	-4.9	39.5	54.0	52.0	69.2	65.2	126.7	120.9	45.2	18.7	575.7	610.2	0.4	0.12	8	9	5	5	1	1	1	1	2	2
41306-A	33.2	51.2	2.8	8.1	0.9	3.5	23.6	39.7	8.6	31.0	49.7	53.0	56.6	72.6	156.4	136.2	78.2	49.6	766.0	584.5	0.6	0.63	5	6	4	3	1	1	1	1	2	2
41307-D	39.5	40.6	3.7	5.3	1.0	3.8	26.8	80.5	5.5	38.4	54.4	52.7	57.9	73.6	139.4	128.9	97.7	49.5	1024.0	444.5	0.8	0.68	4	6	1	3	1	1	1	1	2	2
41307-E	41.2	48.5	3.1	4.8	1.9	5.2	33.3	69.0	9.2	40.0	49.7	55.0	57.6	74.6	125.4	129.2	86.2	49.6	1389.0	520.6	0.7	0.68	4	4	2	3	1	1	1	1	2	2
41308-A	43.2	39.8	5.1	6.2	4.9	3.4	45.3	47.3	15.2	28.2	52.7	55.0	60.6	72.9	129.4	130.2	55.3	53.3	1161.0	661.5	0.8	0.41	4	5	1	4	1	1	1	1	2	2
41309-B	32.4	33.7	4.5	5.5	0.4	2.2	24.2	38.0	7.3	40.5	53.4	51.0	58.9	61.9	131.0	144.2	44.4	45.0	1014.0	130.2	0.5	0.14	8	9	4	5	1	1	1	1	2	2
41310-A	41.1	53.6	6.9	9.9	6.5	6.8	49.9	65.8	19.9	55.4	56.4	54.7	62.6	73.6	132.0	137.9	79.2	67.2	1010.0	779.8	0.7	0.69	4	6	3	3	1	1	1	1	2	2
41311-A	36.8	38.8	4.2	4.0	2.1	1.4	21.8	38.9	6.1	15.8	51.7	51.7	61.2	77.2	133.7	123.2	78.7	43.1	904.9	639.4	0.7	0.63	4	4	4	3	1	1	1	1	2	2
41311-B	39.8	44.7	4.9	3.8	1.7	1.9	23.5	45.0	6.4	33.5	51.7	52.0	61.2	81.9	139.7	136.2	95.5	53.0	998.3	671.5	0.7	0.71	5	5	3	3	1	1	1	1	2	2
41320-A	36.8	44.8	6.2	5.0	2.4	4.1	23.8	47.2	5.4	16.8	51.7	51.7	63.2	75.2	134.7	123.2	96.3	65.3	1457.0	824.4	0.6	0.62	4	4	2	3	1	1	1	1	2	2
41320-B	46.2	41.8	3.5	5.0	2.7	4.1	24.8	58.5	5.1	38.8	54.7	53.7	63.2	75.2	138.7	123.2	84.3	56.1	1167.0	538.6	0.6	0.65	4	5	3	3	1	1	1	1	2	2
41322-A	38.7	41.3	6.8	3.1	4.2	2.2	18.5	26.7	-1.9	37.5	57.0	55.0	60.2	72.9	126.7	136.2	78.6	54.0	1359.0	580.2	0.8	0.81	4	5	2	3	1	1	1	1	2	2
41323-A	38.0	36.6	4.2	6.2	4.2	4.9	33.3	68.8	2.9	44.5	52.7	50.0	60.6	67.2	128.4	120.9	77.8	41.4	873.8	237.3	0.6	0.42	5	5	4	5	1	1	1	1	2	2
41323-B	37.2	47.6	4.2	6.9	1.0	4.2	26.1	41.8	5.2	27.5	53.7	57.0	62.6	71.2	136.7	130.9	88.1	42.4	777.7	361.0	0.5	0.51	6	6	4	4	1	1	1	1	2	2

Appendix 2: Continued....

Genotypes	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	PLH		**NPB**		**NSB**		**FPPP**		**IPPP**		**DTF**		**DTP**		**DTM**		**TSW**		**GY**		**SR**		**FTR**		**SSS**		**FC**		**SLP**		SC	
41324-B	35.7	36.9	4.5	8.2	2.9	4.2	13.5	101.8	0.4	13.5	54.0	52.0	60.2	66.2	129.7	120.9	88.6	89.4	1614.0	1314.0	0.9	0.71	3	3	1	2	1	1	3	3	2	2
41324-C	41.1	37.2	7.2	5.7	9.9	6.9	78.9	115.8	14.3	9.8	55.4	52.0	64.6	61.2	133.0	115.2	107.0	108.9	1590.0	1341.0	0.9	0.74	3	4	1	1	1	1	3	2	2	2
9646-A	44.6	47.1	4.4	5.2	3.2	3.1	47.0	44.0	11.8	33.2	53.7	55.0	61.9	80.9	139.4	130.2	84.0	60.4	1235.0	822.4	0.8	0.81	4	6	2	3	1	1	1	1	2	2
9646-C	39.9	34.9	7.7	9.6	8.2	6.8	61.0	78.3	19.8	52.2	55.7	55.0	62.9	75.9	141.4	130.2	78.8	53.2	904.8	523.0	0.6	0.38	5	6	4	4	1	1	1	1	2	2
Acos	43.0	40.5	1.5	1.5	-0.3	-1.0	5.5	-7.1	3.0	-3.6	52.4	54.0	57.9	62.6	126.0	127.5	87.8	91.3	1537.0	1354.0	0.8	0.81	5	5	4	3	1	1	1	1	2	2
Akaki	46.8	43.3	4.6	5.5	3.6	5.2	19.1	45.4	7.9	45.5	57.7	57.0	64.6	74.9	136.7	154.2	181.3	180.0	1329.0	842.7	0.8	0.78	3	3	1	3	1	1	1	1	2	2
akuri	44.3	43.8	3.8	3.8	4.7	4.0	44.8	32.2	38.3	11.7	61.4	63.0	69.9	74.6	128.0	129.5	69.4	72.9	819.6	2036.0	0.8	0.77	4	4	4	2	0	0	0	0	3	3
Arerti	33.5	35.8	4.3	5.5	2.3	4.0	22.5	34.9	12.8	11.7	68.4	76.0	73.9	84.6	130.4	145.5	80.7	81.9	995.6	1985.0	0.7	0.61	6	5	5	3	0	0	0	0	3	3
BBARCR	31.2	34.6	4.9	5.3	3.4	3.8	40.7	42.0	23.7	37.8	54.8	53.5	63.1	66.3	135.6	138.7	37.8	32.0	368.5	311.5	0.2	0.15	8	9	4	4	1	1	1	1	2	2
Chefe	43.5	38.6	5.0	6.0	3.0	3.1	23.5	21.3	10.5	7.2	53.4	54.0	59.9	66.6	130.4	132.2	134.5	131.8	1016.0	1037.0	0.9	0.81	3	3	4	1	0	0	0	0	3	3
Dalota	47.0	51.2	3.8	6.0	2.4	4.4	41.2	37.9	11.6	8.8	50.4	61.7	59.9	86.2	137.0	145.2	206.2	183.1	1492.0	1163.0	0.9	0.78	3	4	1	2	1	1	1	1	2	2
Dhara	49.0	39.2	4.8	4.8	4.4	3.7	23.8	11.2	10.0	3.4	52.4	54.0	57.9	62.6	140.0	141.5	217.8	221.3	830.4	1747.0	0.8	0.85	3	3	4	3	0	0	0	0	3	3
Dimta	47.0	56.6	4.2	5.7	0.7	5.4	25.2	63.3	9.3	14.5	50.4	53.0	58.9	75.6	134.0	129.2	220.6	167.0	2274.0	1419.0	0.8	0.71	3	3	1	1	1	1	3	3	2	2
dubie	42.7	39.7	5.2	4.5	2.0	1.5	34.5	17.7	23.3	37.5	52.4	55.0	71.9	70.9	137.0	144.2	146.4	147.0	1536.0	919.0	0.9	0.71	3	4	1	3	1	1	1	1	2	2
DZ-10-11	46.0	53.3	4.8	6.0	1.4	5.7	44.8	95.6	8.0	64.5	51.4	51.0	57.9	76.6	134.0	127.2	113.4	113.0	1545.0	932.6	0.9	0.78	3	4	1	3	1	1	1	1	2	2
DZ-10-4	43.6	50.5	4.7	5.2	1.3	2.3	43.4	38.9	14.7	10.7	55.4	63.0	63.6	81.6	131.4	129.5	77.7	79.9	1500.0	2036.0	0.8	0.81	2	2	3	2	0	0	0	0	3	3
Ejere	47.0	38.9	3.8	4.1	2.7	3.2	37.2	41.4	10.3	9.0	51.4	54.0	57.9	62.6	131.0	130.2	103.4	101.6	1699.0	1623.0	0.8	0.78	4	4	4	2	0	0	0	0	3	3
EnewariR	31.7	36.2	4.7	4.9	3.2	3.0	38.0	44.2	13.2	34.9	50.8	53.6	58.2	71.6	133.8	143.8	40.0	37.1	457.1	191.5	0.2	0.14	9	9	5	4	1	1	1	1	2	2
fetenech	49.7	50.0	3.2	4.7	2.0	2.7	38.5	37.6	27.3	13.5	52.4	54.0	72.9	78.6	137.0	138.2	101.4	101.0	1106.0	1054.0	0.8	0.75	4	4	3	3	1	1	2	2	2	2
Harbu	44.5	45.5	3.1	6.1	1.2	2.9	25.6	41.7	11.6	33.0	51.7	58.0	62.6	77.6	125.4	129.2	300.2	296.9	952.7	990.1	0.8	0.81	3	3	2	2	0	0	0	0	3	3
Hora	44.0	42.5	4.2	6.2	5.7	5.0	18.2	9.9	3.6	40.7	52.4	76.0	58.9	91.6	137.0	150.5	139.4	142.9	927.1	843.6	0.8	0.71	2	2	4	3	0	0	0	0	3	3
kobo	49.0	36.0	4.8	4.1	6.7	6.2	26.5	9.7	20.3	24.5	55.4	58.0	71.9	70.9	129.0	136.2	68.4	69.0	954.6	1838.0	0.8	0.75	4	4	4	2	0	0	0	0	3	3
kutaye	41.3	38.3	3.5	2.8	2.4	1.9	60.5	43.7	11.3	15.5	50.4	53.0	62.9	61.9	138.0	145.2	137.4	138.0	1437.0	1320.0	0.9	0.78	3	4	1	1	1	1	2	2	2	2
mariye	41.0	40.5	5.8	5.8	3.7	3.0	73.5	60.9	43.3	36.7	52.4	54.0	75.9	80.6	128.0	129.5	188.4	191.9	1393.0	976.8	0.9	0.76	3	3	1	3	1	1	1	1	2	2
Mastewal	48.0	48.5	6.2	5.3	1.4	4.8	18.8	53.5	6.3	8.8	58.4	75.7	63.9	90.2	138.0	147.2	195.4	196.1	2457.0	1488.0	0.8	0.76	3	3	1	1	1	1	3	3	2	2
Minjar	42.0	47.3	4.5	4.5	0.0	3.5	16.5	46.7	5.6	44.5	52.4	55.0	60.9	74.9	131.0	154.2	135.4	134.0	1397.0	694.7	0.8	0.73	3	4	1	3	1	1	1	1	2	2
Natoli	50.9	44.5	4.4	3.8	5.0	4.7	64.0	27.6	5.1	29.7	63.4	74.0	74.6	89.6	142.4	129.5	207.5	192.1	1412.0	869.9	0.9	0.75	3	3	1	3	1	1	1	1	2	2
Shahso	29.8	34.8	5.1	3.0	2.9	3.5	26.2	69.5	9.7	42.8	61.0	51.0	66.2	64.2	136.7	121.2	81.0	73.9	595.7	465.7	0.2	0.20	6	6	4	4	0	0	0	0	3	3
Shola	43.5	40.3	2.3	2.3	0.3	0.1	21.5	25.9	6.1	-4.9	51.4	51.7	57.9	64.2	126.4	124.2	248.1	246.5	1783.0	2282.0	0.9	0.84	2	2	2	1	0	0	0	0	3	3
tegulet-R	31.8	34.6	5.0	5.4	3.7	3.5	37.1	35.3	17.9	37.7	53.5	55.0	61.4	65.8	136.7	141.1	38.5	32.8	197.3	156.8	0.2	0.12	9	8	5	5	1	1	1	1	2	2
Teji	47.5	46.3	2.5	6.2	3.4	6.9	7.2	58.5	6.7	16.5	53.7	55.0	60.2	75.2	132.7	118.9	148.7	109.4	905.4	1967.0	0.9	0.85	3	3	2	1	0	0	0	0	3	3
Teketay	47.0	37.3	4.5	6.0	2.9	3.4	20.0	23.6	0.2	33.5	52.7	54.0	59.6	65.6	131.4	132.2	124.6	123.4	695.4	768.4	0.8	0.75	4	3	2	3	1	1	1	1	2	2
worku	53.0	50.0	6.8	6.1	6.7	6.2	85.2	68.4	26.3	20.5	52.4	55.0	76.9	75.9	137.0	144.2	151.4	152.0	1093.0	976.5	0.8	0.75	4	3	1	2	1	1	2	2	2	2
yelebe	44.7	44.2	4.2	4.2	4.0	3.3	45.5	32.9	36.3	9.7	60.4	62.0	79.9	84.6	126.0	127.5	157.4	160.9	1590.0	1806.0	0.8	0.81	2	2	2	1	0	0	0	0	3	3
Mean	38.4	41.4	4.9	5.5	3.7	3.9	40.3	53.8	10.9	31.8	52.7	54.7	61.2	72.2	133.0	130.7	87.1	61.4	1119.0	720.0	-	-	-	-	-	-	-	-	-	-	-	-
LSD	1.8	3.3	1.3	2.4	1.5	3.3	19.0	27.9	9.6	10.6	4.5	4.6	5.8	7.9	7.0	14.5	5.4	8.4	195.3	201.7	-	-	-	-	-	-	-	-	-	-	-	-
CV	3.4	5.5	16	27	26	57	28.7	40.2	30.6	16.7	5.0	5.0	5.5	6.7	3.0	6.0	8.1	14.5	33.4	53.5	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 3. List of 60 chickpea genotypes (51 from ICARDA, 8 EBI collections and one improved variety) killed by frost stress during pre-flowering and reproductive stages grown at Debre Birhan Ethiopia for 2018 to 2020 rowing seasons

No	**Genotype**	**SR**	**FTR**	**SSS**	**FC**	**SLP**	**SC**	**Kiling stage**
1	41285-B	0.00	9	5	1	1	2	Pre-flowering
2	207175-C	0.00	9	5	1	1	2	Reproductive
3	207893-B	0.00	9	5	1	1	2	Reproductive
4	209022-B	0.00	9	5	1	1	2	Reproductive
5	225882-A	0.00	9	5	1	1	2	Reproductive
6	227158-C	0.00	9	5	1	1	2	Reproductive
7	228301-B	0.00	9	5	1	1	2	Reproductive
8	207726	0.00	9	5	1	1	2	Reproductive
9	141720	0.00	9	5	1	1	2	Reproductive
10	69690	0.00	9	5	0	0	3	Pre-flowering
11	8191	0.00	9	5	1	1	2	Reproductive
12	8522	0.00	9	5	0	0	3	Pre-flowering
13	9003	0.00	9	5	0	0	3	Pre-flowering
14	8934	0.00	9	5	0	0	3	Reproductive
15	70551	0.00	9	5	0	0	3	Pre-flowering
16	70503	0.00	9	5	0	0	3	Pre-flowering
17	69417	0.00	9	5	0	0	3	Reproductive
18	6416	0.00	9	5	0	0	3	Pre-flowering
19	72051	0.00	9	5	0	0	3	Pre-flowering
20	5909	0.00	9	5	0	0	3	Pre-flowering
21	9402	0.00	9	5	0	0	3	Reproductive
22	9412	0.00	9	5	0	0	3	Pre-flowering
23	74000	0.00	9	5	0	0	3	Reproductive
24	71861	0.00	9	5	0	0	3	Pre-flowering
25	72130	0.00	9	5	0	0	3	Pre-flowering
26	9049	0.00	9	5	0	0	3	Pre-flowering
27	72151	0.00	9	5	1	1	2	Reproductive
28	8357	0.00	9	5	0	0	3	Pre-flowering
29	139930	0.00	9	5	0	0	3	Reproductive
30	72034	0.00	9	5	0	0	3	Pre-flowering
31	71897	0.00	9	5	0	0	3	Pre-flowering
32	73241	0.00	9	5	0	0	3	Pre-flowering
33	128407	0.00	9	5	0	0	3	Pre-flowering
34	9628	0.00	9	5	0	0	3	Pre-flowering
35	72121	0.00	9	5	0	0	3	Pre-flowering
36	6454	0.00	9	5	0	0	3	Pre-flowering
37	71873	0.00	9	5	0	0	3	Pre-flowering
38	125187	0.00	9	5	1	1	2	Reproductive
39	75335	0.00	9	5	0	0	3	Pre-flowering
40	140119	0.00	9	5	0	0	3	Pre-flowering
41	8935	0.00	9	5	0	0	3	Pre-flowering
42	74920	0.00	9	5	0	0	3	Pre-flowering
43	72125	0.00	9	5	0	0	3	Pre-flowering
44	73397	0.00	9	5	0	0	3	Pre-flowering
45	72124	0.00	9	5	0	0	3	Pre-flowering
46	8349	0.00	9	5	0	0	3	Pre-flowering
47	73221	0.00	9	5	1	1	2	Reproductive
48	72038	0.00	9	5	0	0	3	Pre-flowering
49	69604	0.00	9	5	0	0	3	Pre-flowering
50	132880	0.00	9	5	0	0	3	Pre-flowering
51	69733	0.00	9	5	0	0	3	Pre-flowering
52	72016	0.00	9	5	0	0	3	Pre-flowering
53	72036	0.00	9	5	0	0	3	Pre-flowering
54	5903	0.00	9	5	0	0	3	Pre-flowering
55	9058	0.00	9	5	0	0	3	Pre-flowering
56	71853	0.00	9	5	0	0	3	Pre-flowering
57	Kasech	0.00	9	5	0	0	3	Pre-flowering
58	9427	0.00	9	5	0	0	3	Pre-flowering
59	75095	0.00	9	5	0	0	3	Pre-flowering
60	10163	0.00	9	5	0	0	3	Pre-flowering

Appendix 4. Ethiopian chickpea seed coat color diversity and assignment of homogeneous genotypes developed from by splitting the individual accession of current (2017) and previous (1979 to 1983) collections based on seed color, texture and size

No	ACC	Black (1)	Brown (2)	Light brown (3)	Dark brown (4)	Ivory white (5)	Homogeneous Group	New Accession code	Zone	District
1	30287	0	1	0	1	0	2	30287-A, 30287-B	East Gojjam	Dejen
2	30288	0	0	1	0	0	1		East Gojjam	Dejen
3	30289	0	0	0	1	0	1		East Gojjam	Dejen
4	30293	0	1	1	0	0	2	30293-A, 30293-B	East Gojjam	Dejen
Sub Total	**Current**	0	2	2	2	0	6			Dejen
5	41222	0	0	1	1	0	2	41222-A, 41222-B	East Gojjam	Dejen
6	41223	1	0	1	0	0	2	41223-A, 41223-B	East Gojjam	Dejen
7	41322	1	1	1	0	0	3	41322-A,41322-B,41322-C	East Gojjam	Dejen
8	41324	1	1	1	1	1	5		East Gojjam	Dejen
Sub Total	**Previous**	3	2	4	2	1	12			Dejen
9	30301	0	1	0	1	0	2	30301-A, 30301-B	East Gojjam	Enarji Enawiga
10	30302	0	1	0	0	0	1		East Gojjam	Enarji Enawiga
11	30304	0	0	1	0	0	1		East Gojjam	Enarji Enawiga
Sub Total	**Current**	0	2	1	1	0	4			
12	41229	1	1	1	0	0	3	41229-A, 41229-B, 41229-C	East Gojjam	Enarji Enawiga
13	41231	1	1	1	0	0	3	41231-A, 41231-B, 41231-C	East Gojjam	Enarji Enawiga
14	236493	1	0	1	1	0	3	236493-A, 236493-B, 236493-C	East Gojjam	Enarji Enawiga
Sub Total	**Previous**	3	2	3	1	0	9			
15	30308	0	1	0	0	0	1		East Gojjam	Hulet ej enese
16	30309	0	0	1	0	0	1		East Gojjam	Hulet ej enese
17	30310	0	1	0	0	0	1		East Gojjam	Hulet ej enese
18	30311	0	1	0	1	0	2	30311-A, 30311-B	East Gojjam	Hulet ej enese
19	30312	0	1	1	0	0	2	30312-A, 30312-B	East Gojjam	Hulet ej enese
Sub Total	**Current**	0	4	2	1	0	7			Hulet ej enese
20	41026	1	1	1	1	1	5	41026-A, 41026-B, 41026-C, 41026-D, 41026-E	East Gojjam	Hulet ej enese
21	41078	1	1	1	1	0	4	41078-A, 41078-B, 41078-C, 41078-D	East Gojjam	Hulet ej enese
22	41267	1	1	1	1	1	5	41267-A, 41267-B, 41267-C, 41267-D, 41267-E	East Gojjam	Hulet ej enese
23	41268	1	1	1	1	0	4	41268-A, 41268-B, 41268-C, 41268-D	East Gojjam	Hulet ej enese
24	41269	1	1	1	0	0	3	41269-A, 41269-B, 41269-C	East Gojjam	Hulet ej enese
Sub Total	**Previous**	5	5	5	4	2	21			Hulet ej enese
25	30322	0	0	1	0	0	1		North Gonder	Belesa
26	30317	0	0	1	0	0	1		North Gonder	Belesa
27	30318	0	1	0	1	0	2	30318-A, 30318-B	North Gonder	Belesa
28	30319	0	0	1	0	0	1		North Gonder	Belesa
29	30320	0	1	0	0	0	1		North Gonder	Belesa
30	30321	0	0	0	1	0	1		North Gonder	Belesa
Sub Total	**Current**	0	2	3	2	0	7		North Gonder	Belesa
31	207166	1	1	1	1	0	4	207166-A, 207166-B, 207166-C, 207166-D	North Gonder	Belesa
32	207170	1	1	1	1	1	5	207170-A, 207170-B, 207170-C, 207170-D, 207170-E	North Gonder	Belesa
33	241800	1	1	0	1	0	3	241800-A, 241800-B, 241800-C	North Gonder	Belesa
34	241801	1	1	1	0	0	3	241801-A, 241801-B, 241801-C	North Gonder	Belesa
Sub Total	**Previous**	4	4	3	3	1	15		North Gonder	Belesa
35	30327	0	0	1	0	0	1		North Gonder	Takusa
36	30329	0	1	0	0	0	1		North Gonder	Takusa
Sub Total	**Current**	0	1	1	0	0	2		North Gonder	Takusa
37	227160	1	1	1	0	0	3	227160-A, 227160-B, 227160-C	North Gonder	Takusa
38	227161	1	0	1	1	0	3	227161-A, 227161-B, 227161-C	North Gonder	Takusa
Sub Total	**Previous**	2	1	2	1	0	6		North Gonder	Takusa
39	30331	1	1	0	0	0	2	30331-A, 30331-B	North Gonder	Chilga
41	30332	0	1	1	0	0	2	30332-A, 30332-B	North Gonder	Chilga
Sub Total	**Current**	1	2	1	0	0	4		North Gonder	Chilga
43	41046	1	1	0	0	0	2	41046-A, 41046-B	North Gonder	Chilga
45	207143	1	1	1	0	0	3	207143-A, 207143-B, 207143-C	North Gonder	Chilga
Sub Total	**Previous**	2	2	1	0	0	5		North Gonder	Chilga
47	30323	1	0	0	1	0	2	30323-A, 30323-B	North Gonder	Dembia
48	30324	0	0	1	1	0	2	30324-A, 30324-B	North Gonder	Dembia
49	30325	0	1	0	0	0	1		North Gonder	Dembia
50	30326	0	0	0	1	0	1		North Gonder	Dembia
Sub Total	**Current**	1	1	1	3	0	6		North Gonder	Dembia
51	41306	1	1	1	1	1	5	41306-A, 41306-B, 41306-C, 41306-D, 41306-E	North Gonder	Dembia

Appendix 4: Continued....

No	ACC	Black (1)	Brown (2)	Light brown (3)	Dark brown (4)	Ivory white (5)	Homogeneous Group	New Accession code	Zone	District
52	41310	1	1	0	1	0	3	41310-A, 41310-B, 41310-C	North Gonder	Dembia
53	41311	1	1	1	1	1	5	41311-A, 41311-B, 41311-C, 41311-D, 41311-E	North Gonder	Dembia
54	225887	1	1	1	1	0	4	225887-A, 225887-B, 225887-C, 225887-D	North Gonder	Dembia
55	227158	0	1	1	0	0	2	227158-A, 227158-B	North Gonder	Dembia
Sub Total	**Previous**	4	5	4	4	2	19		North Gonder	Dembia
56	30339	0	1	1	1	0	2	30339-A, 30339-B	South Gonder	Fogera
57	30340	0	1	0	0	0	1		South Gonder	Fogera
58	30341	0	0	1	0	0	2	30341-A, 30341-B	South Gonder	Fogera
Sub Total	**Current**	0	2	2	1	0	5		South Gonder	Fogera
59	41295	1	1	1	0	0	3	41295-A, 41295-B, 41295-C	South Gonder	Fogera
60	207145	**0**	**1**	**1**	**1**	**0**	**3**	207145-A, 207145-B, 207145-C	South Gonder	Fogera
Sub Total		1	2	2	1	0	6		South Gonder	Fogera
Grand Total		26	39	37	26	6	**134**			

Appendix 5. Mean value significant test of quantitative traits between current (2017) and previous collections (1979 to 1983) of eight districts of Amhara Regional State using two tailed t-test

Location	**Traits**	**Current Collection**				**Previous Collection**				**P-Value**	**DF**
		N	**Mean**	**StDev**	**SE Mean**	**N**	**Mean**	**StDev**	**SE Mean**		
East Gojjam-Dejen	DTF	16.0	43.0	1.8	0.5	32.0	45.2	4.5	0.8	0.0	44.0
	DTM	16.0	100.9	1.7	0.4	32.0	98.1	4.7	0.8	0.0	43.0
	DTP	16.0	55.6	1.4	0.4	32.0	55.9	5.2	0.9	0.8	39.0
	GY	16.0	2488.0	617.0	154.0	32.0	1835.0	636.0	112.0	0.0	30.0
	NPB	16.0	6.8	0.6	0.2	32.0	5.6	1.7	0.3	0.0	43.0
	NPPP	16.0	67.2	5.8	1.4	32.0	45.2	10.8	1.9	0.0	45.0
	NSB	16.0	10.0	0.4	0.1	32.0	7.5	2.3	0.4	0.0	35.0
	PLH	16.0	35.4	1.0	0.2	32.0	33.7	4.6	0.8	0.1	36.0
	TSW	16.0	165.4	2.4	0.6	32.0	150.7	15.8	2.8	0.0	33.0
East Gojjam-Enarji Enawiga	**DTF**	**16.0**	**44.9**	**1.8**	**0.5**	**16.0**	**45.6**	**4.1**	**1.0**	**0.6**	**20.0**
	DTM	16.0	98.8	1.9	0.5	16.0	99.9	4.5	1.1	0.4	20.0
	DTP	16.0	54.5	3.1	0.8	16.0	57.6	5.0	1.2	0.0	24.0
	GY	**16.0**	**1915.0**	**625.0**	**156.0**	**16.0**	**2000.0**	**828.0**	**207.0**	**0.7**	**27.0**
	NPB	16.0	6.6	0.6	0.2	16.0	5.8	2.0	0.5	0.2	17.0
	NPPP	16.0	51.4	3.4	0.8	16.0	47.3	12.2	3.0	0.2	17.0
	NSB	**16.0**	**7.6**	**0.9**	**0.2**	**16.0**	**8.0**	**2.5**	**0.6**	**0.6**	**19.0**
	PLH	16.0	33.5	1.3	0.3	16.0	35.9	5.9	1.5	0.1	16.0
	TSW	**16.0**	**148.8**	**7.3**	**1.8**	**16.0**	**149.5**	**11.3**	**2.8**	**0.8**	**25.0**
East Gojjam-Hulet Ej Enses	DTF	20.0	42.4	2.3	0.5	36.0	45.7	5.6	0.9	0.0	50.0
	DTM	**20.0**	**97.2**	**1.8**	**0.4**	**36.0**	**96.5**	**6.8**	**1.1**	**0.6**	**42.0**
	DTP	20.0	53.3	3.0	0.7	36.0	57.4	6.2	1.0	0.0	53.0
	GY	20.0	2464.0	762.0	170.0	36.0	1523.0	560.0	93.0	0.0	30.0
	NPB	20.0	6.8	0.6	0.1	36.0	5.5	2.0	0.3	0.0	44.0
	NPPP	20.0	62.2	6.2	1.4	36.0	46.8	16.6	2.8	0.0	48.0
	NSB	20.0	9.0	0.7	0.2	36.0	6.8	3.0	0.5	0.0	42.0
	PLH	**20.0**	**35.1**	**1.1**	**0.2**	**36.0**	**34.5**	**6.2**	**1.0**	**0.6**	**38.0**
	TSW	20.0	153.5	7.1	1.6	36.0	146.1	17.6	2.9	0.0	50.0
North Gonder -Belesa	DTF	24.0	44.0	2.3	0.5	24.0	47.1	5.4	1.1	0.0	30.0
	DTM	24.0	99.4	2.5	0.5	24.0	97.4	6.7	1.4	0.2	29.0
	DTP	24.0	56.2	2.1	0.4	24.0	59.3	5.4	1.1	0.0	29.0
	GY	24.0	2658.0	676.0	138.0	24.0	1857.0	635.0	130.0	0.0	45.0
	NPB	24.0	8.4	1.5	0.3	24.0	6.0	1.5	0.3	0.0	45.0
	NPPP	24.0	71.9	7.7	1.6	24.0	48.4	11.4	2.3	0.0	40.0
	NSB	24.0	10.3	0.9	0.2	24.0	8.1	2.3	0.5	0.0	3.0
	PLH	24.0	35.7	1.3	0.3	24.0	35.1	4.7	1.0	0.5	26.0
	TSW	24.0	163.0	4.6	0.9	24.0	153.9	16.1	3.3	0.1	26.0
North Gonder -Takusa	DTF	8.0	43.3	1.7	0.6	12.0	46.4	4.3	1.2	0.0	15.0
	DTM	8.0	103.2	2.6	0.9	12.0	92.8	7.6	2.2	0.0	14.0
	DTP	8.0	54.8	2.3	0.8	12.0	59.3	3.8	1.1	0.0	17.0
	GY	8.0	2656.0	685.0	242.0	12.0	1421.0	416.0	120.0	0.0	10.0
	NPB	8.0	9.6	1.2	0.4	12.0	4.6	2.2	0.6	0.0	17.0
	NPPP	8.0	67.3	3.6	1.3	12.0	42.0	18.5	5.3	0.0	12.0
	NSB	8.0	10.0	0.8	0.3	12.0	5.6	3.3	1.0	0.0	12.0
	PLH	8.0	38.8	0.9	0.3	12.0	33.8	6.5	1.9	0.0	11.0
	TSW	8.0	164.5	2.5	0.9	12.0	144.9	15.5	4.5	0.0	11.0
North Gonder -Chiliga	DTF	12.0	42.0	2.0	0.6	16.0	45.6	4.2	1.1	0.0	22.0
	DTM	12.0	96.8	4.0	1.2	16.0	94.4	6.8	1.7	0.2	24.0
	GY	12.0	2006.0	113.0	32.0	16.0	1883.0	623.0	156.0	0.4	16.0
	NPB	12.0	6.8	0.9	0.3	16.0	6.0	1.9	0.5	0.1	22.0
	NPPP	12.0	59.2	5.4	1.6	16.0	49.5	12.2	3.0	0.0	21.0
	NSB	12.0	8.3	1.0	0.3	16.0	6.9	2.8	0.7	0.1	20.0
	PLH	**12.0**	**35.4**	**1.0**	**0.3**	**16.0**	**35.7**	**2.9**	**0.7**	**0.7**	**19.0**
	TSW	12.0	157.6	10.2	2.9	16.0	148.3	13.6	3.4	0.1	25.0
North Gonder -Denbia	**DTF**	**20.0**	**43.4**	**1.5**	**0.3**	**36.0**	**43.5**	**3.2**	**0.5**	**0.8**	**53.0**
	DTM	20.0	98.2	1.7	0.4	36.0	92.3	5.2	0.9	0.0	46.0
	DTP	20.0	53.8	2.1	0.5	36.0	54.9	4.7	0.8	0.2	52.0
	GY	20.0	2380.0	753.0	168.0	36.0	1287.0	393.0	65.0	0.0	24.0
	NPB	20.0	6.9	0.8	0.2	36.0	4.4	1.5	0.3	0.0	53.0
	NPPP	20.0	50.4	5.7	1.3	36.0	38.5	11.1	1.8	0.0	53.0
	NSB	20.0	9.1	0.9	0.2	36.0	5.7	1.9	0.3	0.0	53.0
	PLH	20.0	36.4	1.6	0.4	36.0	30.6	2.8	0.5	0.0	53.0
	TSW	20.0	165.2	2.0	0.5	36.0	148.4	16.2	2.7	0.0	36.0
South Gonder - Fogera	DTF	12.0	46.8	2.5	0.7	16.0	42.8	2.7	0.7	0.0	24.0
	DTM	12.0	99.4	2.1	0.6	16.0	91.9	7.0	1.8	0.0	18.0
	DTP	12.0	57.2	2.1	0.6	16.0	54.6	2.5	0.6	0.0	25.0

Appendix 5: Continued....

Location	Traits	Current Collection				Previous Collection				Current Collection	Previous Collection
		N	Mean	N	Mean	N	Mean	N	Mean		
South Gonder - Fogera	GY	12.0	2465.0	775.0	224.0	16.0	1306.0	590.0	147.0	0.0	19.0
	NPB	12.0	9.8	1.0	0.3	16.0	4.8	1.5	0.4	0.0	25.0
	NPPP	12.0	76.4	8.9	2.6	16.0	34.5	10.1	2.5	0.0	25.0
	NSB	12.0	10.7	1.1	0.3	16.0	5.3	2.4	0.6	0.0	21.0
	PLH	12.0	37.3	0.8	0.2	16.0	29.7	4.1	1.0	0.0	16.0
	TSW	12.0	168.0	3.7	1.1	16.0	139.2	15.4	3.9	0.0	17.0

PLH=Plant Canopy Height (cm), DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, NPB=Number of primary branches, NSB=Number secondary branches, NPPP=Number of pods per plant, TSW=Thousand Seed weight, GY in kg ha^{-1}=Grain yield

Appendix 6. Mean value of quantitative traits of 79 chickpea genotypes and two improved chickpea grown at Debre Zeit Ethiopia, in 2018-2020 cropping seasons

No	Genotype	PLH	NPB	NSB	NPPP	DTF	DTP	DTM	TSW	GY	Zone	District
1*	30287-A	35.49	6.96	9.85	65.47	43.12	55.82	100.27	164.75	2435.13	E/Gojjam	Dejen
2*	30288-A	35.45	6.71	9.92	63.54	44.11	55.96	101.09	162.99	2611.14	East Gojjam	Dejen
3*	30289-A	34.68	6.67	9.50	65.08	43.12	55.38	100.24	163.64	2666.86	East Gojjam	Dejen
4*	30293-A	35.39	6.53	9.63	69.84	44.11	56.25	100.06	164.67	1983.98	East Gojjam	Dejen
5	41222-A	33.07	4.80	6.34	37.88	42.63	54.23	94.88	159.34	1895.70	East Gojjam	Dejen
6	41222-B	38.48	7.19	9.10	54.81	47.62	55.10	101.95	160.84	2227.68	East Gojjam	Dejen
7	41223-A	38.31	7.73	9.70	59.19	48.88	60.30	99.03	159.40	2462.92	East Gojjam	Dejen
8	41223-B	34.91	5.65	7.93	41.88	44.27	55.38	95.95	154.66	1718.97	East Gojjam	Dejen
9	41322-A	34.58	5.20	8.22	37.79	45.58	56.97	103.48	155.47	2061.45	East Gojjam	Dejen
10	41322-B	30.20	5.56	5.75	44.56	44.15	55.96	94.39	141.81	1324.63	East Gojjam	Dejen
11	41324-B	27.86	4.19	6.08	50.36	42.47	53.65	96.19	133.56	1441.99	East Gojjam	Dejen
12	41324-C	33.40	5.41	7.60	39.85	44.92	56.69	97.67	145.96	1650.02	East Gojjam	Dejen
13*	30301-A	33.44	6.33	7.49	50.68	44.27	53.94	98.76	149.89	1698.88	East Gojjam	Enarji Enawiga
14*	30301-C	34.57	7.32	8.09	54.37	45.09	56.54	99.42	154.03	2536.81	East Gojjam	Enarji Enawiga
15*	30302-A	33.28	6.49	7.45	49.04	44.92	55.24	98.95	148.49	1721.42	East Gojjam	Enarji Enawiga
16*	30304-A	33.17	6.17	7.51	50.97	45.25	55.24	97.24	146.54	1719.14	East Gojjam	Enarji Enawiga
17	236493-A	35.02	5.77	8.00	49.06	43.62	55.24	97.04	149.20	1723.45	East Gojjam	Enarji Enawiga
18	41229-A	28.66	3.24	4.75	32.79	42.96	55.24	96.81	138.98	1123.42	East Gojjam	Enarji Enawiga
19	41231-A	39.56	7.03	9.48	54.47	47.38	59.00	101.41	157.23	2209.27	East Gojjam	Enarji Enawiga
20	41231-B	39.50	7.46	9.70	54.89	47.21	58.71	102.81	156.01	2920.38	East Gojjam	Enarji Enawiga
21*	30308-A	35.32	6.83	9.28	60.26	42.47	53.94	97.80	158.87	2722.21	East Gojjam	Hulet Ej Enses
22*	30309-A	34.87	6.56	8.66	60.94	42.80	54.08	97.17	155.26	2017.64	East Gojjam	Hulet Ej Enses
23*	30310-A	34.84	7.19	9.52	63.87	42.80	53.94	97.48	156.87	3264.40	East Gojjam	Hulet Ej Enses
24*	30311-A	35.11	6.69	8.77	62.24	45.41	56.54	96.06	146.19	2300.68	East Gojjam	Hulet Ej Enses
25*	30312-A	34.76	6.60	8.35	59.92	42.47	54.23	97.71	151.39	1710.52	East Gojjam	Hulet Ej Enses
26	41026-A	33.66	5.59	7.40	44.04	41.82	52.21	93.61	154.83	1755.21	East Gojjam	Hulet Ej Enses
27	41026-B	35.77	6.51	8.77	58.99	48.19	58.42	102.65	151.44	1728.15	East Gojjam	Hulet Ej Enses
28	41026-C	28.08	3.37	3.65	27.12	45.74	58.42	95.29	126.58	1072.85	East Gojjam	Hulet Ej Enses
29	41078-A	30.05	4.57	4.69	33.42	44.43	57.84	93.83	144.73	1217.57	East Gojjam	Hulet Ej Enses
30	41078-B	34.03	5.20	7.18	43.78	42.31	53.80	92.52	147.08	1815.90	East Gojjam	Hulet Ej Enses
31	41267-A	31.50	4.75	5.22	31.62	43.94	57.70	94.23	133.09	1237.32	East Gojjam	Hulet Ej Enses
32	41268-A	31.83	4.75	5.30	51.45	42.63	53.36	89.91	165.70	1192.41	East Gojjam	Hulet Ej Enses
33	41268-B	42.62	7.62	10.01	63.60	50.16	60.73	103.72	148.00	2103.95	East Gojjam	Hulet Ej Enses
34	41269-B	42.80	8.11	10.16	69.36	49.01	60.01	103.87	154.79	2033.77	East Gojjam	Hulet Ej Enses
35*	30317-A	35.97	8.43	10.43	75.31	43.65	55.59	98.53	163.53	2554.86	North Gonder	Belessa
36*	30318-A	36.37	9.65	10.45	77.28	44.60	56.54	101.34	163.12	2791.69	North Gonder	Belessa
37*	30319-A	35.87	8.95	10.41	69.63	43.08	54.85	99.43	165.35	3192.67	North Gonder	Belessa
38*	30320-A	35.49	7.55	10.32	72.46	43.62	55.88	98.98	161.27	2558.72	North Gonder	Belessa
39*	30321-A	35.72	7.50	10.30	63.71	43.78	56.40	100.05	160.18	2405.47	North Gonder	Belessa
40*	30322-B	33.49	7.26	8.37	62.26	46.95	58.16	96.19	158.05	1942.93	North Gonder	Belessa
41	207166-A	38.55	7.03	9.59	49.21	51.46	62.18	102.00	156.16	2447.51	North Gonder	Belessa
42	207170-A	30.14	4.66	5.81	41.41	44.27	57.41	87.65	151.61	1145.71	North Gonder	Belessa
43	241800-A	34.74	5.77	6.76	43.16	44.60	56.25	98.75	151.31	1645.48	North Gonder	Belessa
44	241800-B	33.37	5.72	8.22	43.57	43.94	55.24	98.31	148.90	1909.47	North Gonder	Belessa
45	241801-A	38.23	7.01	9.88	55.47	47.13	58.85	98.97	159.55	2332.07	North Gonder	Belessa
46	241801-C	35.03	6.17	8.42	59.45	46.39	57.99	98.59	157.04	1723.49	North Gonder	Belessa
47*	30327-A	38.17	9.87	10.23	68.30	42.96	55.38	101.34	164.32	3024.61	North Gonder	Takusa
48*	30329-A	38.07	8.74	9.35	64.09	44.68	55.38	103.37	162.07	2120.28	North Gonder	Takusa
49	227160-B	39.51	7.26	9.72	65.54	49.01	60.30	100.23	152.29	1930.84	North Gonder	Takusa
50	227161-B	30.91	3.47	4.53	34.22	44.27	57.84	92.72	145.16	1403.55	North Gonder	Takusa
51	227161-C	31.28	3.58	3.49	29.39	44.27	55.96	87.37	141.56	1113.13	North Gonder	Takusa
52*	30331-A	35.31	6.38	7.82	56.15	42.31	54.23	95.51	155.75	1944.94	North Gonder	Chiliga
53*	30331-B	34.67	6.13	7.62	55.17	43.78	56.83	94.48	150.88	1938.78	North Gonder	Chiliga
54*	30332-A	35.69	7.84	9.26	64.64	42.80	54.52	100.69	165.14	2114.17	North Gonder	Chiliga
55	207143-A	32.92	4.01	4.24	37.02	42.80	53.94	86.29	143.49	1303.33	North Gonder	Chiliga
56	207143-B	36.44	6.58	7.49	58.31	47.05	58.71	98.34	142.84	1927.97	North Gonder	Chiliga
57	41046-A	38.01	6.76	9.08	49.48	47.41	59.00	99.37	160.20	2358.37	North Gonder	Chiliga
58	41046-B	34.84	6.67	7.38	54.63	44.11	55.24	95.06	150.70	1971.49	North Gonder	Chiliga
59*	30323-A	34.90	6.67	8.35	51.84	44.43	55.82	97.70	163.31	1649.27	North Gonder	Denbia
60*	30323-B	36.11	6.26	8.04	49.04	43.62	55.10	98.56	164.49	1819.50	North Gonder	Denbia
61*	30324-A	36.73	7.12	9.37	47.11	44.92	55.38	97.24	165.35	3128.21	North Gonder	Denbia
62*	30325-A	36.52	7.59	9.39	54.49	42.96	54.23	98.10	162.50	2700.30	North Gonder	Denbia
63*	30326-A	36.07	6.44	9.70	50.16	43.45	53.65	98.52	163.27	2350.65	North Gonder	Denbia
64	225887-A	31.79	5.47	5.79	46.31	44.60	58.42	89.29	147.83	1263.65	North Gonder	Denbia
65	225887-B	33.05	5.86	7.38	37.98	44.76	56.83	96.77	162.35	1810.56	North Gonder	Denbia
66	227158-A	27.72	3.53	4.31	29.56	42.96	54.95	92.76	130.34	1107.26	North Gonder	Denbia
67	227158-C	28.27	2.97	4.44	28.85	43.78	54.37	87.81	160.24	1088.66	North Gonder	Denbia

Appendix 6: Continued....

No	Genotype	PLH	NPB	NSB	NPPP	DTF	DTP	DTM	TSW	GY	Zone	District
68	41306-B	30.96	4.35	5.90	50.29	44.11	55.96	91.25	148.26	1245.79	North Gonder	Denbia
69	41310-A	31.10	4.66	5.77	43.47	44.04	55.10	94.68	146.22	1265.39	North Gonder	Denbia
70	41310-B	30.33	3.01	5.22	27.79	43.29	53.22	89.26	143.49	1071.13	North Gonder	Denbia
71	41311-A	33.99	5.18	7.00	43.18	44.92	56.40	96.83	155.00	1684.21	North Gonder	Denbia
72	41311-B	33.82	5.92	7.47	49.70	43.29	53.94	98.30	150.96	1744.70	North Gonder	Denbia
73[*]	30339-A	36.78	10.01	11.09	77.21	45.58	56.69	99.38	166.66	2476.69	South Gonder	Fogera
74[*]	30340-A	37.46	10.14	10.58	79.88	45.41	56.11	100.24	165.42	3029.73	South Gonder	Fogera
75[*]	30341-A	36.38	8.29	9.46	65.31	47.21	57.55	97.68	166.66	1707.20	South Gonder	Fogera
76	207145-A	27.93	5.81	5.66	34.38	43.45	55.38	86.72	136.19	1158.40	South Gonder	Fogera
77	207145-B	31.83	4.39	5.28	37.09	43.29	54.95	92.31	137.99	1223.28	South Gonder	Fogera
78	41295-A	27.07	3.42	3.14	25.29	43.12	54.81	94.46	137.02	1101.19	South Gonder	Fogera
79	41295-B	34.58	6.04	8.40	45.80	43.94	55.96	97.04	154.61	2041.91	South Gonder	Fogera
80	**Mastewal**	38.9	11.4	13.7	56.6	51.8	62.8	111.2	236.2	2641.0		
81	**Worku**	35.7	12.6	13.1	50.1	49.3	60.3	103.1	222.9	2255.3		
	Mean	**34.46**	**6.38**	**7.99**	**51.71**	**44.78**	**56.25**	**97.24**	**155.31**	**1946.56**		
	LSD	**2.72**	**1.26**	**1.63**	**7.79**	**3.16**	**3.62**	**3.58**	**12.66**	**437.17**		

PLH=Plant Canopy Height (cm), DTF=Days to 50% flowering, DTP=Days to 50% podding, DTM=Days to 90% maturity, NPB=Number of primary branches, NSB=Number secondary branches, NPPP=Number of pods per plant, TSW=Thousand Seed weight, GY in kg ha^{-1}=Grain yield *=2017 collection and the remaining are 1979 to 1983 collections